This is to certify that the

dissertation entitled

Lysine Adequacy of the Lactating Cow

presented by

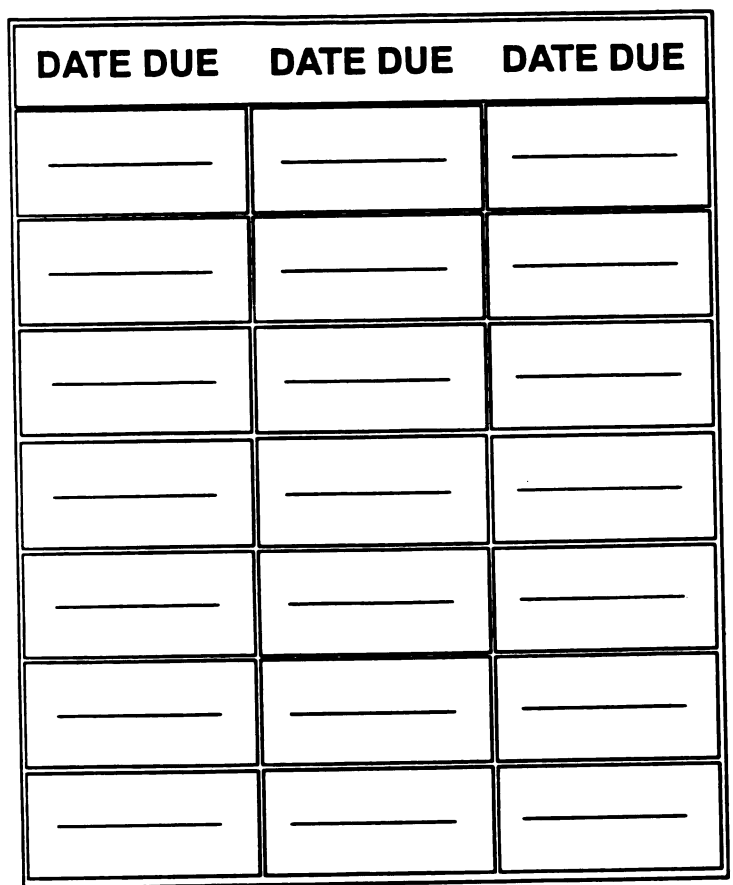
Kenneth J. King

has been accepted towards fulfillment
of the requirements for

Ph.D. degree in Animal Science

W.G. Bergen
Major professor

Date 5/12/1989



Lysine Adequacy of the Lactating Cow

By

Kenneth J. King

A DISSERTATION

Submitted to Michigan State University
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Department of Animal Science

1989

ABSTRACT

LYSINE ADEQUACY OF THE LACTATING COW

By

Kenneth J. King

The objectives of this research were to determine the influence of dietary protein source on amino acids available for digestion and metabolism, and to quantify post-ruminal lysine required by the lactating cow for high milk production. Three lactation experiments were conducted. In experiment 1, six lactating Holsteins, with duodenal T-cannulae, were used in a double 3 X 3 Latin square experiment to determine the influence of dietary protein source (blood (BM), corn gluten (CGM), and cottonseed meal (CSM)) on amino acid profiles of duodenal chyme and plasma. Experiments 2 and 3 were conducted to determine the quantity of post-ruminal lysine necessary to meet the lysine requirements for lactating cows. In experiment 2, six lactating Holsteins, with abomasal cannulae, were used in a double 3 X 3 Latin square experiment containing an extra period. Experiment 3 used twelve lactating Holsteins, fitted with abomasal cannulae, in a 5 X 5 Latin square experiment with two replacement animals. Cows were fed a

corn grain-corn silage diet containing a corn gluten meal supplement similar to that of experiment 1. L-lysine·HCl was infused abomasally at levels of 0, 45, and 90 g/d in experiment 2, and 0, 22.5, 45, 90 and 180 g/d in experiment 3. BM and CGM increased protein passage to the duodenum (23%) above that which was consumed ($P<.05$). Patterns of amino acids flowing into the duodenum closely reflected diet differences. Apparent digestible nitrogen ($P<.05$), net protein utilization ($P<.10$) and protein biological value ($P<.10$) was greatest for CSM, intermediate for CGM, and least for BM. The first three limiting amino acids measured by mammary extraction coefficients for BM were methionine, threonine, phenylalanine; for CGM they were threonine, methionine, lysine; and for CSM they were lysine, methionine, histidine. Thus rumen undegraded dietary protein influenced quantity and quality of amino acids available for digestion and metabolism. A positive linear response to lysine infusion occurred for milk protein synthesis and plasma lysine concentration ($P<.05$). In experiment 3 a quadratic response to infusion was found for venous lysine concentration ($P<.05$). The lysine requirement for cows in experiment 3 was estimated at 225 g of digested lysine/d. I conclude that lysine was the limiting amino acid for milk production in Holstein cows fed predominantly corn proteins.

Dedicated in loving memory to my father,

Dudley Clement King, Jr.

ACKNOWLEDGEMENTS

This research would not have been possible without the contributions of many people. The coordinated skills of Dr. Mary Schmidt and the dairy farm employees is gratefully acknowledged. I am indebted to Alan Grant and Doug Grieve, for their aid in all aspects of this research.

The guidance of my graduate committee, Drs. H. A. Tucker, K. Ames, J. T. Huber, J. W. Thomas and W. G. Bergen, is appreciated. I was particularly fortunate to have the input of Drs. J. Tal Huber, Werner G. Bergen and J. W. Thomas, my three major advisors.

The support from my mother, Dorothy King, and my siblings, Deborah, James, Steven, Donald, Gary, Robert, and Sandra was more than I deserved.

Last, I would like to acknowledge my wife, Vickie who provided statistical and emotional support, and my daughter, Chantal Elizabeth, whose life has just begun.

TABLE OF CONTENTS

LIST OF TABLES

Table | Page

LIST OF FIGURES

LIST OF ABBREVIATIONS

AA	amino acid
ADF	acid detergent fiber
ATP	adenosine triphosphate
AV	arteriovenous
BM	blood meal
BV	biological value = (ingested N - feces N - urine N)/(ingested N - feces N)*100
BW	body weight
°C	degree centigrade
CGM	corn gluten meal
cm	centimeter
CP	crude protein
CSM	cottonseed meal
cv	coefficient of variation
d	day
DHI	Dairy Herd Improvement
dl	deciliter
DM	dry matter
DMI	dry matter intake
DN	apparently digested N = (ingested N - fecal N) / ingested N * 100.
EAA	essential amino acid
FCM	fat corrected milk

Abbreviations (con't.)

g	gram
h	hour
HPLC	high pressure liquid chromatography
IN	ingested N
kg	kilogram
m	meter
ME	metabolic energy
mg	milligram
min	minute
MJ	megajoule
mm	millimeter
mRNA	messenger ribonucleic acid
NAN	nonammonia N
NDF	neutral detergent fiber
NEAA	nonessential amino acid
nM	nanomolar
NPN	nonprotein N
NPU	net protein utilization = (ingested N - feces N - urine N) / ingested N * 100
P	probability
PAA	plasma amino acid
PFF	protein free filtrate
R2	coefficient of determination
RDOM	rumen digested organic matter
RN	duodenal N
SCC	somatic cell count

Abbreviations (con't.)

SCM solids corrected milk

SNF solids not fat

tRNA transfer ribonucleic acid

UDN ruminally undegraded N

uM micromolar

VFA volatile fatty acid

INTRODUCTION

Dairy cows in early lactation need amino acids, which are supplied from three sources - 1) microbial protein synthesized in the rumen, 2) feed proteins resistant to rumen microbial degradation but potentially digested in the small intestine (rumen undegraded feed proteins), and 3) protein stored in body reserves. Computer-models have been developed which quantitatively define protein requirements for lactating dairy cows (102, 241); however, individual amino acid requirements are unknown. Low milk production can get sufficient protein from source #1 alone, but as milk production increases the other two protein sources are brought into action. Cows then rely more on proteins which pass through the rumen undegraded (102, 161, 201). Thus, quantity and/or quality of feed protein reaching the small intestine might influence milk yields under conditions such as in early lactation and very high milk yields.

Several feed and/or food by-products have the characteristic of supplying proteins which pass through the rumen undegraded. These protein sources have often increased growth efficiency of young cattle (57, 183, 219) and sheep (1, 44), and resulted in higher milk production in dairy cows (109, 119, 215). Information is needed on how the

quality of undegraded proteins in the ruminant diet might influence the array and quality of absorbed amino acids, since the amino acid complement of the undegraded fraction could contribute greatly to the total amino acid supply.

Many attempts have been made to determine the limiting amino acid for milk production and/or milk protein synthesis (33, 41, 49, 53, 66, 68, 185, 237). Few attempts have been made, however, to determine the quantity of an amino acid necessary to support a given level of milk production by lactating cows (45, 78, 184, 209). This, in part, is due to the difficulty of controlling the array of amino acids available for digestion (due to rumen microbial protein degradation and protein synthesis) and the difficulty in quantifying the amino acids passing to the intestine.

Casein infused abomasally increased plasma concentrations of essential amino acids in cows, which was associated with increased production of milk protein (49, 53, 66, 184, 185, 237). Cows infused abomasally with specific combinations of amino acids had similar milk production responses as casein-infused cows (208). Methionine, phenylalanine, threonine, lysine, and histidine have been suggested most often as the limiting amino acids for milk production in these infusion studies.

When corn products are substituted for other feed protein sources, duodenal flow and plasma concentration of lysine are reduced (38, 153, 162, 215). Therefore diets

containing corn gluten meal may result in lysine as the limiting amino acid for animal performance and present a logical animal model for determining lysine adequacy of lactating cows.

The objectives of these experiments were:

1) to determine the influence of three protein supplements (blood, corn gluten, and cottonseed meals) on duodenal and plasma amino acid profiles of lactating cows.
2) to quantify post-ruminal lysine required by the lactating cow for high milk production.

Research for objective 1) was conducted in conjunction with Mohammed Sadik's Masters of Science research project (198). Sadik quantified microbial protein passage to the small intestine of cows fed the three protein supplements. This entailed feeding ^{15}N-ammonium sulfate to cows and measuring ^{15}N enrichment of the microbial fraction in the duodenal chyme. Selected data from Sadik's thesis will be presented to facilitate understanding of experiment 1.

LITERATURE REVIEW

Protein nutrition for the dairy cow has been the subject of countless investigations, reviews, and symposiums. Research in this area continues to lead to improvement in understanding nutrient digestion and metabolism for milk production. Recently, improving protein quality for dairy cows by addition of amino acids (AA) has been a subject of several investigations. This review will examine the AA nutrition for the lactating dairy cow.

Protein quality

A good quality protein results in a balanced complement of AA absorbed from the small intestine which can than be used by body tissues for efficient metabolism. Poor quality proteins are characterized by being excessive or deficient in one or more AA, and (or) protected from enzymatic digestion by physical or chemical cross-links. Protein quality can be improved by one or more processes such as 1) adding one or more essential AA, 2) processing proteins to prevent cross-links, 3) treating proteins to break cross-links. Several methods for determining the relative quality of proteins are described by Hegsted (96). These methods involve chemical assays, microbial assays, *in vitro*

enzymatic digestion followed by assay, or *in vivo* balance studies or growth responses. These methods compare a reference protein, usually egg or casein, to the test protein.

In vivo methods require nitrogen (N) balance studies for determining Biological Value (BV) or Net Protein Utilization (NPU). BV is a measure of the proportion of digested protein retained by the animal (BV = [consumed N -feces N - urine N] / [consumed N - feces N]). The Thomas-Mitchell procedure for BV contains methods to determine the endogenous protein contribution to both the urine and feces, and corrects the equation to give the true BV of the dietary protein source studied. Similar to BV is NPU; however, it is expressed as the proportion of ingested N that is retained (NPU = [ingested N - feces N -urine N] / ingested N]).

If experimental conditions are well defined, changes in plasma AA (PAA) concentrations can be useful for assessing AA status of animals. Bergen (19) reviewed factors which influence PAA concentrations when determining limiting amino acids in animals. The PAA pool is small and can be greatly altered by the physiological as well as the nutritional status of the animal. Low plasma concentrations of essential AA (EAA) may be a result of either a dietary protein deficit or increased uptake of EAA for protein synthesis; high plasma concentration of EAA may be a result of dietary protein excess or extensive net catabolism of body protein.

TABLE 1. Selected plasma amino acid profiles for ruminants.[a]

Amino acids	Sheep[b]	Bulls[c]	Steers[d]	Dairy cows[e]
	umol/dl			
Lysine	10.4	8.8	10.0	6.6
Methionine	3.4	.8	2.9	2.6
Cystine	2.4	5.7	1.2	3.8
Valine	20.0	22.0	11.0	17.6
Isoleucine	8.8	8.7	8.9	8.5
Leucine	15.0	14.3	17.6	14.6
Phenylalanine	6.0	4.4	5.5	3.7
TEAA[f]	91	85	73	84
TNEAA[g]	167	96	125	93
TAA	258	181	198	177

[a] from Bergen (19).
[b] Bergen et al. (21).
[c] Boling et al. (30).
[d] Oltjen and Lehman (155).
[e] Broderick et al. (33).
[f] Total essential amino acids, sum of Lys, His, Arg, Thr, Val, Met, Cystine, Ile, Leu, Tyr, Phe.
[g] Total nonessential amino acids, sum of Ser, Glu, Gln, Asp, Ala, Gly, Pro, Orn, Cit, 3-Methyl His, N Methyl, Lys.

Under these conditions PAA profiles will not necessarily reflect dietary AA patterns or protein status.

Normal PAA profiles of various ruminants are listed in table 1. Variation in AA concentrations by dietary manipulation will influence their availability for organ metabolism (64, 141).

Types of Digested Proteins

Dietary protein for ruminants may include N containing compounds which are not used as protein by nonruminants. Amino acids for ruminant digestion are derived from microbial protein synthesized in the rumen from degraded feed N

and recycled urea, endogenous protein from gastric and intestinal secretions, sloughed epithelial tissue, and feed protein which escapes rumen microbial degradation.

The microbial biomass found in the rumen is comprised primarily of three different populations: bacteria, protozoa, and fungi. Bacteria produce ammonia (NH_3) as a product of fermentation from many types of N compounds and use NH_3 for synthesis of protein (129). Protozoa from the environment populate the rumen and are involved in proteolysis and deamination of dietary and bacterial proteins (82, 123). Little is known about the nitrogen metabolism of fungi, but these organisms appear to have specific AA requirements (87).

The AA profile of ruminally synthesized microbial protein passing to the abomasum is not markedly affected by diet composition (22, 117). Changes in individual species of bacteria by dietary manipulations do not influence the AA profile of the combined bacterial fraction (176); however, the AA complement of the protozoa fraction may be manipulated by diet. Tryptophan content of rumen protozoa may fluctuate with type of diet animals are fed (74). The change in tryptophan content of protozoa did not limit AA utilization or protein metabolism by mature sheep (74). Histidine was the most limiting AA for N balance when rats were fed protozoa protein and cystine was most limiting when fed bacteria protein (23). The potential limiting AA for

rats fed the combination of both populations were histidine, cystine, leucine, arginine and lysine (23). The BV, NPU values for bacteria and protozoa were 85.0, 63.4 and 82.0, 71.4%. respectively, compared with casein which had BV, NPU values of 89.5 and 87.0% (23).

Rumen microorganisms administered abomasally to sheep maintained by intragastric infusions of VFA and minerals, were 81% truly digested and had a NPU value of 54% (223). In companion trials, cystine and histidine were the least digestible of the AA (221), and methionine and lysine were the first and second limiting AAs for N retention (222). Salter and Smith (199) determined, using ^{15}N procedures, that bacteria protein digestibility was only 74% in steers.

Legumes and oil seeds are the most common sources of protein fed to ruminants; however, more competitively priced and (or) better quality proteins can be substituted. Plant and animal by-products from the food industry have been incorporated into diets of ruminants. These sources vary considerably in the proportion of protein which is degraded in the rumen (table 2). Protein quality depends on the AA profile of the fraction resistant to degradation in the rumen, which will be discussed later. Comparisons of AA profiles for commonly fed protein supplements and milk protein are in table 3.

Non-protein N (NPN) sources such as Dehy-100, Starea, biuret, isobutylidene diurea, urea, and NH_3, have also been

TABLE 2. *In vivo* estimates of undegraded protein for common feedstuffs when total DM intake is in excess of two percent of body weight.[1]

Feed	n[2]	Range[3]	mean	Adj. mean[4]
Grains				
Barley	2	.14- .28	.21	.20
Corn	3	.58- .73	.65	.50
Milo	8	.20- .69	.52	.50
Oil meals				
Peanut	2	.22- .37	.30	.25
Sunflower	2	.19- .28	.24	.25
Soybean	13	.10- .61	.27	.30
Cottonseed (solvent)	6	.24- .61	.41	.35
Cottonseed (prepress)	2	.35- .38	.36	.40
Cottonseed (screw press)	2	.43- .57	.50	.45
By-product feeds				
Corn gluten feed	2	.14- .26	.20	.20
Brewers dried grains	5	.27- .66	.53	.50
Corn gluten meal	3	.46- .51	.55	.55
Distillers grains	4	.47- .68	.56	.55
Blood meal	2	.54- .82	.68	.65
Meat and bone meal	2	.49- .70	.60	.65
Fish meal	6	.69-1.00	.80	.70
Forages				
Alfalfa silage	1	...	.17	.25
Alfalfa silage[5]	2	...	.33	.30
Alfalfa hay	6	.09- .41	.23	.25
Corn silage	1	...	.27	.30
Alfalfa (dehydrated)	4	.43- .66	.56	.50
Other				
Soybeans	0			.20
Cottonseed	0	...	...	.30

[1] from Satter (202).
[2] number of measurements.
[3] expressed as a fraction of total CP.
[4] means for *in vivo* measurements have been adjusted to reflect *in vitro* and *in situ* information on protein degradation.
[5] treated with formic acid and formaldehyde.

somewhat successfully incorporated into diets of ruminants (102). These NPN sources must be utilized as NH_3 by rumen bacteria before the ruminant will benefit from these as protein sources (102). Other NPN sources include peptides, nucleic acids, and free AA. These may be incorporated directly into the microbial biomass, converted to NH_3 and then used for protein synthesis; or passed directly to the abomasum for digestion and absorption in the intestine (5, 48, 196). Lactating cows may pass up to 34 g peptide N / d to the small intestine (48).

The contribution of endogenous protein to the AA pool in the intestine varies with the diet, and its contribution

TABLE 3. Essential amino acid composition of the total protein in common feedstuffs fed to ruminants (g/100 g total amino acid).[1,2]

Supplement	Arg	His	Ile	Leu	Lys	Met	Phe	Thr	Val
Milk protein	3.6	2.1	5.6	9.7	7.9	2.5	5.2	4.6	6.6
Alfalfa hay	4.5	2.0	4.0	7.5	4.5	1.5	5.5	4.5	2.5
Blood meal	4.0	5.0	1.1	12.8	7.6	1.2	6.9	4.4	8.1
Brewers grain	5.0	1.6	5.8	8.9	3.5	1.5	5.0	3.5	6.2
Corn grain	5.7	2.3	4.5	12.5	2.3	1.9	5.7	4.5	4.5
Corn gluten meal	3.3	2.3	5.4	17.7	1.9	2.3	6.8	3.3	5.1
Cottonseed meal	9.5	2.5	3.7	5.6	4.2	1.6	5.5	3.4	4.1
Oats grain	6.0	1.5	4.5	7.5	3.0	1.5	5.3	3.1	5.3
Rapeseed meal	5.5	2.7	3.6	6.7	5.3	1.9	3.8	4.2	4.8
Soybean meal	7.0	2.4	5.5	7.4	6.3	1.3	4.8	3.7	5.2
Wheat grain	5.6	2.1	4.2	7.0	3.5	1.4	4.9	2.8	4.2
Whey, dried	2.9	1.4	6.5	10.1	8.0	1.4	2.9	5.8	5.1

[1] from Ensminger and Oletine (72).
[2] Arg=arginine, His=histidine, Ile=isoleucine, Leu=leucine, Lys=lysine, Met=methionine, Phe=phenylalanine, Thr=threonine, Val=valine.

increases as the dietary protein intake increases (73). Endogenous N flowing into the rumen includes salivary secretions containing mucin and urea (115); non-ammonia N (NAN) derived from sloughed epithelial cells from the respiratory tract, mouth, and esophagus; keratinized epithelial tissue of the rumen; and urea entering through the rumen wall (160). These sources are mostly degraded to NH_3 in the rumen; however, Orskov et al. (160) measured high cysteine content in the AA profile of protein flowing to the abomasum in cows maintained with intragastric infusion of VFA. They suggested that the high cysteine content is characteristic of undegraded epithelial tissue.

The quantity of NAN flow to the duodenum derived from endogenous sources was variable (50 to 100 mg/kg $BW^{.75}$) between animals in experiments with cattle and sheep (160). Gastric secretions from the fundic region of the abomasum contributed little to the endogenous AA pool, and were also variable between animals (95). Tamminga et al. (228) estimated the endogenous contribution, originating from secretions in abomasal juice, pancreatic juice, bile, and epithelial cells, was approximately 4 g of N / kg dry matter ingested by cattle. Sklan and Halevy (211) estimated total endogenous contribution entering the small intestine at .9 g of N / kg BW of sheep.

Rumen Protein Metabolism

A goal of dairy cattle nutritionist is to maximize the quantity of amino acids available for digestion in the intestine. This entails maximizing microbial protein yield and undegraded feed protein passage. For this to occur, an optimum balance between degradable and undegradable protein must exist. Factors affecting degradation of feed proteins in the rumen are protein source, physical and chemical methods of processing, passage rate of digesta from the rumen, energy intake, rumen pH, and growth factors for rumen microbes (102).

The quantity of degradable protein necessary to maximize microbial growth in the rumen is determined by the amount of potentially rumen digestible carbohydrate in the diet (24). Microbial organisms require an available source of NPN and carbohydrate for growth. Adenosine triphosphate (ATP) is derived from the fermentation of carbohydrates to VFA. Bacteria utilize energy from ATP for synthesizing microbial cells (156). Efficiency of microbial cell (protein) yield is measured by the amount of energy used in the process of growth (synthesis). *In vitro* systems estimate microbial efficiency by measuring microbial protein yield from ATP generated during fermentation. Microbial protein yield as a proportion of ruminally digested organic matter (RDOM) is the most common measure of efficiency with *in vivo* systems (24).

Mehrez *et al.* (137) determined the optimum rumen NH_3 concentration needed to maximize the rate of dry matter fermentation *in situ* (nylon bags) was 23 mg NH_3/dl in sheep fed a barley diet. Others have shown that 5 mg NH_3/dl was effectively maximized microbial cell yield *in vitro* (197, 204). Schaefer *et al.* (205) observed that many predominant species of rumen bacteria grown in pure culture could achieve 95% of their maximum specific growth rate in a medium containing less than 2 mg NH_3/dl. Pisulewski *et al.* (172) found concentrations for maximal microbial growth *in vivo* varied between diets. Optimum concentration ranged from 2.5 to 10.2 mg NH_3/dl. The different optimum NH_3 concentrations for maximum cell yield could be a attributed to micro-environments within the rumen (156). Higher concentrations of rumen NH_3 may be required at times to insure an optimum concentration of NH_3 in the micro-environments of the rumen.

The tertiary structure of proteins is another important determinant of resistance to microbial degradation (124). Cereal grains and protein supplements contain four major classes of proteins that may be characterized by their solubility in different solvents. These include albumin and globulins which are of low molecular weight and soluble in rumen fluid (51). Prolamines and glutelins have greater molecular weights and contain disulfide bonds, making them less soluble in rumen fluid and more resistant to enzymatic

attack (51). There is generally a good correlation between dietary protein solubility and their potential for microbial degradation; however, proteins such as bovine serum albumin and ovalbumin are exceptions. These proteins are soluble but their numerous disulfide cross-links prevents accessibility by proteases (133).

Various processing methods decrease the extent of protein degradation in the rumen without impairing AA availability further down the tract. These techniques involve formation of a limited number of cross-links. One method is to treat proteins with aldehydes (8, 12). Condensation reactions between the aldehyde and the AA form stable methylene cross-links between protein chains. The methylene cross-links are not hydrolyzed until exposure to pepsin in acidic conditions of the abomasum. Heat processing is another common method of forming cross-links between proteins to reduce degradability. The heat causes carbonyl groups of sugars to combine with free amino groups of proteins in the Maillard reaction (28, 55). Even in the absence of sugars or carbohydrates, extensive heating causes amide bonds to form between the NH_2 group of lysine or other free amino group and carbonyl groups of proteins (27). These linkages are more resistant than peptide bonds to enzymatic hydrolysis. Heat treatment of soybean meal may reduce protein solubility without effecting its degradability (116). Kung (119) showed soybean meal heated to 149

°C for 4 h had a reduced degradability from those heated at lower temperatures or for less time, even though their solubilities were similar. However, care must be taken to avoid excessive alteration of the protein rendering the protein indigestible in the intestine (231).

Diets which influence turnover time of rumen DM will affect protein degradation. The influence of ruminal turnover on protein degradation is described in a scheme proposed by Pichard and Van Soest (170). Protein degradability was calculated by the equation:

$$UDN = A + [B_1 \cdot kB_1/(kB_1+kr)] + [B_2 \cdot kB_2/(kB_2+kr)]$$

where UDN is the undegraded protein, fraction A is a water soluble NPN fraction that includes nitrate, ammonia, amines, and free amino acids, which are degraded rapidly and completely. The insoluble component is composed of a rapidly degraded fraction (B_1), a more slowly degraded fraction (B_2), kB_1 and kB_2 are the degradation rates, and kr is the rate constant for rumen DM turnover. An indigestible fraction (C) is calculated by subtracting the A and B fractions from the total protein. A more simplistic model used by Laycock and Miller (121) is calculated by the equation:

$$UDN = A + (1-A) \cdot [kd/(kr+kd)]$$

where kd is the rate of degradation for the insoluble fraction; A, kr as above, while 1-A equals fractions B_1, B_2 and C in the Van Soest scheme (170). Lindberg (125) reported slower rumen turnover time of protein from cottonseed meal than for either rapeseed meal or soybean meal.

Tamminga et al. (228) fed lactating dairy cows mixed diets consisting of long meadow hay and ground, pelleted concentrates containing a mixture of proteins. As dry matter intake of the same feeds was increased from 8.2 to 12.9 kg/cow/d, the fraction of rumen undegradable protein in the diet increased from 29 to 45%. Zinn and Owen (248) fed high concentrate diets at various levels of intake to steers. Increases were noted for the portion of feed protein reaching the duodenum, the amount of bacteria protein synthesized, and microbial efficiency with increased intake. Feed intake alters passage and supply of intestinally digested protein, need for degradable N in the rumen, and efficiency of microbial growth (20, 248).

Diets containing large amounts of readily fermentable carbohydrates have lower ruminal protease activity (128). It was postulated that lower rumen pH will reduce protein solubility and accessibility of protein for ruminal degradation. Strobel and Russell (224) observed decreased methane production and VFA production when rumen pH was lowered from 6.7 to 6.0. Bacterial protein synthesis was also reduced 34

to 69%. The reduction of bacterial protein synthesis was greater than the decreases in ATP production. These investigators suggested the lower pH diverted energy to non-growth functions (eg. maintenance and futile cycles).

Microbial yields have also been increased with the addition of branched chain fatty acids (62, 163, 164), sulfur (149), and nicotinic acid (181).

Protein systems have been developed to describe protein partitioning in lactating cows in an effort increase efficiency of protein utilization by lactating cows (2, 40, 43, 241). These systems balance the proportion of degradable and undegradable protein in the diet. This balance is dependant on the quantity of ingested RDOM. Differences among protein systems for their requirement of microbial degraded protein are large (59 to 93% of total protein) for the production of 10 kg of milk. Differences remain large (45 to 80% degradable protein) for production of 40 kg of milk. Thus, there is still a need to define what proportion of microbial degraded protein is required. Protein systems which require less degradable protein assume lower microbial efficiency (i.e. microbial protein / RDOM). Protein degradability of many common diets for dairy cows is considered to be near 70%.

Protein Flow to the Duodenum

Several investigators have proposed models to predict the AA flow to the duodenum (39, 110, 111, 188, 189, 194, 203, 214). Amino acid flow to the duodenum in dairy cows has been related to digestible OM (DOM) intake as a measure of fermentable energy for microbial protein synthesis. Coefficients of determination reported by Tamminga and van Hellemond (229) and Rohr et al. (187) were .90 and .85, respectively. The Agriculture Research Council (2) estimated non-ammonia N (NAN) flow to the duodenum using a regression model which contained both metabolic energy (ME) intake and N intake as independent variables. The coefficients of determination for this model were .75 for sheep and .97 for cattle. Estimates for NAN flow based solely on energy and N intakes would be inaccurate, however, when large differences exist in feed protein degradability (241). Journet and Verite (110) related NAN flow to DOM intake and insoluble N intake, which improved the coefficient of determination (R^2=.93). Rohr et al. (188) proposed prediction equations for NAN flow based on ME intake, estimated endogenous N, and undegradable feed N. Variation between their equations and that of published in vivo data was small. There was also little variation in the proportion of NAN present as AA-N. The equations for their models are:

NAN(g/d)= 1.82*ME(MJ/d) + UDN(g/d), c.v.= .091;

NAN(g/d)=1.61*ME(MJ/d) +2.34*DMI(kg/d) +UDN(g/d), c.v.=.090

AAN(g/d)= .70*NAN(g/d) -.50, c.v.=.072.

Wanderley and Theurer (241) showed duodenal flow of N was 30% greater than ingested N in steers fed concentrate diets and N flow to the duodenum was similar to the amount of ingested N when fed forage diets. They also found evidence of greater bacteria synthesis for the concentrate diet. Their data suggested a beneficial effect of non-structural carbohydrates on duodenal N flow and essential AA flow. These data demonstrate that diets providing greater quantities of RDOM will have greater passage of microbial protein to the small intestine.

Degradation of Feed Proteins

To measure dietary protein passage from the rumen it is necessary to surgically prepare animals with cannulae in the omasum, abomasum, or proximal duodenum; have a suitable method to calculate flow rate of digesta; and use a reliable marker for identifying microbial contribution to total protein flow (217). Since the microbial AA profile does not markedly change, estimation of AA passing from the protein source can be determined by subtracting the microbial contribution from the total AA in duodenal chyme. The only flaw in this procedure is that the passage of endogenous amino-N is not considered.

A regression technique to measure protein degradability of individual dietary components was used by Stern *et al.* (215, 217) to determine the degradability of corn gluten

meal. The test protein was added to the ration of cattle in incremental amounts while DMI was held constant. Microbial N contribution to the duodenal digesta was assumed to be constant between diets since the rations were of similar fermentabilities. Duodenal AA flow was regressed on AA intake, and the increased flow of an AA was attributed to the test protein. The slope of the regression line represented the undegraded test protein. This method was also employed to determine the degradability of individual AA of the test protein.

Several *in situ* and *in vitro* procedures were developed which are not as labor intensive or costly as *in vivo* procedures (32). *In situ* procedures involve suspension of nylon bags containing the test protein in the rumen. Bags are retrieved at various intervals until only the undegradable (Van Soest's fraction C) protein remains (150, 158). The rate at which the test protein was degraded from the bags and an estimate for rumen digesta passage rate, allows for the estimation of protein degradability (121). Comparisons of *in situ* and *in vivo* measurements for protein degradability were similar for soybean meal and brewers wet grains but as different for corn gluten meal in a study by Stern *et al* (214). *In situ* methods underestimated degradable protein in this study.

In vitro procedures for determining protein degradation involve incubating test proteins with proteolytic enzymes

and measuring protein loss or NH_3 accumulation. Results are then compared with a reference protein (175). These procedures currently serve as a qualitative assessment for protein or AA degradation, since rumen digesta passage rate is not evaluated.

Ruminal Degradation of Amino Acids

Feed AAs are not degraded equally by microbes (206); therefore, the AA composition of undegraded feed protein is different from the original protein (42). Degradation may also result in a significant change in the AA profile passing to the duodenum. Chalupa (42) observed arginine and threonine were the most rapidly degraded AA *in vitro* by rumen microbes. Regression equations indicated that alanine, arginine, histidine, lysine and phenylalanine were degraded to a greater extent than the total protein. Degradation of threonine, valine, isoleucine, leucine, methionine, glycine and tyrosine were similar to or lower than the total protein. Craig and Broderick (59) suggested that microbial proteases are serine protease-like, cleaving lysyl and arginyl linkages, which leads to greater exposure and destruction of lysine and arginine.

Using *in situ* studies, the undegraded protein of grass silage was determined to be devoid of methionine, lower in lysine than the original protein (190), and determined that branch-chained AA were most resistant to degradation (190, 235). The rate at which different AA were degraded depended

on the protein source (235). An *in situ* trial of Ganev *et al*. (85) found little difference in the AA composition of the original protein and the undegraded protein after incubation. These studies leave for debate whether undegraded feed protein has a similar AA composition as the original feed protein.

Duodenal AA profiles are not consistently influenced by the dietary AA composition. The proportion of microbial N present in the duodenal chyme is a major determining factor of duodenal AA profile; however, as protein needs exceed the microbial supply, residual feed protein becomes more important. Kung *et al*. (120) found no difference in AA profiles entering the duodenum of lactating cows fed normal or heated soybean meal. Cottrill *et al*. (57) fed growing cattle isonitrogenous corn diets containing increased proportions of fish meal in place of urea as the protein supplement. With the exception of reduced proportion of threonine, the AA profile of duodenal chyme was similar for all diets, despite an increased flow of AA from the residual fish meal protein. Significant differences in AA flow to the duodenum were reported for lactating cows fed corn silage diets supplemented with soybean meal, corn gluten meal, wet brewers grains or distillers grains (table 4). While the quantity of lysine reaching the duodenum was not different, the proportion of lysine to the total AA flow was reduced when corn products were fed (200).

TABLE 4. Individual amino acid intake and flow to the duodenum of cows fed four protein supplements.[1,2]

Diet	Arg	His	Ile	Leu	Lys	Met	Phe	Thr
	Intake, g/d							
SBM	122[a]	51[a]	95[a]	207[c]	111[a]	16[c]	115[b]	92[a]
CGM	83[b]	50[a]	97[a]	340[a]	60[c]	37[a]	144[a]	91[a]
WBG	80[b]	30[c]	77[b]	169[d]	65[bc]	20[b]	99[c]	70[b]
DDG	81[b]	45[b]	98[a]	240[b]	72[b]	37[a]	112[b]	84[a]
SE	4	2	3	5	3	1	3	3
	Flow to the duodenum, g/d							
SBM	93[b]	44[b]	137[bc]	194[c]	144	38	105[c]	111[b]
CGM	108[a]	56[a]	151[a]	326[a]	142	53	154[a]	131[a]
WBG	94[b]	42[b]	127[c]	184[c]	136	41	108[c]	109[b]
DDG	111[a]	57[a]	147[ab]	267[b]	147	48	133[b]	129[a]
SE	3	2	4	8	5	5	4	4

[1] From Santos et al. (200).
[2] Means in the same column with different superscripts differ (P<.05).
SBM=soybean meal, CGM=corn gluten meal, WBG= wet brewers grains, DDG=distillers dried grain.
Arg=arginine, His=histidine, Ile=isoleucine, Leu=leucine, Lys=lysine, Met=methionine, Phe=phenylalanine, Thr=threonine.

Stern et al. (215) also showed that diet affected AA passage in lactating cows fed increasing amounts of corn gluten meal in the diet, which reduced the proportion of duodenal lysine. They estimated that only 10% of the lysine in corn gluten meal passed to the duodenum while a greater proportion of all other AA passed. This suggests the AA profile of the duodenal chyme can be modified with choice of dietary protein supplements.

Post-Ruminal Digestion of Protein

The process of protein digestion in ruminants has been reviewed (18, 210). In ruminants, proteases secreted by the abomasum and pancreas hydrolyze dietary protein into small peptides. Amino acids are then released from these digestion products by peptidases within cells of the small intestine wall. Pepsin is a gastric endoprotease which hydrolyzes peptide bonds containing hydrophobic AA residues (phenylalanine, tyrosine, leucine, methionine). The pH optimum for pepsin activity is pH 2.1.

Proteolytic enzymes of the pancreas include endopeptidases (trypsin, chymotrypsin, and pancreatopeptidase E) and exopeptidases (carboxypeptidase A and B). Endoproteases hydrolyze peptide bonds from within protein chains and exoproteases attack terminal bonds of proteins and peptides. Trypsin hydrolyzes peptide bonds containing a carboxyl group of arginine or lysine. Chymotrypsin hydrolyzes peptide

bonds adjacent to carboxyl groups of aromatic and large hydrophobic amino acids (tyrosine, tryptophan, phenylalanine, leucine, and methionine). Carboxypeptidase hydrolyzes C-terminal amino acids from peptide chains. The optimum pH for enzyme activity in the small intestine is between pH 7.5 to 8.0 for trypsin, chymotrypsin, and carboxypeptidase and pH 8.8 for pancreatopeptidase E (18, 89). Proteolytic activity of pepsin occurs throughout the ruminant duodenum due to the slow neutralization of digesta passing from the abomasum, and activity of pancreatic enzymes are not optimum until reaching the mid-jejunum (17).

The intestinal mucosa secretes a number of peptidases which are N-terminal exoproteases (18). Free AA are released by peptide hydrolysis. Peptidase activity is least in the duodenum, increases through the jejunum, peaks in the mid-ileum, then declines to the terminal ileum (18, 210). Longer chain-length peptides (greater than four) are hydrolyzed on the brush-border membrane. Tri-peptide hydrolysis occurs evenly between the cytosol of the enteric cells and the brush-border, and dipeptide hydrolysis is localized in the cytosol (180).

Although the mid-ileum is the site of greatest peptidase activity, it has not been shown to be the area of maximal absorption of nitrogenous components *in vivo*. The mid-jejunum is the area of greatest absorption, accounting for 57% of the loss of free AA and 41% of the loss of peptide

linked AA along the small intestine (17, 226, 245). The mid-jejunum region is also associated with the greatest activity of gamma-glutamyl transpeptidase (15), an enzyme suggested to be involved in AA transport (138).

Intestinal availability of rumen undegradable protein has been estimated by inserting ruminally incubated nylon bags into the intestine and recovering them from the feces (29). Intestinal availability of residual corn gluten meal, fish meal, meat and bone meal, soybean meal, canola meal, and alfalfa hay were 95, 83, 73, 99, 79, and 71%, respectively. However, Sklan and Halevy (211) determined the rate limiting step for peptide and AA absorption is breakdown of soluble protein fractions which have molecular weights of 7,000 to 14,000. This size protein fraction would pass through nylon bags and possibly flaw the procedure used in (29). Effect of peptide chain length effects on N absorption was studied by Grimble et al. (89). Mixtures of tri- to penta-peptides from hydrolyzed egg protein were more slowly absorbed than mixtures of di- or tri- peptides. Sklan and Halevy (211) determined intestinal AA disappearance was approximately 55% in sheep. Apparent digestibility of AA entering the duodenum of lactating cows was approximately 73% when fed whole soybean, soybean meal, or corn gluten meal, and 63% when fed brewers grains or distillers grain (200, 216).

Various protein systems use a set estimate for intestinal AA absorption. Bacteria protein is assumed to be 80% digestible and the undegraded protein fraction ranges from 70 to 90% digested (241).

Cellular Transport of Amino Acids

The small intestine is the primary site of AA absorption into the blood. The process involves: simple diffusion, facilitated diffusion, and "active" transport. Regulation of these transport systems was discussed by Guidotti *et al.* (91), Munck (147) and Stevens *et al.* (218), and is summarized in table 5. Amino acid diffusion contributes significantly to transport across both the brush-border and basolateral membranes. As much as 25% of the phenylalanine may be absorbed via diffusion when present at physiological concentrations (218). Both basolateral and brush-border

Table 5. Amino acid transport pathway in jejunal membrane vesicles.[1]

Pathway	Occurrence[2]	Na dependent	Substrate
Diffusion	BB+BL	---	---
NBB	BB	yes	most neutral AA
IMINO	BB	yes	imino acids, Pro, MeAIB
PHE	BB	yes	Phe, Met
y^+	BB+BL	no	cationic AA
L	BB+BL	no	Leu, branched and ringed AA
A	BL	yes	MeAIB, short-chain polar AA
ASC	BL	yes	3 and 4 carbon neutral AA, Ala, Ser, Cys

[1] From Stevens *et al.* (218).
[2] BB and BL mean found on the brush-border and basolateral membrane, respectively.

membranes possess Na-dependent and Na-independent carriers. Only the L and y^+ systems are common to both membranes. The L system is ubiquitous in eukaryotic plasma membranes. Basolateral-membrane pathways are similar to the those distributed on nonepithelial cell membranes such as hepatocytes, reticulocytes, and fibroblasts (218). These pathways include L, A-like, and ASC-like carriers. The brush-border membrane Na-dependent pathway (NBB, IMINO, PHE) are unique to brush-border membranes of the intestine and kidney (218). There has recently been characterized on the brush-border membrane a specific transporter for acidic AA which is Na- dependent (168).

In vitro intestinal transport of AA has been investigated in sheep (108, 169, 245) and cattle (60, 90, 146). The order of AA uptake in sheep from exteriorized intestinal loops was isoleucine > arginine ≥ valine ≥ leucine > methionine > phenylalanine > lysine > tryptophan > aspartate ≥ serine > alanine > proline > histidine ≥ threonine ≥ glutamate > glycine (245). The order of AA disappearance from the intestinal tract of lactating cows fed soybean meal protein was methionine > arginine > histidine > glycine > lysine > phenylalanine > leucine = aspartate > valine > proline = glutamate > tyrosine > alanine > threonine = cysteine = serine > isoleucine (216). Similar orders of amino acid absorption were demonstrated for lactating cows fed raw soybeans, heated soybeans, corn gluten meal, brewers

grains or distillers grains (200).

Johns and Bergen (108) determined that Vmax for methionine, lysine, and glycine absorption was 5.8, 1.52, and 670 umol AA/100 mg gut wet tissue/.5 h, suggesting glycine had the greatest affinity for absorption. Reichl and Rothschild (178) determined an intestinal absorption rate for 11 AA with a regression equation for entry rate into the tissue (nM / 100 mg gut dry weight / min) and concentration of AA in the medium (uM).

Amino Acid Metabolism

Amino acids and NH_3 are absorbed in the portal blood where large amounts are removed by the liver and others are transported to peripheral tissues for use the turnover of body protein and production processes (25). Amino acids are not stored in the body. Unless used for synthesis of protein or other essential compounds, AA will be catabolized with the amino-N excreted as urea and the carbon skeleton oxidized to CO_2 via catabolic pathways (243). Feeding proteins with a poor AA balance to nonruminants increases excretion of dietary N in the urine, decreases N retention and reduces the BV (6).

Arteriovenous (AV) sampling is a method used to study nutrient flow among and within body organs. The technique involves sampling of blood at pre- and post organ sites and determines the change in concentration of selected nutrients. This information plus a valid measure of blood

flow provides a means of quantifying overall net flux of nutrient per unit of time (126). Baumrucker (14) described the interorgan transfer of AA based on data derived from non-lactating sheep (97, 98), and from mammary gland data of Bickerstaff et al. (26), and Clark et al. (52, 53). This is described in Figure 1.

The intestine and liver are rapidly proliferating tissues and require considerable quantities of nutrients for maintenance. Therefore, the AA which are taken up and released by intestinal tissue will not necessarily be presented to the liver or enter the general circulation. Tagari and Bergman (226) determined that 30 to 80% of those AA disappearing from the lumen of the intestine appeared in portal blood. There is removal of glutamine by the gut tissue for maintenance purposes. Alanine is synthesized from NH_2 and pyruvate within the intestine. Citrulline synthesized by the intestine from NH_3 and CO_2 is the major source of citrulline used by the kidney for synthesis of arginine (104, 246).

Huntington and Prior (103) investigated the influence of feed intake on net AA absorption into the portal-drained viscera of beef heifers fed 85% concentrate diets. Net absorption of individual AA as a percentage of AA intake increased as feed intake increased. Changes in the proportion of dietary protein escaping ruminal fermentation did not change the relative molar proportions of AA in the

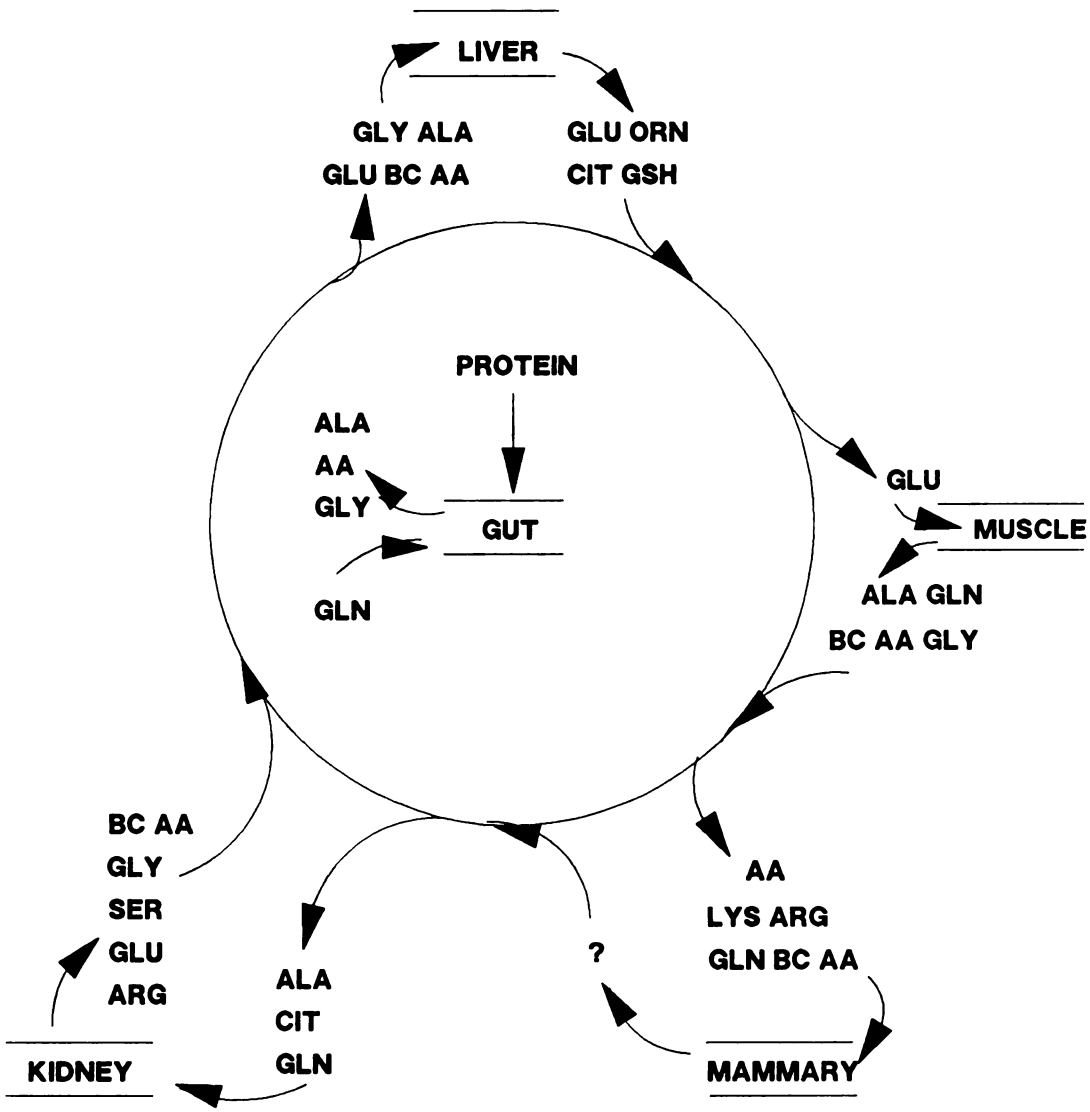


Figure 1. Principal amino acid flows between ruminant tissues. AA = amino acids, GLY = glycine, ALA = alanine, GLU = glutamic acid , ORN = ornithine, CIT = citrulline, GSH = glutathione, GLN = glutamine, SER = serine, ARG = arginine, LYS = lysine. Reproduced from Baumrucker (14).

portal blood. Relative to alanine, which had the highest portal appearance rate at all intakes, respective molar proportions of valine, serine, leucine, lysine, phenylalanine, and isoleucine were .75, .69, .61, .41, .29, and .34. Increased feed intake caused a linear increase in net absorption of lysine, methionine, leucine and valine. Net absorption of glutamate and glutamine was negative at all intakes, indicating their use as metabolic fuels by the gut. Mercer and Miller (143) also found a linear relationship of doudenal and plasma concentration of valine, threonine, lysine, isoleucine, and leucine in sheep.

A qualitative indicator of AA requirements for synthesis of body protein was determined by measuring the quantity of AA taken up from the blood by tissues of steers (103). Order of AA uptake by the round muscle was leucine > lysine > valine > isoleucine > arginine > phenylalanine > histidine > methionine and for the hind-half of the animal was valine > leucine > lysine > isoleucine > arginine > histidine > methionine > phenylalanine (103). These muscles metabolize aspartate to a considerable extent, and they synthesize alanine and glycine (3, 58).

Protein Reserves

Protein reserves are an important source of AA for bodily functions during periods of protein deprivation or stress (6, 31, 79). The cow relies heavily on body stores of energy and protein during early lactation (225).

Bott et al. (31) determined that total protein reserves may be as much as 27% of total body nitrogen. Plasma proteins, liver, and gastrointestinal tract comprise approximately 20% of these reserves and are the major source of AA for short term needs (225). Muscle and skin comprise the remaining 80% of the reserves and are the major sources for long periods of need (225).

Reid et al. (179) estimated that the lactating cow can mobilize up to .36 kg body protein per day to assist in milk production. Protein systems estimate body protein contribution for milk production at values between 12 and 22.5% of the body weight loss during early lactation (241).

Adaptation of muscle metabolism during lactation was investigated by Bryant and Smith (35). In early lactation weights of the longissimus dorsi and semitendinosus muscles were 37 and 28% lower than those for non-lactating ewes. Catabolism of this muscle served as a protein reserve during early lactation. Both muscles regained weight in late lactation. Fractional rates of protein synthesis were similar to that of non-lactating ewes, 2 and 3% for the longissimus dorsi and semitendinosus muscles. Total muscle protein synthesized each day was lower in early lactation because the protein pool was lower at this time.

Mammary Amino Acid Metabolism

Mammary supply of AA is determined by substrate concentration in the blood and rate of mammary blood flow. Amino acid supply to the udder is often inadequate to maintain maximum rate of milk synthesis (49, 127).

Transport

Transport systems for AA uptake by mammary tissue are similar to those discussed for the basolateral membrane of the intestine (14). Although there is no direct experimental evidence for the L or anionic transport systems, these are probably found on the cell membrane (14).

Nonessential AA (NEAA) are taken up by ruminant mammary tissue in amounts often inadequate to account for their output in milk protein. Evidence for intramammary synthesis of NEAA from glycolytic pathway and tricarboxylic acid cycle intermediates exists (139). Essential AA are classified into two groups (Figure 2). Group 1 are those AA where rate of uptake match their output in milk protein, and Group 2 are those where rate of uptake is in excess of milk protein output (80). Large excesses in arginine, and valine are taken up relative to their output into milk proteins (52, 63, 80). Isoleucine, leucine, and lysine may also be taken up in excess of output (26, 52, 140). These AAs are involved in the production of the NEAA in the mammary gland.

Several AAs show a significant positive correlation between arterial concentration and AV difference in ewes

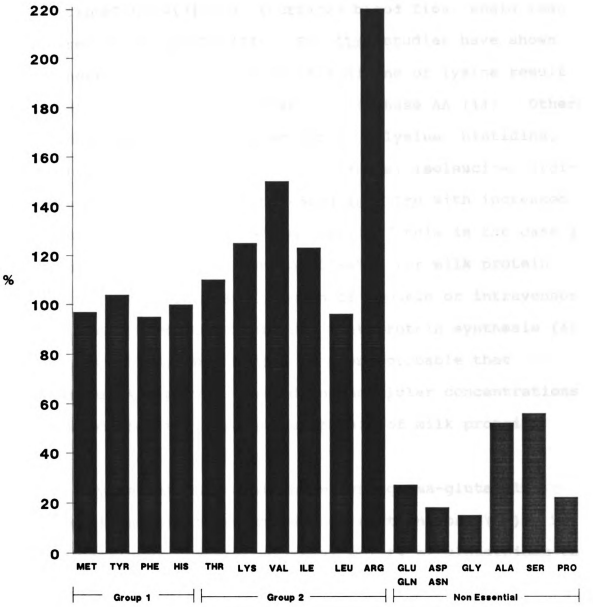


Figure 2. Amino acid uptake by the mammary glands of ewes as a percentage of the output of the same amino acids in milk. Reproduced Fleet and Mepham (80). MET = methionine, TYR = tyrosine, PHE = phenylalanine, HIS = histidine, THR = threonine, LYS = lysine, VAL = valine, ILE = isoleucine, LEU = leucine, ARG = arginine, GLU = glutamate, GLN = glutamine, ASP = aspartate, ASN = asparagine, GLY = glycine, ALA = alanine, SER = serine, PRO = proline.

(80), goats (140), and cows (168). Most transport systems operate well below saturation, and an additional supply (nutritional provision or increased blood flow) would lead to increased AA uptake (14). In vitro studies have shown that increased concentrations of arginine or lysine result in greater cellular concentrations of these AA (14). Others have shown that intracellular pools of lysine, histidine, tyrosine, leucine, valine, phenylalanine, isoleucine, arginine, and threonine were increased in vitro with increased AA concentration in the medium (54). If this is the case in vivo, then more EAA would be avail-able for milk protein synthesis. Post-ruminal infusion of protein or intravenous AA infusions have also increased milk protein synthesis (45, 49, 78, 184, 185, 209, 237). It seems probable that increasing AA supply increased intracellular concentrations of AA which led to increased synthesis of milk protein (141).

It may be possible that the enzyme gamma-glutamyl transpeptidase might be involved in AA transport (16). This enzyme catalyses transfer glutamyl moiety of glutathione to an AA by the equation: Glutathione + AA (extracellular) = gamma-glutamyl-AA + Cysteinyl-glycine (intracellular). This enzyme may also function by hydrolyzing both the extra- and intracellular glutathione to its constituent AA: glutamate, cysteine, and glycine (14). Enzyme activity in rat mammary glands increases at onset of lactogenesis in response to

increased blood prolactin concentration (16). All AA have the capability to be transported via this system; however, it is thought that this pathway is most important for the transfer of its constitutive NEAA (16).

Metabolism

The supply of NEAA to the mammary gland has been shown in some instances to be rate-limiting for milk protein output (95). Improved energy nutrition of dairy cows has increased milk protein content (71, 191). This improvement may be associated with an increased plasma concentration and mammary supply of nonessential AA (92). Mepham and Linzell (142), however, found no improvement in milk protein output when nonessential AA were infused into the mammary artery.

Up to 77% of the arginine metabolized by the bovine mammary gland was converted to ornithine and 20% was metabolized to proline (50). Part of the arginine and ornithine was converted to spermidine, which is involved in RNA synthesis (198). Alumot *et al.* (7) tested the hypothesis that the NEAA proline may limit milk protein synthesis, since it is found present in milk at much greater quantities than is taken up from the blood. The addition of proline post-ruminally decreased mammary uptake of arginine in cows fed low protein diets; however, it did not increase milk protein synthesis (7). The content of milk orotic acid, an end product of mammary NH_3 detoxification (50), was also reduced with proline infusion (7). It appears that the rate

of arginine metabolism by the mammary gland is sufficient to supply proline for milk protein synthesis. Neither arginine supply or metabolism was limiting milk protein synthesis.

Perfusion of guinea pig mammary glands with radiolabeled AA demonstrated the ability of the gland to catabolize AA. Histidine and phenylalanine were not catabolized by the mammary gland, leucine was oxidized to glutamic acid and valine was oxidized to CO_2 and to some extent citrate (65). It was also determined that radioactivity from the catabolism of [^{14}C]valine, leucine and isoleucine was transferred to beta-hydroxybutyrate, isovalerate and methylmalonate, respectively (Wohlt *et al.* as cited in 141). Five percent of the [^{14}C]lysine perfused into the guinea pig gland was recovered as CO_2 (Peeters as cited in 141). When excess lysine is supplied to the mammary gland the potential for lysine oxidation exists.

Baumrucker (14) suggested that alteration in plasma concentration of one AA *in vivo* may competitively inhibit uptake of AA sharing the same transport system. If the concentration of lysine was increased from 80 to 160 umol/ml more lysine would be provided to cells that have the Y^+ system; however, both arginine and ornithine uptake would be reduced (13). It was suggested that all three AA should be increased proportionally to increase lysine uptake without inhibiting uptake of other AA. This consideration assumes that the additions do not saturate the carrier (13).

Physiologically, most AA transport systems are operating at concentrations well below saturation (14). Competition for substrate by specific transport systems may limit milk yield and milk protein output to between 10 and 15% (14).

Synthesis

Once inside the cell, free AA become part of the free intracellular pool which is available for protein synthesis via activation to the amino acyl-t-RNA pool (4). There is a critical threshold concentration that free intracellular AA must exceed before protein synthesis occurs. Once the threshold is exceeded, increased free amino acids have an accompanied increased protein synthesis (69). It is at this level that the limiting AA affects protein synthesis (141).

The synthesis rate of milk proteins is primarily dependent upon the concentration of milk protein mRNAs provided there is a sufficient supply of the other components, including the AA involved in translation (141). Prolactin is responsible for the primary accumulation of casein mRNAs in the mammary gland of the rabbit both *in vivo* and *in vitro* (67, 100), and in the rat mammary gland *in vitro* (92). Prolactin controls accumulation of casein mRNAs by increasing the transcription rate of casein genes and the stabilizing casein mRNA (100). Glucocorticoids alone are unable to induce casein mRNAs accumulation in the mammary, however it is required along with prolactin to have maximal accumulation of casein mRNAs in the rat mammary (134).

Blood flow

Regulation of blood flow could be mediated through mammary production of a vasoactive agent, activity of mammary sympathetic nerves, or indirectly through galactopoietic hormones (64, 126). Blood flow per unit volume of udder tissue varies little throughout lactation, with a mean value of 43.3 ml/100 cm^3·min in sheep (80). Ratios for mammary blood flow to milk production were 300:1 and 400:1 for ewes and goats at peak lactation. Ratios increased to 570:1 in late lactation, when the udder contains a greater proportion of non-secretory tissue (80, 141). Kronfeld et al. (118) determined the ratio of blood flow to milk yield was 680:1 for cows. The rate of blood flow to the mammary gland is critical for supply of AA. Amino acids with high mammary extraction coefficients (AV difference as a percentage of the arterial concentration) make mammary AA supplies sensitive to small changes in blood flow (64).

Amino acid supply

Post-ruminal infusion of casein increased production of milk, milk protein, and milk fat by dairy cows (49, 53, 56, 107, 157, 184, 238, 244). The mechanism by which casein elicits the improved performance is not clear. Orskov et al. (157) and Whitelaw et al., (244) demonstrated that abomasal additions of casein to cows in early lactation increased plasma concentration of non-esterified fatty acids and induced a greater negative energy balance. These

workers postulated that casein infusions facilitated mobilization of body fat in support of milk production and showed that this response was greatest when energy intake was restricted. Infusion of glucose in this same study had no influence on milk yield or body fat mobilization.

Investigations by Whitelaw et al. (244) showed that insulin increased and growth hormone decreased with increased quantities of casein infusion. Istasse et al. (107) found insulin increased only in cows infused with casein in late lactation, and growth hormone did not change at any time. Cohick et al. (56) found no influence of casein infusion on plasma concentrations of growth hormone, insulin, prolactin, triiodothyronine, or thyroxine; plasma glucagon was increased with casein infusion. Oldham et al. (154) observed that growth hormone concentrations were increased by feeding formaldehyde-treated casein; however, they did not show a milk production response. Current information on casein's effect on hormonal status of the cow and how it relates to production is confusing and needs further investigation.

In most studies involving casein infusion total and EAA concentrations in the plasma were increased. This may suggest that casein supplementation increased the availability of AA for protein synthesis in the mammary gland. Lack of a significant increase in late lactation may be due to a reduction in uptake of AA by the mammary gland associated

with reduction in the extraction rate (80).

Limiting amino acid

As mentioned previously, plasma AA measurements can be utilized for the quantitative determination of an AA requirement when experimental conditions are well defined. Administering increasing amounts of a single limiting EAA post-ruminally will result in a two-phase response curve for plasma concentration of the limiting AA (19). Plasma concentration of the limiting AA will not change until the AA requirement is reached and then the plasma concentration will increase, this is considered the break-point (84). This approach has been useful in assessing AA adequacy in functional ruminants (75) as well as nonruminants (23, 81, 234). A two-phase response curve will also occur for production performance, an increase in production until the requirements are met and then it will plateau (145, 181, 234).

If a limiting EAA will not accumulate in the plasma until its requirement is met, then lysine, methionine, and valine were most limiting when incremental amounts of formaldehyde-treated casein were added to a basal diet containing 9% protein to achieve rations for lactating cows containing 11.2, 13.5, 15.7, and 18.0% CP (32). Data for the ratio of AA uptake by the mammary gland to their output in milk protein indicate that phenylalanine, methionine, lysine, histidine, and threonine are the five EAA utilized

most completely by the mammary gland for milk protein synthesis (26, 41, 49, 53, 66, 68, 106, 141, 185). These AA, therefore, may be in the most short supply for milk protein synthesis. If those AA which show the lowest concentration in the arterial plasma coupled with a high percent extraction are the ones which are most likely to be limiting for milk synthesis, then methionine and lysine often rank one and two, respectively (26, 49, 66, 68, 106, 185).

Methionine has often been regarded as a potentially rate-limiting AA for milk production (141). A series of studies by Rogers et al. (184) compared the responses of cows to abomasal infusion of casein, methionine, or glucose. Milk, protein and fat yields were increased similarly with abomasal infusions of casein and methionine, but not glucose. Intravenous administration of L-methionine to cows increased milk protein (78), fat (44) and yield (44). This suggests methionine was a major AA limiting synthesis of milk and its constituents.

Feeding methionine hydroxy analog during the first 4 months of lactation increased yields of milk fat and fat corrected milk (46, 88, 101, 131, 173) and milk yields (88), but not milk protein (131). Others have shown methionine hydroxy analog had no influence on milk production (36, 193).

Oldham (153) suggested that methionine may play a part in regulation of lipid transport through its effect on lipoprotein synthesis (136). Increased blood lipids have been observed when methionine hydroxy analog was fed to lactating cows (101, 167), therefore a methionine deficiency may limit lipid mobilization (244). This may be an explanation for the effect which casein infusion had on increasing blood lipids and milk fat in some experiments.

Other forms of methionine resistant to rumen microbial degradation for lactating cows have also been investigated with conflicting results (11, 41, 106, 114, 186). Milk production, stage of lactation, feeding systems, and methionine nutritional status are factors which may influence the efficacy of post-ruminal methionine. There may be a methionine deficiency when cattle are fed grass silage (45) or when soy products are used as the protein supplement (41, 208). Undegraded protein from grass silage may be devoid of methionine (45, 190, 232), while soybeans contain little methionine (72, 208).

Lysine may be the limiting AA in diets which contain predominantly corn proteins. Lysine content of duodenal digesta may be substantially lower in diets containing corn products than diets containing other feeds (152). Replacing corn with soybean meal decreased plasma lysine concentration in cows (162). Lysine is frequently present at lower concentration in plasma of steers fed corn based, urea

supplemented diets compared with those fed soybean meal (38, 61, 247). The decrease in the level of plasma lysine in cows and steers fed corn or urea as compared with those fed soybean meal suggests that lysine may limit performance in growing steers and lactating cows in which all the supplemental nitrogen is derived from NPN or corn. Abomasal infusion of up to, but not over 24 g lysine/d increased N retention of steers fed a ground corn diet supplemented with urea (37). No improvement in N retention occurred when 36 g lysine/d was infused (37) or when an incremental addition of methionine and 24 g lysine were infused (99). This suggests that lysine was the limiting AA for steers fed corn diets supplemented with urea.

Schwab et al. (209) abomasally infused various combinations of 10 EAA and sodium caseinate to lactating cows fed a 12% CP diet containing predominantly corn products. Infusion of methionine alone had no effect on yields of milk, protein, or fat. Lysine infused alone accounted for 16% of the total response in yield of protein obtained when all 10 EAA or sodium caseinate were infused. The combination of lysine and methionine resulted in 43% of the total response measured for casein. These results suggest that lysine and methionine were the first and second limiting AA. Threonine or isoleucine were suggested to have been the third limiting EAA. The primary response of AA infusion was on milk protein rather than yield, feed intake, milk fat, or

NPN content of milk (209).

Lysine and methionine supplied post-ruminally to cows fed diets containing a soy protein supplement increased milk protein output (186). When lysine was supplied alone, there was a depression in plasma methionine, suggesting an improvement in methionine utilization (186). Plasma concentration of lysine and methionine were increased when supplied post-ruminally.

Since differences between the first and second limiting AA may be small (33, 209), more than one AA may be needed to optimize performance of lactating cows. In other words, the quantity of milk mRNA synthesized may exceed the quantity of activated tRNA for more than one AA, presenting situations in which more than one AA may be limiting for the maximum translation of available mRNA.

Lysine Antagonism

Competitive interrelationships have been reported to exist between lysine and arginine. Excess lysine has been reported to inhibit arginine catabolism and urea excretion. This is due to the inhibitory action of lysine on arginase (207, 212). The competition may be species specific. Fish accumulate plasma arginine with excess dietary lysine, while pigs and dogs do not (70, 112). Neither weight gain nor feed efficiency was lowered by feeding twice the requirement of lysine to pigs; however, at three and four times the requirement, weight gain and feed intake were reduced, but

not feed efficiency (112). An AA imbalance and not an antagonism between lysine or arginine was suggested to be responsible for the reduced performance, since feed efficiency was not reduced (112). Excess lysine fed to pigs did not influence kidney or liver activity of arginase or ornithine transcarboxylase (112).

Basic AA metabolism is influenced by dietary electrolyte balance in pigs (10, 122, 166) and chicks (151). These interactions appear to be species- and tissue-dependent. Alkaline salts of sodium and potassium increased growth of lysine deficient pigs (122, 166), but not growth of lysine adequate pigs (144, 239). Studies by Forsberg (83) demonstrated that lysine utilization may be altered by dietary electrolyte balance or acid-base disturbances. Changes in acid-base balance by feeding acidic and basic AA may alter AA metabolism (83).

OBJECTIVES

Experiment 1 was designed to determine the influence of three protein supplements (blood, corn gluten, and cottonseed meals) on rumen digestion of nutrients, on amino acid profile of duodenal chyme and plasma and to quantify the partitioning of N in dairy cows producing a moderate quantity of milk. Protein supplements selected for this experiment varied considerably in their amino acid composition and degradability in the rumen. Experiments 2 and 3, were designed to feed lactating cows a diet potentially limiting in lysine and determine the amount of post-ruminal lysine required for the cows to be considered adequate in lysine. Dietary, in this case, post-ruminal amino acid adequacy (essential amino acids only) can be assessed based on the substrate excess principle. Thus when an amino acid is available below the requirement, if all other amino acids are available in adequate amounts, the given essential amino acid will be utilized with high efficiency. Reflected by low plasma and tissue pool concentrations (19). When the post-ruminal availability of a given essential amino acid is above that required, increments above needs are not used and will accumulate in tissue fluids which are then catabolized (19, 248). Hence changes in plasma concentration of a given

essential amino acid can be used to assess whether an essential amino acid is limiting, in excess or can be used to determine its requirement (19). In this particular study, if lysine is the limiting amino acid in the diet (post-ruminally), plasma lysine will be low until the requirements are met after post-ruminal infusion of lysine (19, 182). This type of response may also hold true for milk protein synthesis (increasing amount synthesized until requirements are reached). Digestion and metabolism of nitrogen were also evaluated.

The working hypothesis is that lysine is not limiting milk production in lactating cows fed corn - corn silage based diets. Specific aims of this study were 1) to compare the effect of feeding BM, CGM, or CSM supplemented lactation diets on lactation performance, rumen and post-rumen digestion, N partitioning, diet protein escaping rumen degradation and duodenal amino acid flows, and 2) to determine the production response, plasma amino acid profiles, duodenal amino acid flow, coefficient of mammary amino acid extraction and dietary organic matter and protein digestion in cows infused abomasally with 0, 22.5, 45, 90 and 180 g L-lysine HCl daily.

MATERIALS AND METHODS

Experiment 1

Six lactating Holstein cows from the University of Arizona Dairy Research Center were fitted with intestinal T-cannulae and utilized in a double 3 X 3 Latin square experiment. Treatment periods consisted of an 18 d adaptation period followed by a 4 d collection period. Treatments consisted of the three protein supplements mixed with a corn silage, ground corn diet. The supplements were blood meal (BM), corn gluten meal (CGM), and cottonseed meal (CSM). Blood meal was cooker dried at 177 °C. Chromic oxide (Cr_2O_3) was mixed with a wheat flour paste, baked at 100 °C until dry, then coarsely ground. The Cr_2O_3 mixture was mixed with the total diet for 6 d prior to and during the collection periods. This served as an indigestible marker to assess quantitative aspects of digesta flow and partitioning of digestion. Feed ingredients and nutrient composition of the three diets are shown in tables 6 and 7, respectively. The experiment was conducted from March through July, 1986.

Table 6. Feed ingredients of diets supplemented with three protein sources for lactating cows. Experiment 1.[1,2]

Ingredient	BM	CGM	CSM
	(% dry basis)		
Alfalfa hay	8.4	8.3	7.7
Corn silage	29.6	29.0	34.0
Corn, ground shelled	41.5	40.7	38.8
BM	8.7	-	-
CGM	-	11.1	-
CSM	-	-	16.6
Cottonseed, whole	4.4	4.3	-
Molasses	3.4	3.3	-
Dicalcium phosphate	.65	.66	.12
Calcium carbonate	.94	.92	1.16
Potassium chloride	.46	.45	.25
Copper sulfate	.54	-	-
Monosodium phosphate	.11	-	-
Trace mineral salt[3]	.25	.24	.23

[1] BM=blood meal, CGM=corn gluten meal, CSM=cottonseed meal.
[2] Other ingredients were .17% magnesium oxide; .45% sodium bicarbonate; .22% sodium chloride; .27% urea; and 1850, 570, and 4 IU/kg vitamins A,D, and E, respectively.
[3] Contained .013% Ca, .091% Mg, 13.572% K, 12.563% S, .269% Na, 12.792% Cl, .050% Mn, .090% Zn, .075% Fe,.011% Cu, 300 ppm Co, 2624 ppm I, 133 ppm Se.

Table 7. Chemical composition of total mixed diets fed to lactating cows. Experiment 1.[1]

Measurement	BM	CGM	CSM
DM (%)	57.1	57.1	57.4
	(% dry basis)		
CP	16.4	14.5	14.9
NE_1 (Mcal/kg)[2]	1.76	1.79	1.70
DE (Mcal/kg)[2]	3.09	3.44	3.33
ADF[3]	13.8	15.7	17.9
Cellulose[3]	8.0	10.4	11.1
Lignin[3]	2.5	3.0	4.4
NDF	30.0	25.5	27.1
Hemicellulose	16.2	9.8	9.2
Organic matter	92.9	93.4	95.6
Ether extract	2.2	2.5	3.5
Chromic oxide	.16	.16	.16

[1] BM=blood meal, CGM=corn gluten meal, CSM=cottonseed meal.
[2] Calculated (148).
[3] Determined by sequential procedures (86).

Cows were housed in individual, shaded pens with concrete floors (2.2 X 3.7 m) and rubber mats (.9 X 1.8 m X 3 cm). Rations were offered ad lib at 700 and 1800 h and water was available continuously. Cows were milked at 600 and 1800 h daily. A daily milk sample was taken from a.m. and p.m. milk. Milk samples were analyzed separately by Arizona DHI Laboratory for protein, fat, lactose, SNF, and SCC.

Fresh feed and fecal samples were taken twice daily during the 3 d collection period, dried for 72 h at 55 °C in a forced-air oven, composited for the total period, and ground in a Wiley mill through a 1 mm screen. Proximate analyses (9) were determined on dried samples, as were analyses of cellulose, ADF and NDF (86). Chromic oxide was measured by the method of Fenton and Fenton (76). Degradable feed protein was measured in vitro using the ficin protease assay described by Poos-Floyd *et al*. (175) and *in vivo* using ^{15}N enrichment of bacteria in conjunction with other investigations (198). Subsamples of feed were composited for the experiment and hydrolyzed in 6 N HCl. Hydrolysates were analyzed for individual amino acids by HPLC using a reverse phase separation of phenylthiocarbamyl amino acids (171).

Silicon, T-type cannulae were positioned in the proximal duodenum of cows for the collection of chyme. Twenty-four 500 ml duodenal samples (6/d) were collected and immediately

dried in a forced air oven at 75 °C for 96 h. Dried samples were composited for each cow during each treatment period. Composited samples were analyzed for DM, Kjeldahl N, ^{15}N, Cr_2O_3, organic matter, and amino acids similarly to feed samples.

Two of the six cows fitted with rumen cannulae were sampled at 0, 2, 4, 6, and 8 h post-feeding on the final day of collection from several sites of the rumen. Rumen samples were mixed with 2 ml saturated mercuric chloride in a liter container. The pH of the fluid was determined immediately using a glass electrode. Samples were then strained through four layers of cheesecloth and 10 ml portions were deproteinized by mixing with 2 ml of 25% metaphosphoric acid and centrifuging at 1200 X *g* for 20 min. Protein-free filtrates (PFF) were analyzed for VFA by gas chromatography. Another 10 ml of strained rumen fluid was centrifuged 10 min at 1200 X *g* to remove feed particles. The supernatant then was acidified with .5 ml of .1 N HCl and analyzed for rumen ammonia (NH_3) by the method of Chaney and Marbach (47).

Foley catheters were placed in the cows' bladders 12 h prior to collection. Urine was collected in covered, plastic buckets containing 100 ml 18 N H_2SO_4. Urine was measured and sampled twice daily. Daily composites for the treatment period were frozen for later analysis of N by Kjeldahl (9).

Blood was sampled from the coccygeal vessel and subcutaneous abdominal vein in evacuated tubes containing heparin each day of collection. These samples represented arterial and venous blood, respectively. Tubes were chilled, centrifuged at 2000 X *g* in a refrigerated centrifuge, the plasma was frozen for later analyses of free amino acids and urea. Upon thawing, plasma samples were composited for each cow during each treatment period and PFF were prepared by mixing plasma with 1 N HCl in a ratio of 1:2 and centrifuging at 15,000 X *g*. Norleucine was added as an internal standard for amino acid analysis. Amino acid profiles of plasma were determined by HPLC using a reverse phase separation of phenylthiocarbamyl amino acids (174). Plasma urea was determined with a diagnostic kit.[1]

Data were tested for normality and homogeneity of variances. All outliers were removed before analyses. Remaining data were then analyzed as a replicated Latin square design using the General Linear Models procedure of the Statistical Analysis System (205).

Experiments 2 and 3

Two experiments were conducted at the Michigan State University Dairy Research and Teaching Center for this

---

[1]Sigma Chemical Co. Ltd. 1986. Blood Urea Nitrogen Procedure No. 535. Sigma Diagnostics, P.O. Box 14508, St Louis, MO 63178

investigation. In experiment 2, six lactating cows fitted with abomasal cannulae were utilized in a double 3X4 extra period Latin square design experiment. Treatments were 0, 45, and 90 g/d L-lysine·HCl infused abomasally. In experiment 3, twelve lactating cows fitted with abomasal cannulae were utilized in a double 5X5 Latin square design with two animals used for replacement. Treatments for experiment 3 were abomasal infusion of 0, 22.5, 45, 90 and 180 g/d L-lysine·HCl.

Cattle in both experiments were fed a diet which potentially minimized net lysine flow to the duodenum. Feed ingredients and nutrient composition of the diet is in table 8.

In experiment 2, chromic oxide made as in experiment 1 was used as an indigestible marker added to the TMR 7 d prior to and during the collection period. Acid insoluble ash served as the indigestible feed marker for experiment 3. A 21 d preliminary feeding period preceded the start of the experiments, to allow for post-surgical recovery. Each treatment period consisted of a 14 d adaptation period preceding a 3 d collection period. Experiment 2 began in the Fall of 1985 and experiment 3 began the Fall of 1986.

Cows were housed in comfort stalls. For experiment 2, the diet was restricted to the average DM consumed during the first sampling period. Feed was offered ad libitum in experiment 3. Feeding times were 600 and 1800 h during

Table 8. Feed ingredients and nutrient composition of experimental diet fed to lactating cows. Experiment 2 & 3.[1]

Ingredient	(% DM)
Alfalfa haylage	8.3
Corn silage	29.0
Ground shelled corn	40.1
Corn gluten meal	11.4
Soy hulls	4.8
Urea	.3
Molasses	2.8
Limestone	1.03
Dicalcium phosphate	.70
Magnesium oxide	.20
Potassium chloride	.50
Sodium bicarbonate	.50
Vitamin A, D and E premix[2]	.12
Trace mineral salt[3]	.24
Nutrient	
DM (%)	54.3
NE_l (Mcal/kg DM)[4]	1.74
CP (% DM)	15.7
Ficin degradable CP (% CP)	51.6
ADF (% DM)	17.3
Organic matter (% DM)	92.7

[1] Chromic oxide was mixed in the total mixed diet of experiment 2 at 0.5% of diet DM.
[2] Vitamins A, D and E were added to supply 1850, 570, and 4 IU/kg, respectively.
[3] Contained .013% Ca, .091% Mg, 12.56% S, 13.57% K, .30% Na, 12.79% Cl, .050% Mn, .090% Zn, .075% Fe, .011% Cu, 300 ppm Co, 2624 ppm I, and 133 ppm Se.
[4] Calculated (148).

experiment 2 and 100, 700, 1300 and 1800 h during experiment 3. Water was available continuously.

L-lysine·HCl was diluted with 4 l of water and infused into the abomasum by gravitational drip. In experiment 2, infusions were conducted during two 5 h periods/d; while in experiment 3, infusions were during two 10 h periods/d.

Cows were milked daily at 600 and 1700 h. Milk was measured and sampled as in experiment 1. Samples were analyzed by Michigan DHIA laboratory for protein and fat (experiments 2 and 3), and also for lactose, total solids and SCC (experiment 3).

Feed and feces were sampled and analyzed similarly to those of experiment 1.

In experiment 3, Foley catheters were placed in the cows' bladder, 12 h prior to the 3 d collection period. Urine was collected in 20 l covered, buckets containing 150 ml 18 N H_2SO_4. Urine was measured and sampled each day during collection, composited for the period and frozen for later analysis of N by the Kjeldahl procedure (9).

Blood samples were again collected from the coccygeal vessel and subcutaneous abdominal vein as in experiment 1. For experiment 2, PFF were prepared by mixing plasma with .1 ml/ml 50% sulfosalicylic acid, and .1 ml/ml 1mM norleucine and SB-(4 pyridy:ethyl)-1 cysteine were added as internal standards. Precipitated protein was removed by centrifugation at 30,000 X g for 20 min. Amino acids were analyzed in

the PFF by ion exchange chromatography using a lithium citrate buffer system and post column derivatization with ninhydrin. For experiment 3, PFF was analyzed for amino acids similarly to experiment 1. The procedure used in experiments 1 and 3 for amino acid analysis was more sensitive than that of experiment 2, which were analyzed prior to samples of experiment 1 and 3. Plasma urea was determined for both experiments using a diagnostic kit.[2]

All data were tested for normality and homogeneity of variances. Outliers were removed before analyses. Data of experiment 2 were analyzed as a replicated extra-period Latin square design using the method of Lucas (130). Linear and quadratic responses to treatments were calculated and tested for significance using orthogonal contrasts. Data of experiment 3 were analyzed as a replicated Latin square design using the General Linear Models procedure of the Statistical Analysis System (201). Data compiled for the replacement animals were also included in the analyses. Linear, quadratic and cubic responses to treatment were calculated and tested for significance. Broken-line analyses of plasma lysine concentration was determined as described by Robbins et al. (182).

---

[2]Sigma chemical Co. Ltd. 1986. Blood Urea Nitrogen Procedure No. 535. Sigma Diagnostics, P.O. Box 14508, St. Louis, MO 63178.

RESULTS AND DISCUSSION

Experiment 1

Diets of corn silage, ground shelled corn and a protein supplement were formulated to be isonitrogenous with 50% of the protein supplied by the supplement. However, the CGM and CSM diets were lower in CP concentration than the BM diet. Calculated energy concentrations of the diets were sufficient to meet the requirement of cows producing 35 kg milk/d (148).

Yield of milk and SCM were not significantly influenced by treatment (table 9), nor was efficiency of production (SCM/DMI). Cows fed CSM tended to produce more milk of lower protein and fat content than did the cows fed BM or CGM diets. Yield of milk lactose and SCC's were similar between treatments.

Measurements of rumen fermentation characteristics are in table 10. Rumen contents were sampled from two cows fed BM and CGM diets and one cow fed CSM diet. Analysis of variance was not conducted on this data. Concentrations of VFA were greater for the cow fed CSM diet compared to BM, with CGM intermediate. Molar ratios of acetate : propionate were 2.41, 1.78, and 2.29 for BM, CGM, and CSM, respectively. The lower ratio for cows fed CGM diet did not influence milk fat

TABLE 9. Influence of feeding three protein sources on daily production variables of lactating cows. Experiment 1.[1]

Measurement	BM	CGM	CSM	SE
DMI[2] (kg)	15.0[b]	16.4[ab]	17.0[a]	.68
Milk (kg)	20.2	20.3	24.0	1.5
protein (%)	2.90[ab]	3.06[a]	2.78[b]	.10
protein (g)	591	605	662	30
fat (%)	3.75[a]	3.75[a]	3.38[b]	.13
fat (g)	758	776	823	50
lactose (%)	5.25	5.34	5.33	.07
lactose (kg)	1.06	1.08	1.29	.08
SCC ($x10^3$)	220	192	149	31
SCM[3] (kg)	19.1	20.2	20.9	1.5
Milk/DMI	1.36[AB]	1.27[B]	1.41[A]	.03
SCM/DMI	1.3	1.3	1.3	.2

[1] BM=blood meal, CGM=corn gluten meal, CSM=cottonseed meal.
[2] DMI = DM intake.
[3] Calculated (233).
[a,b;A,B] Values not sharing a similar superscript are different (P<.10);(P<.05), respectively.

TABLE 10. Concentration of VFAs (mM), ammonia (mg/dl), and pH in rumen fluid of lactating cows fed three protein sources. Experiment 1.[1]

Measurement	BM	SE	CGM	SE	CSM	SE
number of cows	2		2		1	
Acetate	61.2	4.3	69.0	3.2	86.4	4.7
Propionate	22.2	2.2	33.6	3.4	33.8	1.2
Isobutyrate	.2	.2	ND[2]		.7	.7
Butyrate	12.7	1.4	15.4	1.2	14.7	.7
Isovalerate	1.1	.1	1.0	.1	2.0	.3
Valerate	.8	.1	1.7	.3	1.6	.1
Total	98		120		139	
Acetate:propionate	2.8		2.1		2.5	
Ammonia	1.00	.65	1.60	.61	8.51	.34
pH	6.62	.07	5.64	.07	6.57	.02

[1] BM=blood meal, CGM=corn gluten meal, CSM=cottonseed meal.
[2] Not detected.

percent. Rumen NH_3 was least for BM, and below critical concentrations necessary for a maximum rate of rumen fermentation (132, 137, 204, 205), which may explain the lower concentration of VFAs for BM diet.

Dry matter intake, was least for cows fed BM, but CP intake was similar for all diets (table 11). Ruminal-reticular digestibility of DM and organic matter did not differ significantly between treatments, but was numerically greater for BM and CGM than CSM. The large SE for digestibility of DM and organic matter may be a result of the short collection period. The large coefficient of variability made dietary differences hard to detect. Extent of rumen digestion increases when intake or passage rate is reduced (77), which is consistent with the lowest rumen digestibility of CSM. Different ingredients among diets could have also influenced rumen utilization of nutrients (125).

The BM and CGM diets increased CP passage to the duodenum ($P<.05$), about 23% above that which was consumed, while no increase was shown for the CSM diet. The protein supplements which were relatively resistant to microbial degradation (BM and CGM) resulted in greater flow of protein from the rumen. Similar increases have been reported for ruminants fed low protein diets (174, 215, 230) or byproduct proteins (77, 200). Increased protein flow was due to decreased rumen degradation.

Ruminal-reticular degradation of feed proteins was greatest for CSM, intermediate for CGM, and least for BM

TABLE 11. Intake and ruminal-reticular digestibility of nutrients by cows fed three protein sources. Experiment 1.[1]

Item	BM	CGM	CSM	SE
Intake (kg/d)				
Dry matter	15.0^{b}	16.4^{ab}	17.0^{a}	.7
Organic matter	13.9^{b}	15.3^{ab}	16.3^{a}	.6
Crude protein	2.36	2.32	2.51	.28
Ruminal-Reticular digestion (%)[5]				
Dry matter[2]	52.4	54.4	46.7	6.2
Organic matter[2]	56.5	61.2	50.6	6.5
Crude protein[3]	-22.7^{B}	-22.9^{B}	-3.1^{A}	5.2
Crude protein[2]	31.2^{Bb}	43.3^{ABa}	48.4^{Aa}	4.1
Crude protein[4]	18.1	41.1	49.7	ND
Duodenal crude protein (kg/d)				
Total	2.89	2.84	2.54	.14
Feed and endogenous[5]	1.61^{a}	1.31^{b}	1.29^{b}	.07
Microbial[5]	1.28^{ab}	1.53^{a}	1.24^{b}	.09
Microbial growth efficiency[5]				
g microbial N/kg RFOM	25.6	25.7	24.7	2.0
g microbial CP/Mcal RDE[6]	48.1	44.0	44.3	3.5

[1] BM=blood meal, CGM=corn gluten meal, CSM=cottonseed meal, ND=not determined, RFOM=rumen fermented organic matter, CP=crude protein.
[2] Truly digested calculated by correcting for bacterial fraction in duodenal digest as estimated by the ^{15}N marker method (198).
[3] Apparently digested.
[4] In vitro ficin degradable.
[5] Calculated from data of Sadik (198).
[6] RDE = digestible energy intake * rumen OM digestibility.
[a,b;A,B] Values not sharing a similar superscript are different (P<.10);(P<.05), respectively.

(table 11). Estimates for CGM and CSM protein ruminally degraded were similar for the *in vitro* ficin assay method and the *in vivo* ^{15}N marker method (198). However, the ficin assay underestimated *in vivo* rumen protein degradation for BM. The ficin procedure for determining degradable protein has been used previously by investigators (116, 174). Resulting estimates for rumen protein degradation are similar to *in vivo* determinations for many proteins (202).

Microbial protein production was greatest for CGM diet and similar for BM and CSM diets (198). Microbial protein synthesis tended to increase as the quantity of organic matter and energy digested in the rumen increased. This is consistent with published equations which estimate microbial protein passage to the intestine as a function of ruminally digested energy (188). Efficiencies of microbial growth were similar between diets and are in agreement with those found by others for lactating cows (215).

The three diets differed in amino acid content (table 12). Dietary concentration of histidine, valine, lysine, and phenylalanine were greatest and isoleucine least for BM diet. Lysine was least for CGM diet; while leucine was least and arginine most for CSM diet. Variation in patterns of amino acids flowing to the duodenum closely reflected diet differences (figure 3). This suggests that quality of dietary protein has a profound effect on the quality of protein available for digestion and absorption in the small intestine.

TABLE 12. Amino acids present in the diets and duodenal chyme of lactating cows receiving three protein sources. Experiment 1.[1]

Diet	His	Thr	Arg	Val	Met	Ile	Leu	Phe	Lys	Asp	Glu	Ser	Gly	Ala	Pro	Tyr
	Intake, g/kg total AA															
BM	40.9^{Aa}	36.0^{a}	59.0^{B}	70.4^{a}	1.9	23.3^{B}	108.8^{B}	58.0^{Aa}	50.1^{Aa}	92.0^{a}	124.9^{A}	39.7^{ab}	140.4^{a}	74.6^{ABa}	55.0^{Bc}	23.5^{C}
CGM	22.4^{Bc}	31.4^{b}	60.1^{B}	47.3^{b}	1.1	40.4^{A}	125.2^{A}	54.6^{ABb}	25.5^{Bb}	67.1^{b}	169.5^{B}	41.0^{a}	118.0^{b}	78.5^{Aa}	84.6^{Aa}	31.2^{A}
CSM	25.5^{Bb}	32.5^{ab}	95.8^{A}	51.8^{b}	1.0	39.4^{A}	82.3^{C}	51.4^{Bc}	36.8^{ABb}	83.2^{a}	169.4^{B}	38.8^{b}	139.9^{a}	60.6^{Bb}	60.7^{Bb}	26.9^{B}
SE	.5	.7	3.0	1.7	.2	.8	.6	.5	2.1	2.6	2.6	.3	2.1	1.3	1.2	1.6
	Intake, g/d															
BM	107^{A}	94^{A}	155^{B}	185^{A}	5^{A}	61^{B}	285^{A}	150^{Aa}	131^{A}	241^{A}	328^{B}	104^{a}	368^{Aa}	196^{A}	144^{B}	62^{ABb}
CGM	53^{B}	75^{B}	143^{B}	113^{B}	2^{B}	97^{A}	299^{A}	130^{ABb}	61^{C}	161^{B}	405^{A}	98^{ab}	282^{Bb}	188^{A}	203^{A}	75^{Aa}
CSM	57^{B}	72^{B}	213^{A}	115^{B}	2^{B}	88^{A}	183^{B}	114^{B}	82^{B}	185^{B}	377^{AB}	86^{b}	311^{ABb}	135^{B}	135^{B}	60^{Bb}
SE	3	3	7	6	.2	4	12	6	4	8	16	4	13	8	8	3
	Flow to duodenum, g/kg total AA															
BM	38.5^{A}	49.5^{a}	80.8^{ab}	82.4^{A}	2.0	40.4^{B}	111.5^{A}	61.0^{A}	77.4^{A}	96.5	122.3^{B}	46.4	78.7	81.3^{a}	52.1^{B}	37.8
CGM	24.5^{B}	44.5^{b}	70.8^{b}	65.5^{B}	2.0	54.4^{A}	113.1^{A}	55.3^{B}	64.0^{B}	90.9	156.7^{A}	43.8	75.0	79.7^{ab}	64.7^{A}	44.2
CSM	27.8^{B}	47.0^{ab}	93.7^{a}	69.4^{B}	1.7	55.9^{A}	91.9^{B}	55.0^{B}	76.3^{A}	91.5	156.2^{A}	43.5	87.8	72.8^{b}	54.7^{B}	41.8
SE	1.1	1.3	7.0	1.9	.3	.8	.2	.9	1.3	6.5	5.5	.9	4.5	2.1	1.3	1.1
	Flow to duodenum, g/d															
BM	99^{a}	124^{a}	191	206^{Aa}	5	100^{Bb}	280^{Aa}	154^{a}	143^{a}	250	314	116^{a}	187	202^{Aa}	129^{AB}	94^{AB}
CGM	59^{b}	106^{ab}	160	155^{ABb}	5	129^{Aa}	266^{ABa}	130^{ab}	110^{ab}	216	370	104^{ab}	176	188^{ABa}	152^{A}	105^{A}
CSM	54^{b}	91^{b}	175	134^{Bb}	4	108^{ABb}	178^{Bb}	106^{b}	106^{b}	177	302	84^{b}	169	141^{Bb}	106^{B}	81^{B}
SE	10	7	21	13	1	6	21	12	12	26	29	8	10	12	8	5

[1] BM=blood meal, CGM=corn gluten meal, CSM=cottonseed meal, His=histidine, Thr=threonine, Arg=arginine, Val=valine, Met=methionine, Ile=isoleucine, Leu=leucine, Phe=phenylalanine, Lys=lysine, Asp=aspartic acid, Glu=glutamic acid, Ser=serine, Gly=glycine, Ala=alanine, Pro=proline, Tyr=tyrosine.

a,b,c;A,B,C Values in same column not showing a similar suberscript are different (P<.10);(P<.05), respectively.

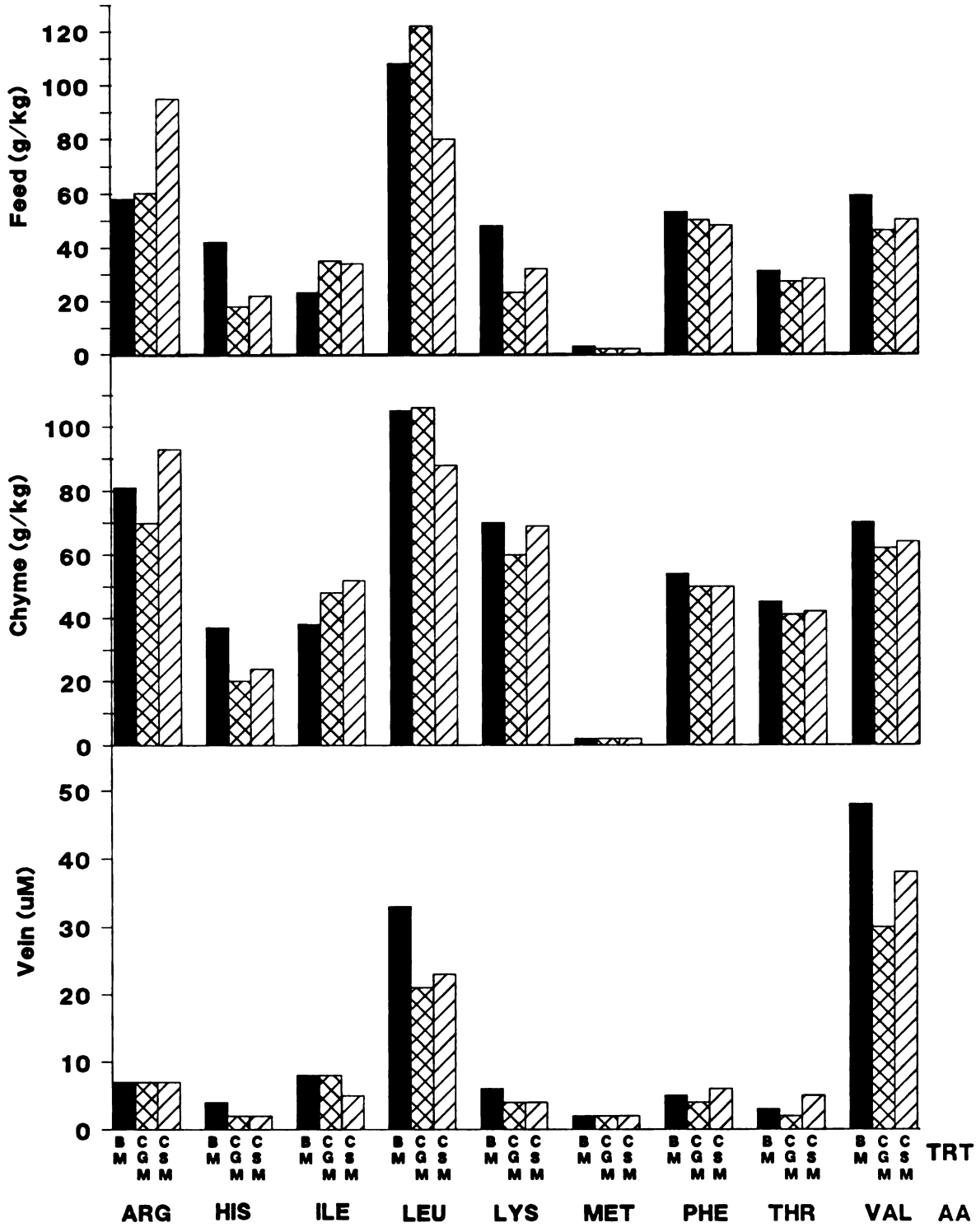


Figure 3. Profiles for selected amino acids of diets, duodenal chyme, and chyme, and venous plasma of cows fed 3 protein sources. Experiment 1. TRT = diet fed, BM = blood meal, CGM = corn gluten meal CSM = cottonseed meal, AA = amino acid.

Yet, there was some examples of no direct relation between venous concentration and that in diet or chyme, ie. isoleucine or lysine.

Rumen bacteria do not greatly differ in their amino acid profile (22, 117), therefore the feed and endogenous amino acid contribution to the duodenal chyme can be calculated by subtracting the bacteria contribution from the total passage of each amino acid. This was done for values in table 13. Lysine and threonine appear to be preferentially digested in the rumen. This is indicated by a reduced concentration of these amino acids in the duodenal chyme compared to that which was ingested (row 4 of table 13). Lysine and threonine were preferentially degraded by mixed cultures of rumen bacteria in vitro (42, 59). Therefore, estimation of amino acid passage to the duodenum can not be calculated by simply multiplying the rumen nondegradable protein value of feed to

TABLE 13. Essential amino acid profile of undegraded feed and endogenous protein passing to the duodenum of cows fed three protein sources. Experiment 1.

Diet	His	Thr	Arg	Val	Met	Ile	Leu	Phe	Lys
				(g/kg total AA)					
BM	51	28	89	98	-10	26	126	65	41
CGM	29	8	81	76	-16	48	139	56	14
CSM	34	13	122	81	-16	49	96	54	29
				(% of ingested AA)					
Mean[1]	129	39	137	151	-934	118	114	106	70

BM=blood meal, CGM=corn gluten meal, CSM=cottonseed meal.
[1]Mean= ((chyme AA_x - estimated microbial AA_x)/total chyme AA)/(ingested AA_x/total ingested AA)*100.

by the feed's amino acid content, as was done by other investigators (51).

The influence of protein supplements on plasma profiles and mammary extraction of amino acids is in table 14. Mammary extraction coefficients were determined after removing outlying venous and arterial amino acid concentrations. Mammary extraction values were not subjected to a least-squares analysis due to the occurrence of inestimatable values. The CSM diet had the greatest arterial concentration of threonine and alanine. The BM diet had the greatest venous concentration of histidine, and numerically greater venous concentrations of valine, leucine, and lysine than CGM or CSM diets. Whether these differences reflect the quantity of the amino acid absorbed from the intestine is not known; however, the BM diet did provide a greater quantity of histidine, valine, leucine, and lysine to the intestine.

Mammary extraction coefficient may have potential as an indicator of limiting amino acids for milk production (141). Those amino acids which show the lowest concentration in the arterial plasma coupled with a high percent extraction may limit milk protein synthesis. In general mammary extraction coefficients were large for threonine, methionine, phenyl-alanine and lysine, low for valine, leucine, and tyrosine, and mostly negative for aspartate, serine, and glycine. First, second and third amino acids extracted in greatest proportions by the mammary gland for the BM diet were

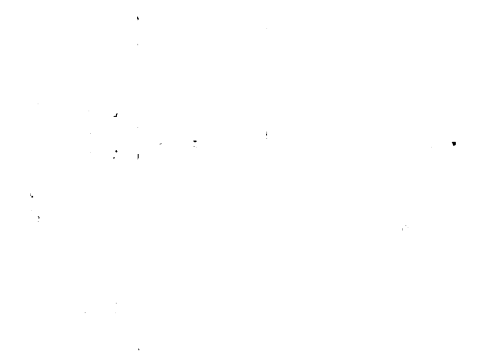
TABLE 14. Plasma amino acid concentrations and mammary extraction coefficients of lactating cows fed three protein sources. Experiment 1.[1]

Diet	His	Thr	Arg	Val	Met	Ile	Leu	Phe	Lys	Asp	Glu	Ser	Gly	Ala	Pro	Tyr
	Coccygeal AA, umoles/dl															
BM	4.5	5.8^{AB}	8.9	43.3	1.6	13.0	39.4	9.0	8.6	1.5	5.1	16.2	46.5	25.5^{B}	16.0	6.7
SE	.9	.6	1.2	9.1	.2	1.6	7.5	1.2	1.6	.2	.5	2.2	14.9	1.5	3.2	.6
CGM	4.4	4.3^{B}	9.6	36.2	1.4	9.4	25.5	6.8	7.6	1.7	7.1	14.8	54.0	25.9^{B}	14.3	5.4
SE	.9	.5	1.2	9.1	.2	1.6	7.5	1.2	1.6	.2	.6	2.2	12.0	2.0	2.5	.6
CSM	5.3	7.4^{A}	8.8	45.2	1.2	8.7	29.3	8.5	8.6	1.6	5.2	15.8	57.2	34.0^{A}	12.8	5.3
SE	.9	.7	1.2	9.1	.2	1.6	7.5	1.2	2.1	.2	.6	2.2	11.3	1.2.5	.6	
	Venous AA, umoles/dl															
BM	4.5^{A}	3.1	7.1	49.8	.8	8.9	34.8	5.4	6.2	1.9	4.1^{ab}	17.8	66.7	27.9	12.4	5.8
SE	.1	2.3	1.6	9.7	.3	1.7	7.0	1.0	1.0	.4	.7	2.6	11.0	4.1	2.1	1.7
CGM	3.3^{B}	1.5	6.5	32.7	.6	9.0	22.8	3.7	3.5	1.9	4.3^{a}	17.0	60.6	29.2	18.0	5.4
SE	.1	3.1	1.6	9.7	.3	1.7	7.0	1.0	1.0	.4	.7	2.6	11.0	4.1	1.6	2.2
CSM	3.1^{B}	5.2	7.1	39.8	.7	5.9	24.1	5.9	3.9	1.6	2.7^{b}	15.8	56.1	32.7	13.1	4.5
SE	.1	2.5	1.6	9.7	.3	1.7	7.0	1.0	1.3	.4	.7	2.6	11.0	4.1	1.6	1.7
	Mammary extraction coefficient[2]															
BM	1.5	46.9	19.7	-15.1	50.6	31.2	11.7	40.0	28.0	-26.7	19.6	-9.8	-44.3	-9.4	22.5	13.4
CGM	24.5	64.8	32.3	9.7	54.3	4.3	10.6	45.7	54.1	-11.8	39.4	-14.9	-12.2	-12.7	-25.9	0.0
CSM	41.6	29.9	18.7	11.9	45.1	32.5	19.4	29.7	55.3	0.0	48.1	0.0	1.9	3.8	-2.3	15.1

[1] BM=blood meal, CGM=corn gluten meal, CSM=cottonseed meal, His=histidine, Thr=threonine, Arg=arginine, Val=valine, Met=methionine, Ile=isoleucine, Leu=leucine, Phe=phenylalanine, Lys=lysine, Asp=aspartic acid, Glu=glutamic acid, Ser=serine, Gly=glycine, Ala=alanine, Pro=proline, Tyr=tyrosine.

[2] Mammary extraction coefficient = (coccygeal - venous) / coccygeal x 100.

[a,b;A,B] Values in the same column not sharing a similar superscript are different (P<.10);(P<.05), respectively.



methionine, threonine, phenylalanine; for CGM, threonine, methionine, lysine; and for CSM, lysine, methionine, histidine. Extraction coefficients of histidine, valine and lysine were less for the BM than other diets and these three amino acids had the greatest intestinal concentration and supply (table 12). Together the above data may reflect the supply of individual amino acids at the duodenum.

Large mammary extraction of lysine is evidence that a critical need for lysine in cows fed the CGM and CSM diets exists. Other evidence suggesting a critical need for lysine by cows fed CGM and CSM over those fed BM is the expression of milk lysine as a proportion of intestinal lysine or milk lysine as a proportion of lysine apparently digested. These values are 43.4, 49.3, and 32.6%; 72.2, 94.4, and 54.4% for CGM, CSM and BM, respectively.

Apparent digested N, net protein utilization (NPU) and protein biological value (BV) tended to be greater for CSM, intermediate for CGM, and lowest for BM (table 15). Urinary N was least for CSM when expressed as a portion of digested N or ingested N. The proportion of ingested N in milk was not different between diets, however N balance was negative for BM, zero for CGM and positive for CSM. These data suggest CSM provided a better quality protein for digestion than BM or CGM. Estimates of NPU and BV did not sum to 100% due to removal of outlier. The relatively large SE for retained, and fecal N may be a result of the short collection period.

There were no differences for digestion of N entering the small intestine among treatments (table 16). However, chyme N was used more efficiently for synthesis of milk protein with the CSM diet than with BM or CGM diets. This suggests a better array of amino acids were absorbed with the CSM diet than with BM or CGM diets.

Digestion of microbial N as measured by the ^{15}N method was not different among the diets and averaged 53% (198). This value was derived by the difference of ^{15}N appearance at the duodenum and that which passed to the feces. Digestion of

TABLE 15. Influence of three protein sources on nitrogen digestion and metabolism by lactating cows. Experiment 1.[1]

Measurement	BM	CGM	CSM	SE
Ingested N (g/d)	371	369	400	19
Digested (%)[2]	44.2	50.9	56.1	5.6
NPU (%)[3]	17.9[b]	24.7[ab]	32.6[a]	5.3
Milk (%)	25.2	26.2	26.0	.54
Retained (%)	-6.4	-1.5	6.1	5.6
Urine (%)	26.3	26.3	23.5	3.0
Digested N (g/d)[2]	161[B]	191[AB]	224[A]	15
BV (%)[4]	35.1[b]	43.8[ab]	57.5[a]	8.2
Milk (%)	47.7	41.0	47.3	1.9
Retained (%)	-7.1	19.8	10.6	12.0
Urine (%)	64.9[a]	56.2[ab]	42.9[b]	8.2
Milk N (g/d)	93	95	104	4
Urine N (g/d)	95	97	93	10
Fecal N (g/d)	210	178	177	26
Retained N (g)[5]	-25	-1	25	20

[1] BM=blood meal, CGM=corn gluten meal, CSM=cottonseed meal.
[2] Apparently digested N.
[3] NPU = (ingested N - fecal N - urine N)/ ingested N * 100.
[4] BV = (digested N - urine N)/ digested N * 100.
[5] Retained N = ingested N - milk N - urine N - fecal N.
[a,b;A,B] Value not sharing similar superscript are different (P<.10);(P<.05), respectively.

TABLE 16. Influence of three protein sources on digestion and metabolism of intestinal nitrogen (N) by lactating cows. Experiment 1.[1]

Measurement	BM	CGM	CSM	SE
Intestinal N (g/d)	458	454	406	22
Digested (%)[2]	60.1	60.2	57.0	4.9
Milk (%)[3]	20.8[A]	21.5[A]	25.7[B]	.5

[1] BM=blood meal, CGM=corn gluten meal, CSM=cottonseed meal.
[2] Apparently digested N entering the small intestine.
[3] milk N / intestinal N *100.
[A,B] Values not sharing similar superscript are different (P<.05).

undegraded dietary and endogenous N passing to the intestine was calculated to be 52, 72 and 64% for BM, CGM, and CSM diets, respectively. The BM diet provided the most poorly intestinally digested protein, although it supplied the greatest proportion of feed protein to the duodenum. The blood meal used in this study was batch dried at a high temperature which may have decreased the availability of the protein. Values for acid detergent insoluble N (indicator of heat damage) for blood meal used in this experiment ranged from four to 10 % of the total N. Processing methods such as spray or roller drying might have diminished heat damage and produced a more digestible product capable of promoting better performance (165, 240).

Experiments 2 and 3

Plasma free amino acid measurements can be utilized for the quantitative determination of an amino acid requirement

when experimental conditions are well defined (19). Administion of increasing amounts of a single limiting essential amino acid will result in a two-phase response curve for the plasma concentration of the limiting amino acid (19). Plasma concentration of the limiting amino acid will not change until the amino acid requirement is reached and then the plasma concentration will increase, this is considered the break-point (84). This approach has been useful in assessing amino acid adequacy in ruminants (75) and nonruminants (23, 81, 234) as well as for other numerous biological responses. A two-phase response curve will also occur for production, an increase in production until the requirements are met and than it will plateau (145, 181, 284).

Dry matter intake and milk production parameters for the lactating cows of experiments 2 and 3 are in table 17. There were no differences among treatments within an experiment for DM consumption or for production of milk fat, lactose, total solids and FCM. Milk production increased linearly in experiment 3 ($P<.05$), but due to a decreased fat content there were no differences for SCM. Milk somatic cell counts (SCC) measured in experiment 3 were abnormally high and undoubtedly had an influence on the milk yield and/or composition.

A linear increase to lysine infusion ($P<.05$) was noted for production of milk protein in both experiments. These results suggest that lysine intake may be directly related to milk yield and when lysine intake is low milk yield may be limited.

TABLE 17. Daily intake and production variables of lactating cows abomasally infused with L-lysine·HCl. Experiments 2 and 3.

Parameter		L-lysine·HCl infused (g/d) 0	SE	22.5	SE	45	SE	90	SE	180	SE
Intake	DM (kg)[1]	19.2	.3			19.2	.3	19.5	.3		
	(kg)[2]	23.1	.6	22.7	.5	23.0	.5	22.7	.5	22.7	.5
Milk	Yield (kg)[1]	26.4	1.0			27.4	1.0	27.7	1.0		
	(kg)[2,L]	29.7	.7	31.2[a]	.6	30.1	.6	31.2[a]	.6	32.1[AC]	.6
	Fat (%)[1]	4.03	.8			4.15	.8	3.87[C]	.08		
	(%)[2]	3.42	.14	3.37	.12	3.47	.12	3.38	.12	3.14	.13
	(kg)[1]	1.06	.03			1.14	.03	1.07	.03		
	(kg)[2]	1.01	.04	1.04	.03	1.06	.03	1.01	.03	1.05	.04
	Protein (%)[1]	3.13	.06			3.25	.06	3.32[A]	.06		
	(%)[2]	3.17	.04	3.25	.04	3.28[a]	.04	3.27[a]	.04	3.25	.04
	(kg)[1,L]	.82	.03			.88	.03	.95[A]	.03		
	(kg)[2,L]	.94	.02	1.01[A]	.02	.98	.02	1.02[A]	.02	1.04[Ac]	.02
	Lactose (%)[2]	5.02	.13	4.96	.11	4.96	.11	4.99	.11	5.03	.11
	(kg)[2]	1.50	.06	1.57	.05	1.49	.05	1.57	.05	1.61	.05
	Total solids (%)[2]	12.4	.2	12.2	.2	12.4	.2	12.4	.2	12.2	.2
	(kg)[2]	3.68	.09	3.81	.08	3.72	.08	3.87	.08	3.91	.08
	SCC ($x10^3$)[2]	522	178	379	153	866	153	624	153	400	165
	FCM (kg)[1,3]	26.4	.8			28.0	.8	27.2	.8		
	SCM (kg)[2,4]	27.7	.7	28.7	.6	28.2	.6	29.1	.6	29.0	.6
	Milk / DMI[1]	1.40	.05			1.39	.05	1.48	.05		
	Milk / DMI[2]	1.33	.04	1.39	.04	1.34	.04	1.42[ac]	.04	1.42[a]	.04
	FCM / DMI[1]	1.39	.04			1.46	.04	1.41	.04		
	SCM / DMI[2]	1.24	.04	1.28	.04	1.25	.04	1.32	.04	1.29	.04
Body wt change	(kg)[1]	3	5			-1	5	4	5		
	(kg)[2]	12	5	12	5	12	5	11	5	4	5

[a,c] treatment different from 0 and 45 g L-lysine·HCl infused P<.10, respectively.
[A,C] treatment different from 0 and 45 g L-lysine·HCl infused P<.05, respectively.
[L] linear response to treatment P<.05.
[1,2] experiment 2, 1985 and experiment 3, 1986, respectively.
[3] Fat corrected milk = 15 * fat (kg) + .4 * milk yield (kg) (84).
[4] Solids corrected milk = 12.3 * fat (kg) + 6.56 * solids-not-fat (kg) - .0752 * milk yield (kg) (233).

A quadratic response for milk protein synthesis to infusion of lysine approached significance in experiment 3 ($P<.17$) indicating the requirement for lysine was exceeded with the 180 g L-lysine·HCl infusion. Milk protein production was least of all infusions when 45 g L-lysine·HCl was infused and this was possibly related to the abnormally high SCC for this treatment group. When SCC was used in the statistical analysis as a covered, milk protein production for the 45 g infusion treatment was .99 kg/d and not different from other treatments.

Plasma free amino acid profiles for experiments 2 and 3 are in tables 18 and 19, respectively. As infusion dose increased coccygeal plasma concentration of lysine tended to increase in experiment 2. However, in experiment 3 plasma lysine concentration increased linearly and quadratically in coccygeal plasma, and increased quadratically in plasma from the subcutaneous abdominal vein (figure 4). Both vessels of cows in experiment 3 also exhibited increased arginine and ornithine concentrations with 180 g infusion of lysine. Coccygeal plasma concentrations of both threonine and methionine decreased linearly with increased lysine infusion in experiment 3 (figure 5).

The quadratic response of plasma free lysine in the subcutaneous abdominal vein indicates lysine was the limiting amino acid for milk production (19, 182). At the point where the concentration of plasma lysine increased, the lysine

TABLE 18. Plasma amino acid concentration of lactating cows abomasally infused with L-lysine·HCl. Experiment 2.

Infused (g/d)	His	Thr	Arg	Val	Met	Ile	Leu	Phe	Lys	Asp	Glu	Ser	Gly	Ala	Tyr
	Coccygeal plasma AA, umoles/dl														
0	6.8	6.1	6.6	16.9	2.4	7.8	22.4	8.1	9.9	3.6	13.4	10.6	42.7	26.9	6.6
SE	.4	.7	.8	.8	.2	.4	2.6	.5	4.2	.5	1.1	.8	4.4	3.8	.7
45	6.6	5.1	6.4	15.3	2.3	7.5	26.2	7.4	11.3	2.8	11.0	.5	35.0	28.1	7.0
SE	.4	.7	.8	1.0	.2	.5	3.1	.5	4.7	.5	1.1	.8	4.4	3.8	.7
90	5.9	4.6	6.2	15.9	1.9	8.4	25.6	5.6^{AB}	15.1	2.9	9.0^{A}	6.4^{AB}	27.6^{A}	19.5	6.2
SE	.4	1.0	.9	.8	.2	.5	2.8	.5	4.2	.5	1.3	1.0	4.8	4.1	.7
	Venous AA, umoles/dl														
0	6.8	4.3	5.6	16.9	1.9	6.0	20.8	6.5	9.1	2.9	10.9	11.9	47.2	25.0	4.8
SE	1.1	1.0	.7	1.8	.3	.5	1.2	.5	2.4	.4	1.1	1.6	4.5	2.8	.9
45	6.1	6.7	4.4	18.0	2.1	6.6	22.8	6.3	8.8	3.8^{a}	11.0	14.0	35.7	30.4	7.3^{a}
SE	1.1	1.0	.8	1.9	.3	.6	1.3	.5	2.9	.4	1.1	1.6$_{b}$	4.6	2.5	.8
90	5.1	5.0	5.1	18.3	1.9	6.6	22.0	6.1	8.5	2.9	11.6	8.1^{b}	34.2	22.3	5.7
SE	1.4	1.3	1.0	2.3	.4	.6	1.5	.6	3.3	.5	1.5	2.1	6.0	3.3	1.1

[1] His=histidine, Thr=threonine, Arg=arginine, Val=valine, Met=methionine, Ile=isoleucine, Leu=leucine, Phe=phenylalanine, Lys=lysine, Asp=aspartic acid, Glu=glutamic acid, Ser=serine, Gly=glycine, Ala=alanine, Tyr=tyrosine.

^{a,b} Treatment difference between 0 and 45 g L-lysine·HCl infused P<.10, respectively.

^{A,B} Treatment difference between 0 and 45 g L-lysine·HCl infused P<.05, respectively.

TABLE 19. Plasma free amino acid concentration of lactating cows abomasally infused with L-lysine·HCL. Experiment 3.

Infused (g/d)	His	Thr	Arg	Val	Met	Ile	Leu	Phe	Trp	Lys	Asp	Glu	Ser	Gly	Ala	Pro	Tyr	Orn
	Coccygeal plasma AA, umoles/dl																	
0	10.6	11.3	16.2	61.3	4.4	25.5	67.9	12.9	8.4	8.3	2.4	8.0	25.9	55.2	53.9	35.6	17.4	8.7
SE	1.1	1.2	1.7	5.5	.4	2.1	5.0	.6	.7	5.1	.2	.7	2.0	5.1	6.0	2.7	2.0	4.2
22.5	12.8	13.6	20.2^{a}	75.1^{a}	4.8	26.3	77.4	13.0	10.0	14.9	1.6^{A}	8.8	25.1	55.4	54.8	34.6	20.9	11.2
SE	.9	1.1	1.4	4.7	.3	1.8	4.2	.6	.6	4.3	.2	.6	1.7	4.3	5.1	2.3	1.7	3.5
45	10.5^{b}	10.7^{b}	19.7	67.7	4.4	24.8	72.8	12.6	8.3^{B}	19.2^{a}	2.1^{b}	7.8	23.0	50.1	58.4	31.9	19.2	10.5
SE	1.0	1.1	1.5	4.7	.3	1.8	4.2	.6	.6	4.3	.2	.6	1.7^{AB}	4.3	5.1	2.3	1.7	3.5
90	10.2^{b}	9.1^{b}	19.7	67.2	3.9^{b}	24.2	74.5	12.2	8.1^{B}	24.7^{AB}	1.8^{a}	8.5	20.1^{AB}	46.2	53.3	28.9^{ab}	18.5	10.4
SE	.9	1.1	1.5	4.7	.3	1.8	4.2	.6	.6	4.3	.2	.6	1.7	4.3	5.1	2.3	1.7	3.5
180	10.6	10.0^{b}	24.5^{A-D}	69.0	3.7^{B}	24.8	75.6	11.5^{B}	8.2^{B}	60.0^{A-D}	1.9	8.8	22.3	49.1	55.2	31.2	17.9	20.2^{a-d}
SE	1.0	1.2	1.6	5.1	.3	2.0	4.6	.6	.7	4.7	.2	.6	1.9	4.7	5.5	2.5	1.8	3.8
	Venous plasma AA, umoles/dl																	
0	8.5	11.4	8.0	47.6	3.3	14.6	49.2	8.3	5.6	8.7	1.8	6.8	20.4	50.0	45.6	31.2	12.3	5.3
SE	1.3	2.0	1.6	5.4	.8	3.3	6.8	.9	.9	4.3	.2	1.0	2.5	7.0	6.8	3.9	1.3	.9
22.5	8.7	10.6	9.0	48.8	2.8	16.7	50.7	8.7	6.7	7.8	1.8	6.9	19.2	50.4	51.9	28.7	13.4	5.7
SE	1.1	1.6	1.47	4.6	.6	2.5	5.1	.7	.7	3.2	.2	.8	2.1	5.9	5.8	3.3	1.1	.7
45	7.3	9.9	8.6	42.1	2.7	13.6	43.4	7.9	5.5	6.9	2.2	7.3	17.5	45.6	44.1	24.4	11.2	5.0
SE	1.1	2.0	1.4	4.8	.8	2.6	5.2	.7	.7	3.3	.2	.9	2.2	6.1	6.0	3.4	1.2	.7
90	8.3	10.4	9.4	48.5	2.4	14.2	49.9	7.2	5.1	12.2	1.8	7.8	18.2	46.9	44.6	26.8	11.9	5.2
SE	1.1	1.8	1.3^{Abc}	4.6	.7	2.7	5.5	.8	.8	3.5^{A-D}	.2	.9	2.1	5.9	5.8	3.3	1.1	.8
180	8.3	8.5	12.5	46.3	2.9	17.4	53.8	7.6	5.9	32.3	2.0	6.7	16.7	45.0	46.4	27.4	10.9	7.5^{a-d}
SE	1.2	1.8	1.5	5.0	.7	2.7	5.5	.8	.8	3.5	.2	.9	2.3	6.4	6.3	3.6	1.2	.8

^{a,b,c,d} Treatment different from 0, 22.5, 45 and 90 g L-lysine·HCl infused P<.10, respectively.
^{A,B,C,D} Treatment different from 0, 22.5, 45 and 90 g L-lysine·HCl infused P<.05, respectively.

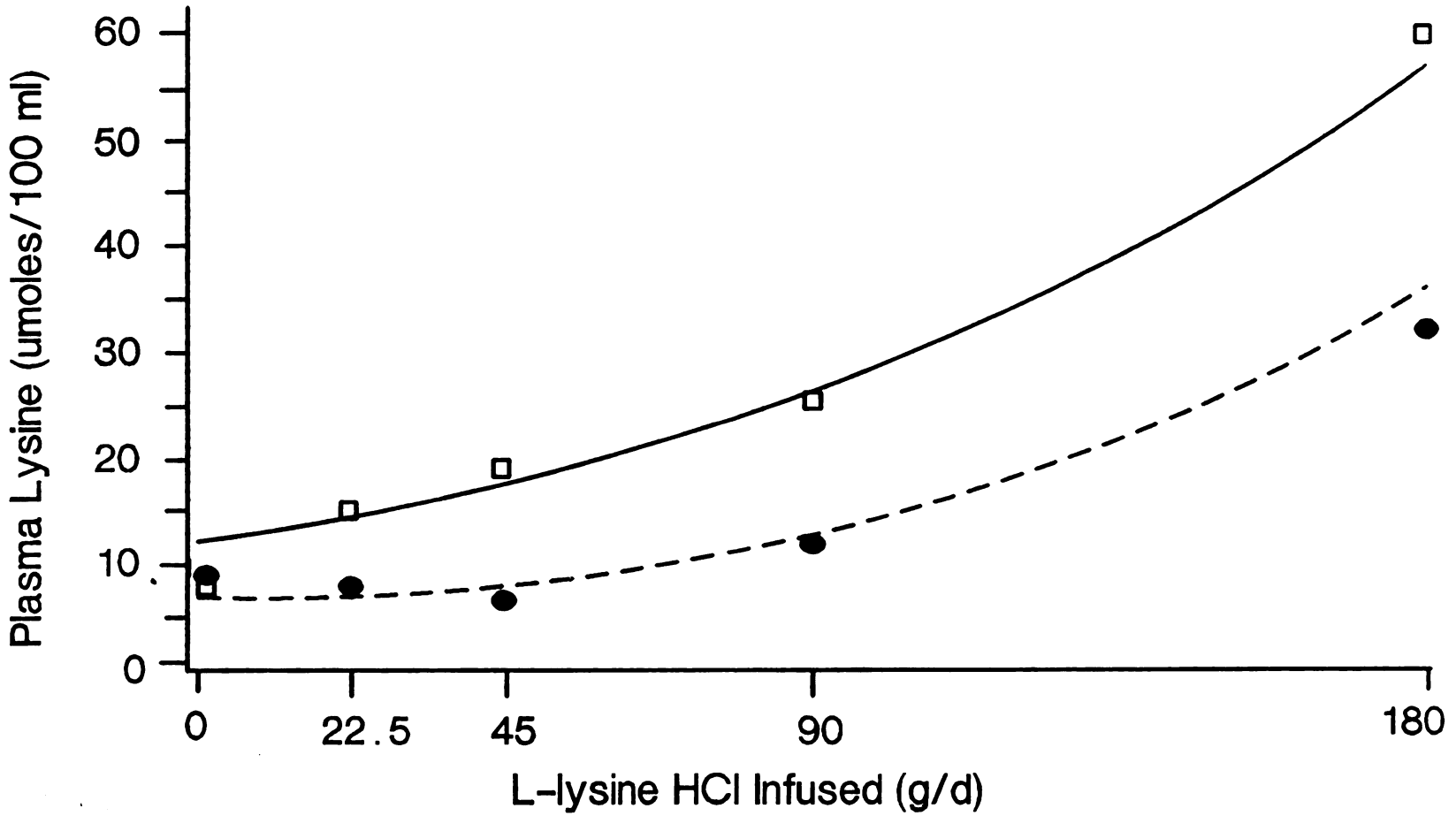


Figure 4. Quadratic response for plasma lysine concentration in cows abomasally infused with L-lysine HCl. □——□ Coccygeal vessel, $Y = 12.16 + .07X + .001X^2$, $P < .14$; ●- - -● Subcutaneous abdominal vessel, $Y = 7.05 - .019X + .001X^2$, $P < .05$. Experiment 3.

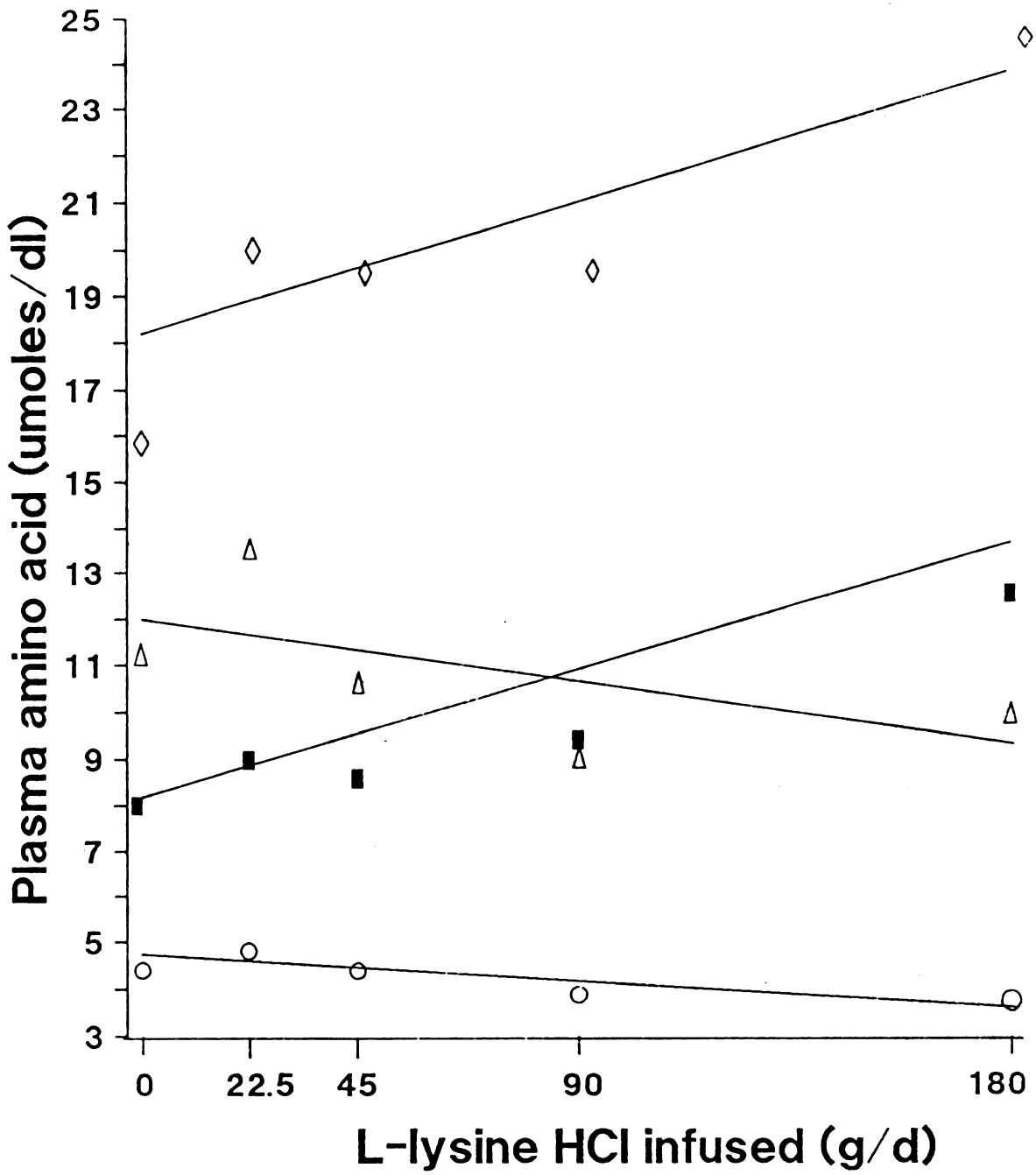


FIGURE 5. Plasma concentrations of free amino acids from lactating cows abomasally infused with L-lysine HCl.

○ Coccygeal methionine, Y = 4.663 - .0055X, P<.02;

△ Coccygeal threonine, Y = 12.022 - .01445X, P<.10;

◊ Coccygeal arginine, Y = 18.140 + .0312X, P<.02;

▮ Subcutaneous abdominal arginine, 8.212 + .0304X, P<.01. Experiment 3.

requirement was met. Broken-line analyses to determine the point at which lysine concentration increased was determined by regressing plasma lysine concentration with lysine infusions on each side of the inflection point (182). The break-point for the coccygeal plasma and subcutaneous abdominal vein plasma occurred at 81.5 and 78.4 g L-lysine HCl/d, respectively (figure 6). Expressed as L-lysine required by cows, the values were from 62.7 to 65.2 g/d of additional L-lysine required.

Mammary extraction of lysine tended to increase until 90 g/d infusion was reached, then it declined (experiment 3), apparently after the mammary requirement was met (table 20). For experiment 2, mammary extraction of plasma lysine increased with 45 g infusion and declined with 90 g infusion. This suggests that 90 g infusion was in excess of the cows' lysine requirement for milk production.

Once the requirement for the first limiting amino acid is exceeded, the plasma concentration of the next limiting amino acid is depressed due to an improved utilization of that amino acid for protein synthesis (19, 186). Therefore, the second limiting amino acid in experiment 3 was either threonine or methionine (table 19). Both have been implicated in several investigations as one of the first three limiting amino acids for milk production (33, 49, 141, 209). Threonine concentrations were less in duodenal digest and plasma (experiment 1, 162) of cows fed corn protein then those fed other sources of

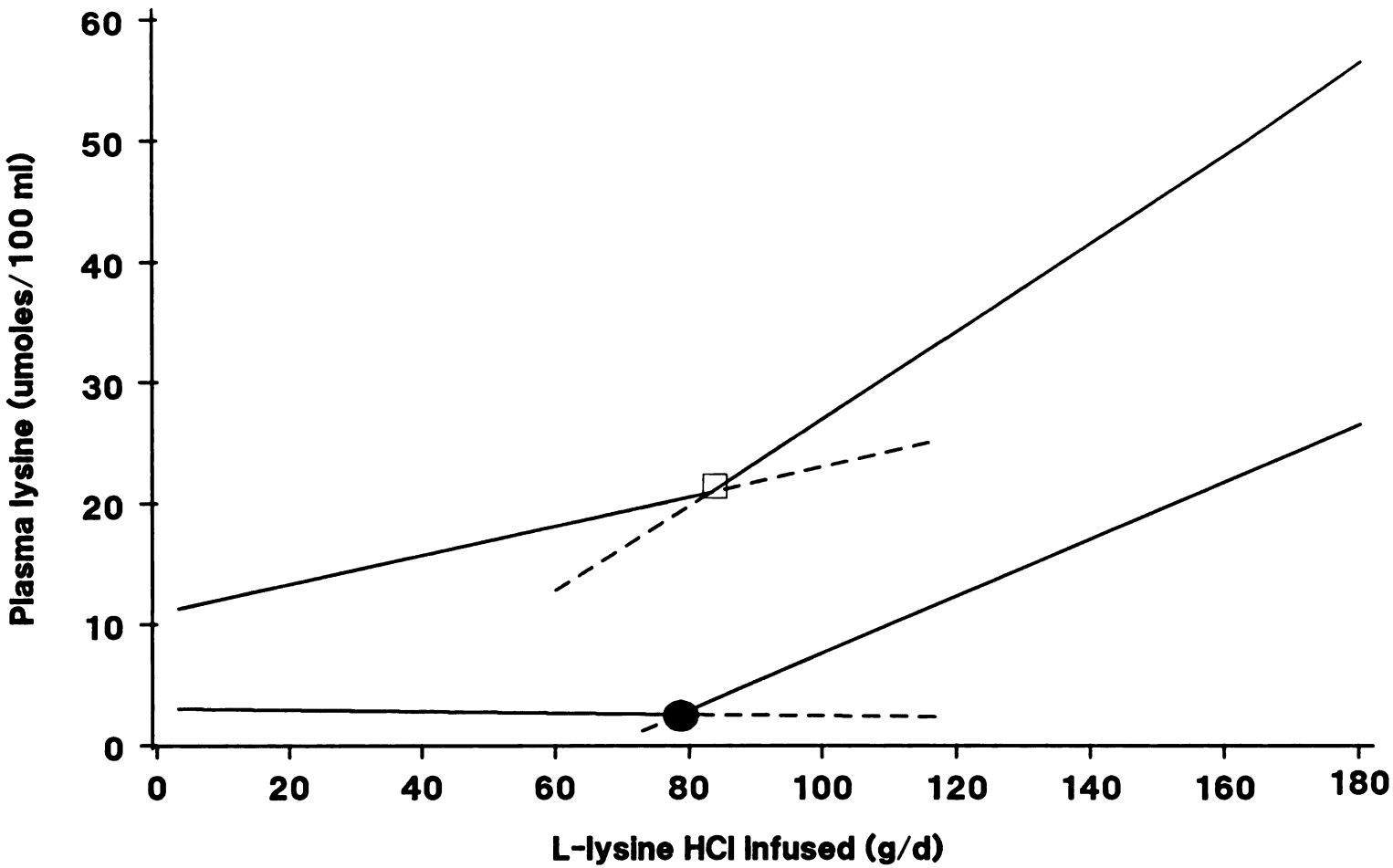


Figure 6. Broken-line analyses of lysine concentration in plasma of cows abomasally infused with L-lysine HCl. □ Coccygeal plasma break-point = 81.5 g/d; ● Subcutaneous abdominal plasma break-point = 78.4 g/d.

TABLE 20. Mammary extraction coefficients of cows abomasally infused with L-lysine HCl. Experiments 2 and 3.[1,2]

Infused (g/d)	His	Thr	Arg	Val	Met	Ile	Leu	Phe	Trp	Lys	Asp	Glu	Ser	Gly	Ala	Pro	Tyr	Orn
	Experiment 2																	
0	17.0	27.8	17.1	9.6	37.2	20.4	18.9	10.2		10.4	26.3	14.6	-17.2	4.8	7.8		24.4	
SE	6.7	16.0	9.0	12.0	7.6	8.5	7.0	2.0		4.4	21.5	8.6	13.9	13.0	14.3		9.1	
45	16.8	-23.9[a]	22.0	.3	12.4[a]	19.5	9.0	14.7		33.8	-50.9[A]	10.1	-33.5	-12.6	-15.4		-2.7[a]	
SE	8.8	17.4	10.8	10.5	8.7	12.5	7.3	1.7		6.1	19.0	10.3	12.3	11.5	12.7		8.7	
90	13.4	-4.5	24.4	7.0	16.1	18.2	13.9	-.3[aC]		22.5	-2.3	-2.4	1.6	-1.6	5.2		13.7	
SE	8.2	30.5	12.4	10.9	9.4	11.5	8.5	2.3		5.6	27.2	14.1	20.8	15.5	17.0		10.9	
	Experiment 3																	
0	3.9	-16.1	44.9	19.2	20.8	43.6	31.0	37.2	33.5	26.9	.7	16.3	17.4	-1.8	9.5	10.3	22.8	42.9
SE	13.3	21.8	8.9	8.6	27.0	11.1	10.3	9.6	13.8	18.7	41.6	14.3	12.2	13.9	14.9	9.9	10.5	11.5
22.5	33.5	19.9	55.5	34.5	27.8	37.0	34.5	31.8	30.6	48.0	-35.4	15.6	22.2	10.5	7.7	17.8	35.6	50.0
SE	10.9	17.6	7.5	7.3	21.9	8.3	7.7	7.2	10.3	14.0	32.3	12.1	10.5	11.8	12.6	8.4	8.9	8.6
45	20.9	1.2	50.8	33.5	26.5	41.8	36.5	33.4	27.0	53.4	-3.4	2.5	17.0	-.5	14.2	18.4	33.5	46.8
SE	11.2	21.8	7.8	7.5	27.1	8.6	8.0	7.5	10.7	14.5	40.6	12.5	10.8	12.2	13.1	8.6	9.2	8.9
90	19.1	-20.4	50.2	27.8	42.8	42.8	34.1	41.3	36.6	59.2	-41.0	3.9	13.0	1.5	14.9	11.1	31.0	51.3
SE	11.2	19.9	7.5	7.3	24.6	9.1	8.5	8.0	11.3	15.3	33.0	12.1	10.5	11.8	12.6	8.4	8.9	9.5
180	21.6	-9.1	49.2	33.0	9.5	31.9	31.8	32.4	24.5	35.1	-12.4	16.5	26.6	10.3	17.4	12.5	32.3	53.3
SE	12.3	19.8	8.1	7.9	24.8	9.1	8.5	7.9	11.3	15.3	40.0	13.2	11.4	12.8	13.7	9.1	9.6	9.5

[1] Mammary extraction = (coccygeal - venous) / coccygeal * 100.

[2] His=histidine, Thr=threonine, Arg=arginine, Val=valine, Met=methionine, Ile=isoleucine, Leu=leucine, Phe=phenylalanine, Trp=trptophan, Lys=lysine, Asp=aspartic acid, Glu=glutamic acid, Ser=serine, Gly=glycine, Ala=alanine, Pro=proline, Tyr=tyrosine, Orn=ornithine.

[a] Treatment different from 0 g L-lysine·HCl infused P<.10.

[A,C] Treatment different from 0 and 45 g L-lysine·HCl infused P<.05, respectively.

protein. Others have determined methionine was the second limiting or a co-limiting amino acid when cows were fed a corn-based diet (33, 37).

Increased plasma arginine concentration may be due to metabolism of excess lysine in the liver (207) or mammary gland (141). Lysine oxidation increases markedly when dietary lysine is above that which is required for maximum performance (34). Increased plasma ornithine may result from the metabolism of arginine. Clark et al (50) showed that up to 77% of the arginine metabolized by the bovine mammary gland was converted to ornithine. Another possible explanation for increased arginine and ornithine concentrations was suggested by Baumrucker (14). Alteration in plasma concentration of one amino acid in vivo may competitively inhibit uptake of amino acids sharing the same cellular transport system. If the concentration of lysine was increased from 8 to 16 umol/dl, more lysine would be provided to cells that have the Y^+ (cationic) transport system; however, both arginine and ornithine uptake would be reduced due to competitive inhibition by lysine for the common transport site (13). This may result in accumulation of arginine and ornithine in the plasma. Investigators have observed that excess dietary lysine inhibited liver arginase activity and increased plasma arginine concentration in some species (207, 212), but not others (70, 112). Plasma urea was not different among treatments and averaged 25.5 and 18.7 mg/dl in coccygeal

plasma and subcutaneous abdominal plasma for experiment 3. Since plasma urea was not effected by treatment and plasma arginine was increased, liver metabolism of arginine may have been reduced.

The apparent digestible lysine required by cows in experiment 3 can be estimated by:

$$(UDLys * DC_1) + (BLys * DC_2) + (infused\ Lys)$$

where UDLys = lysine from undegraded feed protein + endogenous protein, BLys = lysine from bacteria, and $DC_{1\&2}$ represent the digestion coefficients for UDLys and BLys, respectively. Estimates varied according to the assumptions imposed for the calculations. Using a prediction equation for non-ammonia nitrogen passing to the small intestine (188), 2.73 kg of microbial protein and 2.38 kg of feed and endogenous protein would have passed to the duodenum. Since bacteria are similar in their amino acid profile (22), total microbial contribution of lysine to the duodenum was 162 g/d and had a digestion coefficient of .80 (241). Assuming the composition of the combined feed and endogenous protein was 19 g lysine/kg protein (experiment 1), the contribution from this source was 45 g/d and had a digestion coefficient of approximately .75 (experiment 1). On this basis, cows in experiment 3 were required to digest 228 g of lysine/d. However, if the assumption that the rumen passage of protein was 123% of intake and 54% was of microbial origin (198), the bacterial contribution of lysine was 157 g/d. The combined feed and endogenous

contribution of lysine was 43 g/d. With these assumptions, 222 g of digested lysine were required. The differences between DM consumption in experiment 1 and experiment 3 (17 vs. 22 kg/d) may alter the estimate for lysine passing to the intestine, since increased DM intake results in less ruminal protein degradation and more microbial protein synthesis (228). Greater intake, however, would not greatly alter the proportion of microbial N to feed and endogenous N.

Efficiency of net milk protein secretion from ingested protein increased quadratically ($P<.05$) with increased lysine infusion in experiment 3 and linearly ($P<.10$) in experiment 2 (table 21). This is another indication that lysine was the limiting amino acid for milk protein synthesis, and with 180 g L-lysine·HCl infusion the cows' requirement for lysine was surpassed. Approximately 75 g lysine were secreted in the milk of cows' not infused. Therefore, the conversion efficiency for lysine entering the duodenum to milk lysine was approximately 37%. The estimated efficiency for duodenal lysine conversion into milk for infusions of 22.5, 45, 90 and 180 g L-lysine·HCl are 36, 32, 29, and 23%, respectively. Lysine conversion to milk was lower only for the 180 g infusion than for values obtained for the various proteins fed in experiment 1, indicating lysine supply to the intestine was not excessive in experiment 1. Absorbed protein conversion to milk protein proposed by various computer modeling systems range from 56 to 95%. Estimated lysine conversion efficiency

TABLE 21. Nitrogen (N) partitioning by lactating cows abomasally infused with L-lysine·HCl.Experiments 2 and 3.

	L-lysine·HCl infused (g/d)									
Parameter	0	SE	22.5	SE	45	SE	90	SE	180	SE
Ingested N (g)[1]	490	10			490	10	500	10		
(g)[2]	658	24	632	21	615	21	660	22	610	21
digestible (%)[1]	66.9	2.6			69.4	2.6	69.0	3.6		
(%)[2]	72.7	3.6	77.2	2.8	80.4	2.8	79.1	2.6	76.5	3.1
NPU (%)[2,3]	48.3	3.2	44.2	2.3	51.7	2.7	48.6	2.6	47.9	2.6
milk (%)[1]	26.6	.8			27.8	.8	28.9[a]	.8		
(%)[2,Q]	23.1	1.1	26.4[A]	.9	25.0	.9	26.8[A]	.9	25.2	1.0
retained (%)[2]	24.6	4.0	17.8	3.5	26.1	3.3	23.5	3.2	22.7	3.2
urine (%)[2]	34.5	1.5	35.9	1.3	32.2	1.4	34.6	1.4	33.8	1.4
Digested N (g)[1]	326	17			343	17	343	24		
(g)[2]	488	33	478	26	508	26	494	24	528	29
BV (%)[2,4]	58.7	2.8	54.6	2.2	62.1	2.3	59.2	2.3	59.6	2.2
milk (%)[1]	40.2	2.3			40.4	2.3	43.0	3.2		
(%)[2]	35.3	3.3	35.2	2.7	33.1	2.7	33.8	2.4	32.8	2.9
retained (%)[2]	29.3	4.6	20.8	4.02	31.3	3.8	28.6	3.7	37.9	3.6
urine (%)[2]	45.9	3.5	46.7	2.7	38.1[ab]	3.1	39.9	3.1	40.8	3.0
Metabolic N (g)[2,5]	269	33	266	26	315	29	286	26	310	29
milk (%)[2]	50.6	8.5	68.4	7.4	51.3	7.0	52.2	6.9	52.1	6.7
retained (%)[2]	49.4	8.5	31.6	7.4	48.7	7.0	47.8	6.9	47.8	6.7
Milk N (g)[1,L]	127	5			138	5	146[A]	5		
(g)[2,L]	147	4	158[A]	3	154	3	160[A]	3	163[Ac]	3
Urine N (g)[2]	226	9	216	8	204[a]	8	208	8	220	8
Retained N (g)[2]	120	33	107	26	159	29	131	26	142	29

[1] Experiment 2, 1985, chromic oxide was digesta marker.
[2] Experiment 3, 1986, acid insoluble ash was digesta marker.
[3] Net protein utilization = (ingested N - fecal N - urine N) / ingested N *100.
[4] Biological value = (ingested N - fecal N - urine N) / (ingested N - fecal N) * 100.
[5] Metabolic N = ingested N - fecal N - urine N.
[a,b,c] Treatment different from 0, 22.5, and 45 g L-lysine·HCl P<.10, respectively.
[A,B,C] Treatment different from 0, 22.5, and 45 g L-lysine·HCl P<.05, respectively.
[L,Q] Linear or quadratic response to treatment P<.05, respectively.

for apparently digested lysine to milk lysine is 47, 45, 39, 35, and 26% for 0, 22.5, 45, 90, and 180 g L-lysine·HCl infused. The discrepancy between values for estimated conversion of absorbed protein and that of apparently digested lysine suggests intestinal tissue and/or intestinal bacteria metabolize from 10 to 50 % of the digested lysine (0 g L-lysine infused). Increasing the quantity of lysine infused also increased the quantity of lysine metabolized by the small intestine, if we assume no change in conversion efficiency of absorbed lysine to milk.

General Discussion

Data from experiment 1 show that protein sources of varying degradabilities in ruminant diets influence the complement of amino acids available for digestion and metabolism. For instance, when corn gluten meal or cottonseed meal comprised 50% of the dietary protein, lysine was one of the amino acids least abundant in the plasma but on the blood meal diet, lysine was one of the most abundant. Dietary N was degraded to a lesser extent in the rumen for cows receiving BM and CGM diets than those fed CSM.

From these data it appears that when greater than 50% of the dietary protein is from corn products, lysine may be a limiting amino acid. Protein sources which are relatively high in lysine and resistant to microbial degradation may be

useful in alleviating this deficiency.

Blood meal (high lysine, low degradability) increased the lysine concentration of feed and endogenous protein passing to the duodenum compared with corn gluten (low lysine, low degradability) and cottonseed meals (low lysine, high degradability), 4.5 vs. 1.9 vs. 2.9%, respectively. Blood meal also increased the proportion of feed and endogenous protein to total protein in the duodenal chyme compared with corn gluten and cottonseed meals, 56 vs. 46. vs. 51%, respectively. The estimated passage of lysine to the duodenum would be 240 g/d for the blood meal diet and 195 g/d for both the corn gluten and cottonseed meal diets, assuming a dietary intake equivalent to that of experiment 3, and passage of protein and amino acids proportional to experiment 1. Based on the titration value for lysine requirements determined by plasma amino acid concentration (experiment 3), blood meal appears to supply adequate post-ruminal lysine to meet the cows' requirements, while corn gluten and cottonseed meals do not.

Although blood meal may alleviate lysine deficiency in cows fed conventional diets, other amino acids may be found in more critical supply for milk protein secretion by cows fed relatively high amounts of blood meal in place of conventional protein sources.

SUMMARY

The response of lactating cows to abomasal infusion of L-lysine·HCl in these experiments demonstrate the validity of this procedure and that lysine is the limiting amino acid when cattle are fed diets containing predominately corn products. Milk protein production and efficiency of milk protein synthesis from feed protein was improved with the addition of an adequate quantity of lysine. A break-point response to lysine infusion was demonstrated for plasma lysine concentration from the subcutaneous abdominal plasma and to a lesser extent for coccygeal plasma. Excess lysine resulted in increased plasma concentrations of arginine and ornithine. It was estimated that a 610 kg lactating cow, producing 32 kg of milk containing 3.3% protein, requires 225 g of digested lysine per day.

BIBLIOGRAPHY

BIBLIOGRAPHY

1 Aderibigbe, A. O., and D. C. Church. 1983. Feather and hair meals for ruminants. II. Comparative evaluation of feather and hair meals as protein supplements. J. Anim. Sci. 57:473.

2 Agricultural Research Council. 1984. The Nutrient Requirements of Ruminant Livestock, Suppl. 1. HMSO, London, pp. 45.

3 Ahmed, B. M., W. G. Bergen, and N. K. Ames. 1983. Effect of nutritional state and insulin on hind-limb metabolism in steers. J. Nutr. 113:1529.

4 Airhart, J. J., A. Virdrich, and E. A. Khairallah. 1974. Compartmentation of free amino acids for protein synthesis. Biochem. J. 140:539.

5 Allison, M. J. 1970. Nitrogen metabolism of ruminal microorganisms. Pages 456-473 in Physiology of digestion and metabolism in the ruminant. A. T. Phillipson, ed. Oriel Press, New Castle upon Tyne, England.

6 Allison, J. B. and R. W. Wannemacher, Jr. 1965. The concept and significance of labile and over-all protein reserves of the body. Amer. J. Clin. Nutr. 16:445.

7 Alumot, E. I. Bruckental, A. Tadmor, C. Kennit, and P. Holstein. 1983. Effect of proline on arginine uptake and nitrogen metabolism of lactating goats. J. Dairy Sci. 66:1243.

8 Ashes J. R., J. L. Mangan, and G. S. Sidhu. 1984. Nutritional availability of amino acids from protein cross-linked to protect against degradation in the rumen. Br J. Nutr. 52:239.

9 Association of Official Analytical Chemists. 1975. Official methods of analysis. 12th ed. Washington, DC.

10 Austic, R. E., and C. C. Calvert. 1981. Nutritional interrelationships of electylytes and amino acids. Fed. Proc. 40:63.

11 Ayoade, A., P. J. Buttery, and D. Lewis. 1982. Studies on methionine derivatives as possible sources of protected methionine in ruminant rations. J. Sci. Food Agric. 33:949.

12 Barry, T. N. 1976. The effectiveness of formaldehyde treatment in protecting dietary protein from rumen microbial degradation. Proc. Nutr. Soc. 35:221.

13 Baumrucker, C. R. 1984. Cationic amino acid transport by bovine mammary tissue. J. Dairy Sci. 67:2500.

14 Baumrucker, C. R. 1985. Amino acid transport systems in bovine mammary tissue. J. Dairy Sci. 68:2436.

15 Baumrucker, C. R., and C. L. Davis. 1980. Gamma-glutamyl transpeptidase activity along the small intestine of sheep: Potential areas of amino acid and peptide transport. J. Dairy Sci. 63:379.

16 Baumrucker, C. R., P. A. Pocius, and T. L. Riss. 1981. Glutathione utilization by lactating bovine mammary secretory tissue. Biochem. J. 198:243.

17 Ben-Gehedalia, D., H. Tagari, and A. Bondi. 1974. Protein digestion in the intestine of sheep. Brit. J. Nutr. 31:125.

18 Bergen, W. G. 1978. Postruminal digestion and absorption of nitrogenous components. Fed. Proc. 37:1223.

19 Bergen, W. G. 1979. Free amino acids in blood of ruminants-physiological and nutritional regulation. J. Anim. Sci. 49:1577.

20 Bergen, W. G., D. B. Bates, D. E. Johnson, J. C. Waller, and J. R. Black. 1982. Ruminal microbial protein synthesis and efficiency. Pages 99-112 in: Protein requirements of cattle: Symposium. F. N. Owens, ed. Oklahoma State Univ., Misc. Proc. 109.

21 Bergen, W. G., H. A. Henneman, and W. T. Magee. 1973. Effect of dietary protein level and protein source on plasma and tissue free amino acids in growing sheep. J. Nutr. 103:575.

22 Bergen, W. G., D. B. Purser, and J. H. Cline. 1968. Effect of ration on the nutritive quality of rumen microbial protein. J. Anim. Sci. 27:1497.

23 Bergen, W. G., D. B. Purser, and J. H. Cline. 1968. Determination of limiting amino acids of rumen-isolated microbial proteins fed to rat. J. Dairy Sci. 51:1698.

24 Bergen, W. G., and M. T. Yokoyama. 1977. Productive limits to rumen fermentation. J. Anim. Sci. 46:573.

25 Bergman, E. N., and R. N. Heitmann. 1978. Metabolism of amino acids by the gut, liver, kidneys, and peripheral tissues. Fed. Proc. 37:1228.

25 Bickerstaffe, R., E. F. Annison, and J. L. Linzell. 1974. The metabolism of glucose, acetate, lipids and amino acids in lactating cows. J. Agric. Sci. (Camb.) 82:71.

27 Bjarnason, J., and K. J. Carpenter. 1969. Mechanism of heat damage in protein. I. Models with acylated lysine units. Br. J. Nutr. 23:859.

28 Bjarnason, J., and K. J. Carpenter. 1970. Mechanism of heat damage in protein. II. Chemical changes in pure proteins. Br. J. Nutr. 24:313.

29 de Boer, G., J. J. Murphy, and J. J. Kennelly. 1987. Mobile nylon bag for estimating intestinal availability of rumen undegradable protein. J. Dairy Sci. 70:977.

30 Boling, J. A., N. W. Bradley, and J. C. Willard. 1972. Amino acid patterns in the blood plasma of the young bovine. Internat. J. Vit. Nutr. Res. 42:306.

31 Botts, R. L., R. W. Hemken, and L. S. Bull. 1979. Protein reserves in the lactating dairy cow. J. Dairy Sci. 62:433.

32 Broderick, G. 1982. Estimation of protein degradation using in situ and in vitro meithods. Pages 72-80 in Protein requirements of cattle: Symposium. F. N. Owens, ed. Oklahoma State Univ., Misc. Publ.

33 Broderick, G. A., L. D. Satter, and A. E. Harper. 1974. Use of plasma amino acid concentration to identify limiting amino acids for milk production. J. Dairy Sci. 57:1015.

34 Brookes, I. M., F. N. Owens, R. E. Brown, and U. S. Garrigus. 1973. Amino acid oxidation and plasma amino acid levels in sheep with abomasal infusions of graded amounts of lysine. J. Anim. Sci. 36:965.

35 Bryant, D. T. W., and R. W. Smith. 1982. The effect of lactation on protein sythesis in ovine skeletal muscle. J. Agric. Sci. (Camb.) 99:319.

36 Burgos, A., and H. H. Olson. 1970. Effect of 40 g of methionine hydroxy analog on yield and composition of milk. J. Dairy Sci. 53:647.

37 Burris, W. R., J. A. Boling, N. W. Bradley, and A. W. Young. 1976. Abomasal lysine infusion in steers fed a urea supplemented diet. J. Anim. Sci. 42:699.

38 Burris, W. R., N. W. Bradley, and J. A. Boling. 1975. Growth and plasma amino acids of steers fed different nitrogen sources at restricted intake. J. Anim. Sci. 40:714.

39 Burroughs, W., D. Nelson, and D. Mertens. 1975. Protein physiology and its application in the lactating cow: The metabolizable protein standard. J. Anim. Sci. 41:933.

40 Buttery, P. J., and A. N. Foulds. 1985. Amino acid requirements of ruminants. Pages 257-271 in Recent advances in animal nutrition. W. Haresign and D. J. A. Cole, ed. Butterworths, London, England.

41 Casper, D. P., D. J. Schingoethe, C-M. J. Yang, and C. R. Mueller. 1987. Protected methionine supplementation with extruded blend of soybeans and soybean meal for dairy cows. J. Dairy Sci. 70:321.

42 Chalupa, W. 1976. Degradation of amino acids by the mixed rumen microbial population. J. Anim. Sci. 43:828.

43 Chalupa, W. 1980. Methods for estimating protein requirements and feed protein values for ruminants. Feedstuffs 52:(26):18.

44 Chamberlain, D. G., and P. C. Thomas. 1979. Ruminal nitrogen metabolism and the passage of amino acids to the duodenum in sheep receiving diets containing hay and concentrates in various proportions. J. Sci. Food Agric. 30:677.

45 Chamberlain, D. G. and P. C. Thomas. 1982. Effect of intravenous supplements of L-methionine on milk yield and composition in cows given silage-cereal diets. J. Dairy Res. 49:25.

46 Chandler, P. T., C. A. Brown, R. P. Johnston, Jr., G. K. Macleod, R. D. McCarthy, B. R. Mos, A. H. Rakes, and L. D. Satter. 1976. Protein and methionine hydroxy analog for lactating cows. J. Dairy Sci. 59:1897.

47 Chaney, A. L., and E. P. Marbach. 1962. Determination of urea and ammonia. Clin. Chem. 8:130.

48 Chen, G., C. J. Sniffen, and J. B. Russel. 1987. Concentration and estimated flow of peptides from the rumen of dairy cattle: Effects of protein quantity, protein solubility, and feeding frequency. J. Dairy Sci. 70:983.

49 Clark, J. H. 1975. Lactational responses to postruminal administration of proteins and amino acids. J. Dairy Sci. 58:1178.

50 Clark, J. H., R. G. Derrig, C. L. Davis, and H. R. Spires. 1975. Metabolism of arginine and ornithine in the cow and rabbit mammary tissue. J. Dairy Sci. 58:1808.

51 Clark, J. H., M. R. Murphy, and B. A. Crooker. 1987. Supplying the protein needs of dairy cattle from by-product feeds. J. Dairy Sci. 70:1092.

52 Clark, J. H., H. R. Spires, and C. L. Davis. 1978. Uptake and metabolism of nitrogenous compounds by the lactating mammary gland. Fed. Proc. 37:1233.

53 Clark, J. H., H. R. Spires, R. G. Derrig, and M. R. Bennink. 1977. Milk production, nitrogen utilization and glucose synthesis in lactating cows infused post-ruminally with sodium caseinate and glucose. J. Nutr. 107:631.

54 Clark, R. M., P. T. Chandler, C. S. Park, and A. W. Norman. 1980. Extracellular amino acid effects on milk protein synthesis and intracellular amino acid pools with bovine mammary cells in culture. J. Dairy Sci. 63:1230.

55 Cleale, R. M., IV, T. J. Klopfenstein, R. A. Britton, L. S. Satterlee, and S. R. Lowry. 1987. Induced non-enzymatic browning of sybean meal. I. Effects of factors controlling non-enzymatic browning on in vitro ammonia release. J. Anim. Sci. 65:1312.

56 Cohick, W. S., J. L. Vicini, C. R. Stables, J. H. Clark, S. N. McCutcheon, and D. E. Bauman. 1986. Effects of intake and postruminal casein infusion on performance and concentrations of hormones in plasma of lactating cows. J. Dairy Sci. 69:3022.

57 Cottrill, B. R., D. E. Beever, A. R. Austin, and D. F. Osbourn. 1982. The effect of protein- and non-protein-nitrogen supplements to maize silage on total amino acid supply in young cattle. Br. J. Nutr. 48:527.

58 Coward, B. J. and P. J. Buttery. 1982. Metabolism of perfused ruminant muscle. J. Agric. Sci. (Camb.) 93:307.

59 Craig, W. M. and G. A. Broderick. 1984. Amino acids released during protein degradation by rumen microbes. J. Anim. Sci. 58:436.

60 Crooker, B. A., and J. H. Clark. Inhibition of L-alanine uptake in bovine jejunal brush border membrane vesicles by L-amino acids. J. Dairy Sci. 70:963.

61 Cross, D. L., R. L. Ludwick, J. A. Boling, and N. W. Bradley. 1974. Plasma and rumen fluid components of steers fed two sources and levels of nitrogen J. Anim. Sci. 38:404.

62 Cummins, K. A., and A. H. Papas. 1985. Effect of isocarbon-4 and isocarbon-5 volatile fatty acids on microbial protein synthesis and dry matter digestibility in vitro. J. Dairy Sci. 68:2588.

63 Davis, S. R., R. Bickerstaffe, and D. S. Hart. 1978. Amino acid uptake by the mammary gland of the lactating ewe. Aust. J. Biol. Sci. 31:123.

64 Davis, S. R., and R. J. Collier. 1985. Mammary blood flow and regulation of substrate supply for milk synthesis. J. Dairy Sci. 68:1041.

65 Davis, S. R. and T. B. Mepham. 1976. Metabolism of L-[U-^{14}C] valine, L-[U-^{14}C]leucine, L-[U-^{14}C]histidine and L-[U-^{14}C] phenylalanine by the isolated perfused lactating guinea-pig mammary gland. Biochem. J. 156:553.

66 Derrig, R. G., J. H. Clark, and C. L. Davis. 1974. Effect of abomasal infusion of sodium caseinate on milk yield, nitrogen utilization and amino acid nutrition of the dairy cow. J. Nutr. 104:151.

67 Devinoy, E., L. M. Houdebine, and C. Delouis. 1978. Role of prolactin and glucocorticoids in the expression of casein genes in rabbit mammary gland organ culrure. Quantification of casein mRNA. Biochem. Biophys. Acta 517:360.

68 Drackley, J. K., and D. J. Schingoethe. 1986. Extruded blend of soybean meal and sunflower seeds for dairy cattle in early lactation. J. Dairy Sci. 69:371.

69 Eagle, H., A. K. Piez, and M. Levy. 1961. The intracellular amino concentration required for protein synthesis in cultured human cells. J. Biol. Chem. 236:2039.

70 Edmonds, M. S., and D. H. Baker. 1987. Failure of excess dietary lysine to antagonize arginine in young pigs. J. Nutr. 117:1397.

71 Emery, R. S. 1978. Feeding for increased milk protein. J. Dairy Sci. 61:825.

72 Ensminger, M. E., and C. G. Olentine, Jr. 1978. Feeds and Nutrition- complete. The Ensminger Publishing Co., Clovis, CA.

73 Fauconneau, G, and M. C. Michel. 1970. In Mammalian protein metabolism. ed H. N. Munro, Vol. 4, pp 481-516. Academic Press, New York.

74 Fenderson, C. L., and W. G. Bergen. 1972. Effect of ration composition and protein level on plasma free tryptophan and ruminal microbial tryptophan content in sheep. J Anim. Sci. 35:896.

75 Fenderson, C. L., and W. G. Bergen. 1975. An assessmemt of amino acid requirements of growing steers. J. Anim. Sci. 41:1759.

76 Fenton, T. W., and M. Fenton. 1979. An improved procedure for the determination of chromic oxide in feed and feces. Can. J. Anim. Sci. 59:631.

77 Firkins, J. L., S. M. Lewis, L. Montgomery, L. L. Berger, N.R. Merchen, and G.C. Gahey. 1987. Effects of feed intake and dietary urea concentration on ruminal dilution rate and efficiency of bacterial growth in steers. J. Dairy Sci. 70:2312.

78 Fisher, L. J. 1972. Response of lactating cows to the intravenous infusion of amino acids. Can. J. Anim. Sci. 52:377.

79 Fisher, N., J. Grun, R. Shapiro, and J. Ashley. 1964. Protein reserves: Evidence for their utilization under nutritional and disease stress conditions. J. Nutr. 83:165.

80 Fleet, I. R., and T. B. Mepham. 1985. Mammary uptake of amino acids and glucose throughout lactation in Friesland sheep. J. Dairy Res. 52:229.

81 Foldager, J., J. T. Huber, and W. G. Bergen. 1977. Methionine and sulfur amino acid requirement of the preruminant calf. J. Dairy Sci. 60:1095.

82 Forsberg, C. W., L. K. A. Lovelock, L. Krumholz, and J. G. Buchanan-Smith. 1984. Protease activities of rumen protozoa. Appl. Enviro. Microbiol. 47:101.

83 Forsberg, N. E., R. E. Austic, and R. D. Boyd. 1987. Influence of dietary electrolyte balance and extracellular bicarbonate concentration on lysine metabolism Nutr. Rep. Int. 35:453.

84 Gaines, W. L. 1928. The energy basis of measuring milk yield in dairy cows. Illinois Agr. Exp. Stn., Bull. 308.

85 Ganev, G., E. R. Orskov, and R. Smart. 1979. The effect of roughage or concentrate feeding and rumen retention time on total degradation of protein in the rumen. J. Agric. Sci. (Camb.) 93:651.

86 Goering, H. K., and P. J. Van Soest. 1970. Forage fiber analyses. Agric. Handbook No. 379, Agric. Res. Serv., US Dep. Agric., Washington, DC.

87 Gordon, G. L. R. and J. R. Ashes. 1984. Can. J. Anim. Sci. 64(suppl. 1):156. Abstr.

88 Griel, L. C. Jr., R. A. Patton, R. D. McCarthy, and P. T. Chandler. 1968. Milk production response to feeding methionine hydroxy analog to lactating cows. J. Dairy Sci. 51:1866.

89 Grimble, G. K., R. G. Rees, P. P. Kednane, T. Cartwright, M. Desreumaux, and D. B. A. Silk. 1987. Effect of peptide chain length on absorption of egg protein hydrolysates in normal human jejunum. Gastroenterol. 92:136.

90 Guerino, F., and C. R. Baumrucker. 1985. Lysine and methionine transport systems in the bovine small intestine. J. Anim. Sci. 61 (Suppl. 1):457. (Abstr.)

91 Guidotti, G. G., A. F. Borghetti, and G. C. Gazzola. 1978. The regulation of amino acid transport in animal cells. Biochim. Biophys. Acta 515:329.

92 Halfpenny, A. F., J. A. F. Rook, and G. H. Smith. 1969. Variations with energy nutrition in the concentrations of amino acids of the blood plasma in the dairy cow. Br. J. Nutr. 23:547.

93 Harper, A. E., N. J. Benevenga, and R. M. Wohlhueter. 1970. Effects of ingestion of disproporionate amounts of amino acids. Phys. Rev. 50:428.

94 Harrison, D. G., D. E. Beever, and D. F. Osbourn. 1979. The contribution of protozoa to the protein entering the duodenum of sheep. Br. J. Nutr. 32:341.

95 Harrop, C. J. F. 1974. Nitrogen metabolism in the ovine stomach. 4. Nitogenous components of the abomasal secretions. J. Agric Sci. (Camb.) 83:249.

96 Hegsted, D. M. 1974. Assessment of protein quality. Page 65 in Committee on Amino Acids, Food and Nutrition Board, Improvement of Protein Nutriture. NRC ed. Natl. Acad. Sci., Washington, DC.

97 Heitmann, R. N., and E. N. Bergman. 1980. Transport of amino acids in whole blood and plasma of sheep. Am. J. Physiol. 239:E237.

98 Heitmann, R. N. and E. N. Bergman. 1980. Intergration of amino acid metabolism in sheep: effects of fasting and acidosis. Am. J. Physiol. 239:E248.

99 Hill, G. M., J. A. Boling, and N. W. Bradley. 1980. Postruminal lysine and methionine infusion in steers fed a urea-supplemented diet adequate in sulfur. J. Dairy Sci. 63:1242.

100 Houdebine, L. M. and P. Gaye. 1975. Regulation of casein synthesis in rabbit mammary gland. Titration of mRNA activity for casein under prolactin and progesterone treatments. Mol. Cell. Endocrinol. 3:37.

101 Huber, J. T., R. S. Emery, W. G. Bergen, J. S.Liesman, L. Kung, Jr., K. J. King, R. W. Gardner, and M. Checketts. 1984. Influences of methionine hudroxy analog on milk and milk fat production, blood serum lipids, and plasma amino acids. J. Dairy Sci. 67:2525.

102 Huber, J. T., and L. Kung, Jr. 1981. Protein and nonprotein nitrogen utilization in dairy cattle. J. Dairy Sci. 64:1170.

103 Huntington, G. B., and R. L. Prior. 1985. Net absorption of amino acids by portal-drained viscera and hind half of beef cattle fed a high concentrate diet. J. Anim. Sci. 60:1491.

104 Hurwitz, R., and N. Kretchmer. 1986. Development of arginine-synthesizing enzymes in mouse intestine. Am. J. Physiol. 251:G103.

105 Ibrahim, E. A., J. R. Ingalls, and D. B. Bragg. 1970. Separation and identification of amino acids present in rumen microorganisms. Can. J. Anim. Sci. 50:397.

106 Illg, D. J., J. L. Sommerfeldt, and D. J. Schingoethe. 1987. Lactational and systemic responses to the supplementation of protected methionine in soybean meal diets. J. Dairy Sci. 70:620.

107 Istasse, L., A. C. Brewer, and E. R. Orskov. 1986. Effects of stage of lactation on the response fo dairy cows to abomasal infusions of casein. Livest. Prod. Sci. 15:97.

108 Johns, J.T. and W.G. Bergen. 1973. Studies of amino acid uptake by ovine small intestine. J. Nutr. 103:1581.

109 Johnson, C. O. L. E., J. T. Huber, and K. J. King. 1987. Storage and utilization of brewers wet grains in diets for lactating cows. J. Dairy Sci. 70:98.

110 Journet, M., and R. Verite. 1979. Predicting equations of N duodenal flow in dairy cattle: effects of level of feeding and proportion of concentrate in the diet. Ann. Rech. Vet. 10:303.

111 Kaufmann, W. 1977. Calculation of the protein requirement for dairy cows according to measurements of N metabolism. in Proc. 2nd Int. Symp. Protein Metab. and Nutr. Pages 130-132 S. Tamminga ed. Centre Agr. Pub. Doc., Wageningen, Holland.

112 Kaushik, S. J., and B. Fauconneau. 1984. Effects of lysine administration on plasma arginine and on some nitrogen catabolites in rainbow trout. Comp. Biochem. Physiol. A. Comp. Physiol. 79:459.

113 Keller, P. J., E. Cohen, and H. Neurath. 1958. The proteins of bovine pancreatic juice. J. Biol. Chem. 233:344.

114 Kenna, T. M., and C. G. Schwab. 1981. Evaluation of N-hydroxymethyl-DL-methionine-Ca and Di-hydroxymethyl-L-lysine-Ca in a blended corn based ration for lactating cows. J. Dairy Sci. 64:775.

115 Kennedy, P. M. and L. P. Milligan. 1980. Input of endogenous protein into the forestomachs of sheep. Can. J. Anim. Sci. 60:1029.

116 King, K. J., J. W. Thomas, L. Kung, Jr., and J. T. Huber. 1983. Protein degradability of several protein sources using a ficin protease assay. Proc. 16th Ann. Meeting Midwestern Section ASAS, Chicago, IL.

117 Klooster, A. T. V., and A. A. Boekholt. 1972. Protein digestion in the stomachs and intestines of the cow. Neth. J. Agric. Sci. 20:272.

118 Kronfeld, D. S., F. Raggi, and C. F. Ramberg, Jr. 1968. Mammary blood flow and ketone body metabolism in normal, fasted, and ketotic cows. Am. J. Physiol. 215:218.

119 Kung, L., Jr., and J. T. Huber. 1983. Performance of high producing cows in early lactation fed protein of varying amounts, sources and degradability. J. Dairy Sci. 66:227.

120 Kung, L., Jr., J. T. Huber, W. G. Bergen, and D. Petitclerc. 1984. Amino acids in plasma and duodenal digesta and plasma growth hormone in cows fed varying amounts of protein of differing degradability. J. Dairy Sci. 67:2519.

121 Laycock, K. A., and E. L. Miller. 1981. Nitrogen solubility and protein degradability of commercially and laboratory prepared rapeseed and soya-bean meals. Proc. Nutr. Soc. 40:103A.

122 Leibholz, J. M., J. T. McCall, V. W. Hays, and V. C. Speer. 1966. Potassium, protein and basic amino acid relationships in swine. J. Anim. Sci. 25:37.

123 Leng, R. A. 1982. Dynamics of protozoa in the rumen of sheep. Br. J. Nutr. 48:399.

124 Leng, R. A. and J. V. Nolan. 1984. Nitrogen metabolism in the rumen. J. Dairy Sci. 67:1072.

125 Lindberg, J. E. 1982. Ruminal flow rate of soya-bean meal, rapeseed meal and cottonseed meal in cows fed at maintenance and at three times maintenance. J. Agric. Sci. (Camb.) 98:689.

126 Linzell, J. L. 1974. Mammary blood flow and methods of identifying and measuring precursors in milk. Page 143 in Lactation: A comprehensive treatise. Vol. I. B. L. Larson and V.R. Smith, ed. Academic Press, NY.

127 Linzell, J. L., and T. B. Mepham. 1974. Effects of intramammary arterial infusion of essential amino acids in the lactating goat. J. Dairy Res. 41:101.

128 Loerch, S. C., L. L. Berger, S. D. Plegge, and G. C. Fahey, Jr. 1983. Digestibility and rumenescape of soybean meal, blood meal, meat and bone mealand dehydrated alfalfa nitrogen. J. Anim. Sci. 57:1037.

129 Loosli, J. K., H. H. Williams, W. E. Thomas, F. H. Terris, and L. A. Maynard. 1949. Synthesis of amino acids in the rumen. Science 110:144.

130 Lucas, J. L. 1957. Extra-period latin-square change-over designs. J. Dairy Sci. 40:225.

131 Lundquist, R. G., J. G. Linn, and D. E. Otterby. 1983. Influence of dietary energy and protein on yield and composition of milk from cows fed methionine hydroxy analog. J. Dairy Sci. 66:475.

132 Maeng, W. J., and R. L. Baldwin. 1976. Factors influencing rumen microbial growth rate and yield: Effect of amino acid addition to purified diet with nitrogen from urea. J. Dairy Sci. 59:1648.

133 Manadevan, S., J. D. Erfel, and F. D. Sauer. 1980. Degradation of soluble and insoluble proteins by Bacteroides amylophilus protease and by rumen microorganisms. J. Anim. Sci. 50:723.

134 Matusik, R. J., and J. M. Rosen. 1978. Prolactin induction of casein mRNA in organ culture. A model system for studying peptide hormone regulation on gene expression. J. Biol. Chem. 253:2343.

135 McAllan, A. B., and R. H. Smith. 1984. The efficiency of microbial protein synthesis in the rumen and the degradability of feed nitrogen between the mouth and abomasum in steers given different diets. Br. J. Nutr. 51:77.

136 McCarthy, R. D., G. A. Porter, and L. C. Griel. 1968. Bovine ketosis and depressed fat test in milk: A problem of methionine metabolism and serum lipoprotein aberration. J. Dairy Sci. 51:459.

137 Mehrez, A. Z., E. R. Orskov, and I. McDonald. 1977. Rates of rumen fermentation in relation to ammonia concentration. Br. J. Nutr. 38:447.

138 Meister, A., S. S. Tate, and L. L. Rose. 1976. Interaction of gamma-glutamyl transpeptidase with amino acids, dipeptides, and derivations and analogs of glutathione. J. Biol. Chem. 249:7593.

139 Mepham, T. B. 1971. Amino acid utilization by the lactating mammary gland. Page 297 in Lactation. I.R. Falconer, ed. Butterworths, London, England.

140 Mepham, T. B. 1982. Amino acid utilization by lactating mammary gland. J. Dairy Sci. 65:287.

141 Mepham, T. B., P. Gaye, and J. C. Mercier. 1982. Biosynthesis of milk proteins. Pages 115-156 in Developments in dairy chemistry - 1. Proteins. P. F. Fox, ed. Applied Sci. Publ., London, England.

142 Mepham, T. B., and J. L. Linzell. 1974. Effects of intramammary arterial infusion of nonessential amino acids and glucose in the lactating goat. J. Dairy Res. 41:111.

143 Mercer, J. R., and E. L. Miller. 1982. Effect of diet and infusion of volatile fatty acids into the rumen on the concentration of plasma free amino acids in sheep. Br. J. Nutr. 48:519.

144 Miller, E. R., and J. A. Forseth. 1982. Addition of potassium carbonate to swine diets containing varying levels of lysine. J. Anim. Sci. 55:97.

145 Mitchell, J. R., Jr., D. E. Becker, A. H. Jensen, B. G. Harmon, and H. W. Norton. 1968. Determination of amino acid needs of the young pig by nitrogen balance and plasma-free amino acids. J. Anim. Sci. 27:1327.

146 Moe, A. J., P. A. Pocius, and C. E. Polan. 1987. Transport of L-amino acids by brush border membrane vesicles from bovine small intestine. J. Dairy Sci. 70:290.

147 Munck, B. A. 1981. Intestinal absorption of amino acids. Page 1097 in Physiology of the gastrointestinal tract. L. R. Johnson ed. Raven Press, NY.

148 National Research Council. 1978. Nutrient requirements of dairy cattle. 5th ed. Natl. Acad. Sci., Washington, DC.

149 Njaa, L. R. and A. Aksnes. 1982. The nitogen-sparing effect of methionone sulphoxide and some other sulphur-containing amino acids. Br. J. Nutr. 48:565.

150 Nocek, J. E. and J. E. English. 1986. In situ digestion kinetics: evaluation of rate determination procedures. J. Dairy Sci. 69:77.

151 O'Dell, B. L., and J. E. Savage. 1966. Arginine-lysine antagonism in the chick and its relationship to dietary cations. J. Nutr. 90:364.

152 Oldham, J. D. 1980. Amino acid requirements for lactation in high-yielding dairy cows. Page 33 in Recent advances in animal nutrition. W. Haresign, ed. Butterworths, London, England.

153 Oldham, J. D. 1981. Amino acid requirements for lactation in high-yielding cows. Pages 49-81 in Recent developments in ruminant nutrition. W. Haresign and D. J. A. Cole, eds., Butterworths, London, England.

154 Oldham, J. D., I. C. Hart, and J. A. Bines. 1982. Formaldehyde-treated proteins for dairy cows-effects on blood hormone concentrations. Br. J. Nutr. 48:543.

155 Oltjen, R.R., and R. P. Lehmann. 1968. Effect of diethylstilbestrol on the blood plasma amino acid patterns of beef steers fed finishing diets. J. Nutr. 95:399.

156 Orskov, E. R. 1982. Protein nutrition in ruminants. Academic Press, Inc., London, England.

157 Orskov, E. R., Grubb, D. A., and R. N. B. Kay. 1977. Effect of postruminal glucose or protein supplementation on milk yield on composition in Friesian cows in early lactation and negative energy balance. Br. J. Nutr. 38:397.

158 Orskov, E. R., F. D. DeB. Hovell, and R. Mould. 1980. The use of the nylon bag technique for the evaluation of feedstuffs. Trop. Anim. Prod. 5:195.

159 Orskov, E. R., and I. McDonald. 1970. The estimation of protein degradability in the rumen from incubation measurements weighed according to rate of passage. J. Agric. Sci. (Camb.) 92:499.

160 Orskov, E. R., N. A. MacLeod, and D. J. Kyle. 1986. Flow of nitrogen from the rumen and abomasum in cattle and sheep given protein-free nutrients by intragastric infusion. Br. J. Nutr. 56:241.

161 Owens, F. N., and W. G. Bergen. Nitrogen metabolism of ruminant animals: Historical perspective, current understanding and future implications. J. Anim. Sci. 57:498.

162 Palmquist, D. L., and H. R. Conrad. 1982. Utilization of distillers dried grains plus solubles by dairy cows in early lactation. J. Dairy Sci. 65:1729.

163 Papas, A. M., S. R. Ames, R. M. Cook, C. J. Sniffen, C. E. Polan, and L. Chase. 1984. Production responses of dairy cows fed diets supplemented with ammonium salts of iso C-4 and C-5 acids. J. Dairy Sci. 67:276.

164 Papas, A. M., E. E. Hatfield, and F. N. Owens. 1974. Responses of growing lambs to abomasal infusion of corn oil, starch, casein, and amino acid mixtures. J. Nutr. 12:1543.

165 Parsons, M. J., P. K. Ku, and E. R. Miller. 1985. Lysine availability in flash-dried blood meals for swine. J. Anim. Sci. 60:1985.

166 Patience, J. F., R. E. Austic, and R. D. Boyd. 1984. The effects of sodium bicarbonate on performance of swine fed lysine-deficient diets. J. Anim. Sci. 59(Suppl. 1):279.

167 Patton, R. A., R. D. McCarthy, and L. C. Griel, Jr. 1970. Observations on rumen fluid, blood serum and milk lipids of cows fed methionine hydroxy analog. J. Dairy Sci. 53:776.

168 Peeters, G., A. Houvenaghel, E. Roets, A. M. Massart-Leen, R. Verbeke, G. Dhondt, and F. Verschooten. 1979. Electromagnetic blood flow recording and balance of nutrients in the udder of lactating cows. J. Anim. Sci. 48:1143.

169 Phillips, W. A., K. E. Webb, Jr., and J. P. Fontenot. 1976. In vitro absorption of amino acids by the small intestine of sheep. J. Anim. Sci. 42:201.

170 Pichard, G., and P. J. VanSoest. 1977. Protein solubilities of ruminant feeds. Page 91 in Proc. Cornell Nutr. Conf.

171 Pico-Tag™ Amino Acid Analysis System. 1986. Operator's Manual, Waters Chromatography Division, Millipore Corp. Manual No. 88140.

172 Pisulewski, P. M., A. U. Okorie, P. J. Buttery, W. Haresign, and D. Lewis. 1981. Ammonia concentration and protein synthesis in the rumen. J. Sci. Food Agric. 32:759.

173 Polan, C. E., P. T. Chandler, and C. N. Miller. 1970. Methionine hydroxy analog: Varying levels for lactating cows. J. Dairy Sci. 53:607.

174 Poos, M. I., T. L. Hanson, and T. J. Klopfenstein. 1979. Monensin effects on diet digestibility, ruminal protein bypass and microbial protein synthesis. J. Anim. Sci. 48:1516.

175 Poos-Floyd, M., T. Klopfenstein, and R. A. Britton. 1980. Evaluation of laboratory techniques for predicting ruminal protein degradation. J. Dairy Sci. 68:829.

176 Purser, D. B., and S. M. Buechler. 1966. Amino acid composition of rumen organisms. J. Dairy Sci. 49:81.

177 Rajendrau, V. M., J. M. Harig, M. B. Adams, and K. Ramaswamy. 1987. Transport of acidic AA by human jejunal brush-border membrane vesicles. Am. J. Physiol. 252:G33.

178 Reichl, J. R., and B. Rothschild. 1984. The in vitro entry rates of amino acids into the tisues of the small intestine. 5. Results of rats, pigs, young bulls and cattle. Z. Tierphysiol. Tierernahr Futtermittelkd 51:3.

179 Reid, J. T., P. W. Moe, and H. F. Tyrell. 1966. Symposium: Re-evaluation of nutrient allowances for high producing cows. J. Dairy Sci. 49:215.

180 Richardson, R. I., and A. R. P. Jouan. 1986. The distribution of peptidase activiety in the small intestine of sheep. Br. J. Nutr. 55:149.

181 Riddell, D. O., E. E. Bartley, and A. D. Dayton. 1981. Effect of nicotinic acid on microbial protein synthesis in vitro and on dairy cattle growth and milk production. J. Dairy Sci. 64:782.

182 Robbins, K. R., H. W. Norton, and D. H. Baker. 1979. Estimation of the nutrient requirements from growth data. J. Nutr. 109:1710.

183 Rock, D. W., T. J. Klopfenstein, J. K. Ward, R. A. Britton, and M.L. Mcdonnell. 1983. Evaluation of slowly degraded proteins: Dehydrated alfalfa and corn gluten meal. J. Anim. Sci. 56:476.

184 Rogers, G. L., A. M. Bryant, and L. M. McLeay. 1979. Abomasal infusions of casein, methionine, and glucose, on milk yield and composition. N.Z. J. Agric. Res. 22:533.

185 Rogers, J. A., J. H. Clark, T. R. Drendel and G. C. Fahey, Jr. 1984. Milk production and nitrogen utilization by dairy cows infused postruminally with sodium caseinate, soybean meal, or cottonseed meal. J. Dairy Sci 67:1928.

186 Rogers, J. A., U. Krishnamoorthy, and C. J. Sniffen. 1987. Plasma amino acids and milk protein production by cows fed rumen-protected methionine and lysine. J. Dairy Sci. 70:789.

187 Rohr, K., M. Brandt, O., Castrillo, P. Lebzien, and G. Assmus. 1979. Der einfluss eines teilweisen ersatzes von futterprotein durch harnstoff auf den stickstoff- und amino- saurenfluss am duodenum. Landbauforsch. Volkenrode, 29:32.

188 Rohr, K., P. Lebzien, H. Schafft, and E. Schulz. 1986. Prediction of duodenal flow of non-ammonia nitrogen and amino acid nitrogen in dairy cows. Livest. Prod. Sci. 14:29.

189 Rooke, J. A., B. W. Norton, and D. G. Armstrong. 1982. The digestion of untreated and formaldehyde-treated soybean meals and estimation of their rumen degradability by different methods. J. Agric. Sci. 99:441.

190 Rooke, J. A., H. A. Greife, and D. G. Armstrong. 1984. The effect of in sacco rumen incubation of a grass silage upon the total D-amino acid composition of the residual dry matter. J. Agric. Sci. (Camb.) 102:695.

191 Rook, J. A. F., and C. Line. 1961. The effect of the plane of energy nutrition of the cow on the secretion in milk of the constituents of the solids-not-fat fraction and the concentrations fo certain blood plasma constituents. Br. J. Nutr. 15:109.

192 Rosen, J. M., D. L. O'Neal, J. E. McHugh, and J. P. Comstock. 1978. Progesterone-mediated inhibition of casein mRNA and polysomal casein synthesis in the rat mammary gland during pregnancy. Biochemistry 17:290.

193 Rosser, R. A., C. E. Polan, P. T. Chandler, and T. L. Bibb. 1971. Effects of whey components and methionine analog on bovine milk fat production. J. Dairy Sci. 54:1807.

194 Roy, J. H. B., C. C. Balch, E. L. Miller, E. R. Orskov, and R. H. Smith. 1977. Calculation of the N-requirement for ruminants from nitrogen metabolism studies. Pages 125-129 in Proc. 2nd Int. Symp. Protein Metab. and Nutr. S. Tamminga, ed. Centre Agr. Pub. Coc., Wageningen, Holland.

195 Russell, D. H., and T. A. McVicker. 1972. Polyamine biogenesis in the rat mammary gland during pregnancy and lactation. Biochem. J. 130:71

196 Russel, J. B., C. J. Sniffen, and P. J. Van Soest. 1983. Effect of carbohydrate limitation on degradation and utilization of casein by mixed rumen bacteria. J. Dairy Sci. 66:763.

197 Russel, J. B., and H. J. Stobel. 1987. Concentration of ammonia across cell membranes of mixed rumen bacteria. J. Dairy Sci. 70:970.

198 Sadik, M. S. 1987. Microbial flow to the small intestines of lactating cows fed three protein supplements. M.S. Thesis, Univ. Arizona, Tucson.

199 Salter, D. N., and R. H. Smith. 1984. Protein utilization in the young steer: digestion and nitrogen retention of ^{15}N-labelled rumen bacteria protein. Br. J. Nutr. 51:531.

200 Santos, K. A., M. D. Stern, and L. D. Satter. 1984. Protein degradation in the rumen and amino acid absorption in the small intestine of lactating dairy cattle fed various protein sources. J. Anim. Sci. 58:244.

201 SAS Institute Inc. 1985. SAS User's Guide: Statistics, Version 5 Edition. Cary, NC: SAS Institute Inc.

202 Satter, L. D. 1986. Protein supply from undegraded dietary protein. J. Dairy Sci. 69:2734.

203 Satter, L. D., and R. E. Roffler. 1975. Nitrogen requirement and utilization in dairy cattle. J. Dairy Sci. 58:1219.

204 Satter, L. D., and L. L. Slyter. 1974. Effect of ammonia concentration on rumen microbial protein production in vitro. Br. J. Nutr. 32:199.

205 Schaefer, D. M., C. L. Davis, and M. P. Bryant. 1980. Determination of ammonia saturation constants for predominant species of rumen bacteria. J. Dairy Sci. 63:1248.

206 Scheifinger, C. 1976. Degradation of amino acids by pure cultures of rumen bacteria. J. Anim. Sci. 43:821.

207 Schepartz, B. 1973. Regulation of amino acid metabolism in mammals. Page 98 in Physiological chemistry. E. J. Masoro, ed. W.B. Saunders Co., Philadelphia, PA.

208 Schingoethe, D. J., D. P. Casper, C. Yang, D. J. Illg, J. L. Sommerfeldt, and C. R., Mueller. 1988. Lactational response to soybean meal, heated soybean meal, and extruded soybeans with ruminally protected methionine. J. Dairy Sci. 71:173.

209 Schwab, C. G., L. D. Satter, and A. B. Clay. 1976. Response of lactating dairy cows to abomasal infusion of amino acids. J. Dairy Sci. 59:1254.

210 Sissons, J. W. 1981. Digestive enzymes of cattle. J. Sci. Food Agric. 32:105.

211 Sklan, D., and O. Halevy. 1985. Digestion and absrption of protein along ovine gastrointestinal tract. J. Dairy Sci. 68:1676.

212 Statter, M., and A. Russell. 1978. Competitive inter-relationships between lysine and arginine in rat under normal conditions and in experimental hyperammonia. Life Sci. 22:2097.

213 Steinhour, W. D., M. R. Stokes, J. H. Clark, J. A. Rogers, and C. L. Davis. 1982. Estimation of the proportion of non-ammonia-nitrogen reaching the lower gut of ruminant derived from bacterial and protozoal nitrogen. Br. J. Nutr. 48:417.

214 Stern, M. D., M. E. Ortega, and L. D. Satter. 1983. Retention time in rumen and degradation of protein supplements fed to lactating dairy cattle. J. Dairy Sci. 66:1264.

215 Stern, M. D., L. M. Rode, R. W. Prange, R. H. Stauffacher, and L. D. Satter. 1983. Ruminal protein degradation of corn gluten meal in lactating dairy cattle fitted with duodenal T-type cannulae. J. Anim. Sci. 56:194.

216 Stern, M. D., K. A. Santos, and L. D. Satter. 1985. Protein degradation in rumen and amino acid absorption in small intestine of lactating dairy cattle fed heat treated soybeans. J. Dairy Sci. 68:45.

217 Stern, M. D., and L. D. Satter. 1980. In vivo estimation of protein degradability in the rumen. Page 57 in Protein Requirements for Cattle. F. N. Owens, ed. Oklahoma State Univ.

218 Stevens, B. R., J. D. Kaunitz, and E. M. Wright. 1984. Intestinal transport of amino acids and sugars: Advances using membrane vesicles. Ann. Rev. Physiol. 46:417.

219 Stock, R., T. Klopfenstein, D. Brink, S. Lowry, D. Rock, and S. Abrams. 1983. Impact of weighing procedures and variation in protein degradation rate on measured performance of growing lambs and cattle. J. Anim. Sci. 57:1276.

220 Stockland, W. L., R. J. Meade, and A. L. Melliere. 1970. Lysine requirement of the growing rat: Plasma lysine as a response criterion. J. Nutr. 100:925.

221 Storm, E., D. S. Brown, and E. R. Orskov. 1983. The nutritive value of rumen micro-organisms. 3. The digestion of microbial amino and nucleic acids in, and losses of endogenous nitrogen from, the small intestine of sheep. Br. J. Nutr. 50:479.

222 Storm, E., and E. R. Orskov. 1984. The nutritive value of rumen micro-organisms in ruminants. 4. The limiting amino acids of microbial protein in growing sheep by a new approach. Br. J. Nur. 52:613.

223 Storm, E., E. R. Orskov, and R. I. Smart. 1983. The nutritive value of rumen micro-organisms. 1. Large scale isolation and chemical composition of rumen micro-organisms. Br. J. Nutr. 50:463.

224 Strobel, J. H., and J. B. Russel. 1986. Effect of pH and energy spilling on bacterial protein synthesis by carbohydrate-limited cultures of mixed rumen bacteria. J. Dairy Sci. 69:2941.

225 Swick, R. W., and N. J. Benevenga. 1977. Labile protein reserves and protein turnover. J. Dairy Sci. 60:505.

226 Tagari, H., and E. N. Bergman. 1978. Intestinal disappearance and portal blood appearance of amino acids in sheep. J. Nutr. 108:790.

227 Tamminga, S. 1979. Protein degradation in the fore-stomachs of ruminants. J. Anim. Sci. 49:1615.

228 Tamminga, S., C. J. Van der Koelen, and A. M. Van Vuuren. 1979. Effect of the level of feed intake on nitrogen entering the small intestine of dairy cows. Livest. Prod. Sci. 6:255.

229 Tamminga, S., and K. K. von Hellemond. 1977. The protein requirements of dairy cattle and developments in the use of protein, essential amino acids and non-protein nitrogen, in the feeding of dairy cattle. Page 9 in Protein and Non-Protein Nitrogen for Ruminants. Pergamon Press, Oxford, England.

230 Teller, E., J.-M. Godeau, and R. DeBaere. 1979. The fate of nitrogen in the various segments of the digestive tract of cows. Z. Tierphysiol. Tierernahr. Futtermittelk. 42:263.

231 Thomas, J. W., Y. Yu, T. Middleton, and C. Stallings. 1982. Estimations of protein damage. Pages 81-98 in Protein requirements of Cattle: Symposium. F. N. Owens, ed. Oklahoma State Univ., Misc. Proc. 109.

232 Thomas, P. C., D. G. Chamberlain, N. C. Kelly, and M. K. Wait. 1980. The nutritive value of silages. Digestion of nitrogenous constituents in sheep receiving diets fo grass silage and grass silage and barley. Br. J. Nutr. 43:469.

233 Tyrell, J. F., and J. T. Reid. 1965. Prediction of the energy value of cow's milk. J. Dairy Sci. 48:1215.

234 Tzeng, D., and C. L. Davis. 1980. Amino acid nutrition of the young calf. Estimation of methionine and lysine requirements. J. Dairy Sci. 63:441.

235 Varvikko, T. 1986. Microbially corrected amino acid composition of rumen undegraded feed protein and amino acid degradability in the rumen of feeds enclosed in nylon bags. Br. J. Nutr. 56:131.

236 Verite, R., M. Journet and R. Jarrige. 1979. A new system for the protein feeding of ruminants: The PDI system. Livest. Prod. Sci. 6:349.

237 Vik-Mo, L., J. T. Huber, W. G. Bergen, R. E. Lichtenwalner, and R. S. Emery. 1974. Blood metabolites in cows abomasally infused with casein or glucose. J. Dairy Sci. 57:1024.

238 Vik-Mo, L., R.S. Emery, and J.T. Huber. 1974. Milk protein production in cows abomasally infused with casein or glucose. J. Dairy Sci. 57:869.

239 Wahlstrom, R. C., S. L. Siyoto, and G. W. Libal. 1983. Effect of potassium and lysine supplementation on performance of young pigs fed low potassium diets. Nutr. Rep. Int. 28:1159.

240 Waibel, P. E., M. Cuperlovic, R. F. Hurrell, and K. J. Carpenter. 1977. Processing damage to lysine and other amino acids in the manufacture of blood meal. J. Agric. Food Chem. 25:171.

241 Waldo, D. R. and P. P. Glenn. 1984. Comparison of new protein systems for lactating dairy cows. J. Dairy Sci. 67:1115.

242 Wanderley, R. C., and C. B. Theurer. 1984. Fluxo de proteina do rumen para o duodeno de novilhos alimentados con dieta volumosa e dieta concentrada. Pesq. Agiop. Bras. 18:453.

243 Waterlow, J. C., P. J. Garlick, and D. J. Millward. 1978. Protein turnover in mammalian tissues and in the whole body. North Holland Publ. Co., Amsterdam, Netherlands.

244 Whitelaw, F. G., J. S. Milne, E. R. Orskov, and J. S. Smith. 1986. The nitogen and energy metabolism of lactating cows given abomasal infusions of casein. Br. J. Nutr. 55:537.

245 Williams, V. J. 1969. The relative rates of absorption of amino acids from the small intestine of the sheep. Comp. Biochem. Physiol. 29:865.

246 Windmueller, H. G., and A. E. Spaeth. Source and fate of circulating citrulline. Am. J. Physiol. 241:E473.

247 Young, A. W., J. A. Boling, and N. W. Bradley. 1973. Performance plasma amino acids of steers fed soybean meal, urea, or no supplemental nitrogen in finishing rations. J. Anim. Sci. 36:803.

248 Zimmerman, R. A., and J. M. Scott. 1965. Interrelationship of plasma amino acid levels and weight gain in the chick as influenced by suboptimal and superoptimal dietary concentrations of single amino acids. J. Nutr. 87:13.

249 Zinn, R. A., and F. N. Owens. 1983. Influence of feed intake level on site of digestion in steers fed a high concentrate diet. J. Anim. Sci. 56:471.