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PROTEIN REQUIREMENT AND NON-PROTEIN NITROGEN FOR HIGH PRODUCING COWS IN EARLY LACTATION

By

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ABSTRACT

PROTEIN REQUIREMENT AND NON-PROTEIN NITROGEN
FOR HIGH PRODUCING COWS IN EARLY LACTATION

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Recent reports have suggested that high producing cows should be fed rations containing 15 to 17% crude protein (CP) in dry matter (DM), and that only plant protein should supply the supplementary nitrogen. Several studies have shown that milk yields in cows fed corn silage treated with ammonia or urea at ensiling yielded as much or more milk as those fed equal protein from soybean meal. However, none of these studies were commenced at the beginning of lactation when milk yields are highest and intakes, relative to needs, are lowest.

The requirement of protein and the feasibility of non-protein nitrogen (NPN) as the supplementary nitrogen source were tested in 68 Holstein cows from weeks three through 20 post-partum. Identical rations containing NPN were fed all cows from four weeks pre-partum through the second week post-partum. Experimental rations were: concentrate (.4 kg per kg milk, through week 10 post-partum and then .33 kg from 11 to 20 weeks), corn silage ad libitum, and 2.2 kg hay. Seventeen cows were assigned to each of four treatment groups. The

first two groups received rations of only plant protein; which contained 12 to 13% CP in DM (group NC) and 15 to 16% (group PC), respectively. The other two groups were also fed rations with 15 to 16% CP, but approximately 25% of total nitrogen came from NPN. Corn silage treated with .65% urea (group U) or .40% ammonia (group AU) was fed with concentrate containing 1.25% urea. Rumen and blood were sampled two weeks pre-partum and at weeks 3, 10 and 18 post-partum. Blood was also collected in week 6.

Treatment effects on intake of dry matter and nutrients were not statistically significant, except for total CP, water soluble nitrogen and CP content of DM. However, group NC tended to consume more total DM and had highest intake of DM per 100 kg body weight. Milk yields for all cows and adjusted yields for cows which produced more than 25 kg per day during week two post-partum were 29.6, 32.6; 27.9, 34.8; 27.0, 32.1; and 27.7, 32.8 kg per day for the respective groups. Neither milk yields, milk components, nor body weight gains were significantly affected by treatments, except for slightly lower adjusted yields for high producers in group U during weeks three through six.

Rumen volatile fatty acids, plasma glucose, plasma ammonia and amino acids were not significantly affected by treatment. Rumen ammonia and plasma urea nitrogen (PUN) were lowest for group NC, highest for U, with PC and AU intermediate. Similar PUN in groups PC and AU suggests equal losses of nitrogen from supplementing soybean meal or corn silage treated with ammonia plus urea in the concentrate.

Lower PUN in group AU than U was attributed to prevention by ammonia of plant protein proteolysis in corn silage.

Based on production results, body weight gains, supporting evidence from rumen and blood metabolites, calculations for metabolizable protein, and protein entering the abomasum, it was concluded that high yielding cows fed rations of corn, corn silage, and limited hay require no more than 13% CP in DM, which is equal to only 80 to 90% of presently recommended standards (NRC 1971).

In the above and a previous experiment (also reported herein), lowest plasma concentrations of essential amino acids coincided with highest demands (high yielding, early lactation cows) and/or lowest supply (low protein ration and zero and two hours after feeding). Co-limiting amino acids identified by the two-phase method were histidine, valine, and leucine. Milk yields at 42 days was also positively correlated with these amino acids plus threonine and lysine. Estimates of minimum blood supply to the mammary gland needed for output of amino acids produced in milk identified methionine as the first limiting amino acid in groups NC, PC, and U and phenylalanine for group AU. However, the ranking of limiting amino acids by the latter method could be easily changed by slight errors in arteriovenous differences, since values were nearly equal for a number of amino acids.

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LIST OF ABBREVIATIONS

ADF = acid detergent fiber

ADH = antidiarrhetic hormone

ADN = acid detergent nitrogen

ALA = alanine

ARG = arginine

ASN = asparagine

ASP = aspartate

AU = group fed ration with ammonia

A-V = arterio-venous difference

BHBA = β-hydroxy butyrate

BW = body weight

C_{16} = fatty acid with 16 carbon units

CA = casein

CP = crude protein

CYS = cysteine

DM = dry matter

DNA = deoxyribonucleic acid

DP = total dietary nitrogen

EAA = essential amino acid(s)

GLN = glutamine

GLU = glutamate

GLY = glycine

HIS = histidine

Ig = immunoglobulins

ILE = isoleucine

IV = intravenous

LA = lactalbumin

LG = lactoglobulin

LEU = leucine

LYS = lysine

MAA = metabolizable amino acids

MBF = mammary blood flow

MET = methionine

MP = metabolizable protein

N = nitrogen

NC = group fed the negative control ration

NEAA = non-essential amino acid(s)

NE_{ℓ} = net energy for lactation

NPN = non-protein nitrogen

P = group fed ration containing ProSil (ammonia)

PAA = plasma amino acid(s)

PAN = plasma ammonia nitrogen

PC = group fed the positive control ration

pCO_2 = partial pressure of carbonioxide

$PGF_{2\alpha}$ = prostaglandin $F_{2\alpha}$

PHE = phenylalanine

PP = proteose-pentones

PRO = proline

PUN = plasma urea nitrogen

RAN = rumen ammonia nitrogen

RER = rough endoplasmic reticulum

RNA = ribonucleic acid

SA = serum albumin

SER = serine

SNF = solids-non-fat

SOLN = water soluble nitrogen

TDN = total digestible nutrients

THR = threonine

TN = total nitrogen

TP = true protein

TYR = tyrosine

U = group fed ration with urea

UFP = urea fermentation potential

VAL = valine

VFA = volatile fatty acid

Y_{ATP} = yield of dry microbial cells per mole ATP

2P = group fed ration with twice the recommended level of ProSil (ammonia)

LITERATURE REVIEW

1. Requirements and sources of protein in rations for dairy cows

The synthesis of milk constituents and the milk volume in dairy cows are under physiological and biochemical control, and the controlling factors are to a large degree dependent upon the availability of absorbable nutrients. Studies utilizing abomasal and intravenous (IV) infusion techniques have clearly shown positive effects on the production of milk and milk constituents.

Protein is the most expensive major nutrient in dairy cattle rations and to date has received less attention than energy. In formulating the protein content of dairy rations the important questions are: (1) How much protein does the cow require at various production levels? (2) Which and in what combination can available protein sources be used for the most lucrative production of milk?

1.1 Protein requirements

In Nutrient Requirements of Dairy Cows (1971) recommended amounts of total protein (crude protein (CP) = N x 6.25) are 14, 15 and 16% of dry matter (DM) at milk yields less than 20, 20 to 30, and more than 30 kg per day, respectively. The corresponding recommendations for digestible crude protein are 10.5, 11.4, and 12.3% of dry matter. Based on required daily amounts in relation to body

weight, milk yield, and milk composition, the recommended CP concentration in dry matter (DM) will vary as shown in Table 1 depending upon the daily DM intake per 100 kg body weight (BW). From the calculations in Table 1 a high producing cow (50 kg/day) with a low DM intake may require as much as 27.4 CP in the DM. The requirement decreases with increased DM intake per unit BW, decreased fat percentage and decreased milk yield.

The effect of protein content in DM on milk yield has recently been studied by Schwab et al. (1971), Thomas (1971), Gardner and Parker (1973), Sparrow et al. (1973), Grieve et al. (1974), Huber (1975), Chandler et al. (1976), Wallenius (1976), and Cressman et al. (1977). Cows producing about 30 kg milk per day had normal persistencies when fed rations containing 12.5 to 13.6% CP in DM (Thomas, 1971). For cows producing less than 20 kg milk per day, rations containing 10.5 to 11% CP appearred adequate (Thomas, 1971). Schwab et al. (1971), Huber (1975), Chandler et al. (1976), and Wallenius (1976) obtained equal milk productions in cows fed 12.8 to 14% CP rations versus 17 to 17.7% CP. Gardner and Parker (1973) showed that 305 day milk yield increased when the ration CP was increased from 13.2 to 14.4 and 15.5%. In experiments conducted between parturition and 20 weeks post-partum, milk yields were increased when the dietary protein was increased from 13.5 to 18% of the DM (Sparrow et al., 1973; Grieve et al., 1974; Cressman et al., 1977). Cressman et al. (1977) obtained the above response in multiparous cows. Heifers did not respond to ration CP contents higher than 12.4%. In the trial by Grieve et al. (1974), the DM intake increased with the CP content. Cows fed rations containing 15.1 or 16.1% CP showed no difference in milk yield (Lamb

Table 1.--Required total protein in dry matter of dairy rations by bodyweight, fat percent, dry matter intake, and milk yield.[a]

Fat Percent	DM[b]	Bodyweight 500 kg					Bodyweight 600 kg					
		15[c]	20	25	30	40	15	20	25	30	40	50
3.5	2.80	13.5	16.1	18.9	21.4	26.7	12.0	14.2	16.4	18.6	23.0	27.4
	3.00	12.6	15.1	17.5	20.0	24.9	11.2	13.3	15.3	17.4	21.5	25.6
	3.20	11.8	14.1	16.4	18.8	23.4	10.5	12.4	14.4	16.3	20.2	24.0
	3.40	11.1	13.3	15.5	17.6	22.0	9.9	11.7	13.5	15.3	19.0	22.6
	3.60	10.5	12.6	14.6	16.7	20.8	9.4	11.1	12.8	14.5	17.9	21.3
	3.80	9.9	11.9	13.8	15.8	19.7	8.9	10.5	12.1	13.7	17.0	20.3
	4.00	9.5	11.3	13.2	15.0	18.7	8.4	10.0	11.5	13.0	16.1	19.2
3.0	2.80	13.1	15.6	18.1	20.6	25.6	11.7	13.8	15.8	17.9	22.1	26.3
	3.00	12.2	14.5	16.9	19.2	23.9	10.9	12.8	14.8	16.7	20.6	24.5
	3.20	11.4	13.6	15.8	18.0	22.4	10.2	12.0	13.9	15.7	19.3	23.0
	3.40	10.8	12.8	14.9	16.9	21.1	9.6	11.3	13.0	14.8	18.2	21.6
	3.60	10.2	12.1	14.1	16.0	19.9	9.1	10.7	12.3	13.9	17.2	20.4
	3.80	9.6	11.5	13.3	15.2	18.8	8.6	10.1	11.7	13.2	16.3	19.3
	4.00	9.2	10.9	12.7	14.4	17.9	8.2	9.6	11.1	12.5	15.5	18.4

[a]Calculated according to NRC (1971).

[b]Dry matter intake as percent of bodyweight.

[c]Milk yield, kg per day.

et al., 1974). Cows fed 16% CP rations and then dropped to 13% CP at week 6 postpartum showed marked decreases in milk yield compared to cows maintained at 13% CP throughout the 12 week trials (Huber, 1975). In a review of the protein supply in dairy cows, Satter and Roffler (1975) made the suggestion to feed 17% CP rations in early lactation, but Huber (1975) cautioned that it is unwise to routinely recommend levels higher than 15% CP for dairy herd rations because of expense and waste by low producers.

The described effects of CP content in dairy rations are inconclusive. The variable results might be due to stage of lactation when the study was initiated, dry matter intakes, and effects of protein on energy utilization.

In the first 2 to 3 weeks postpartum the DM intake is lower than during subsequent weeks (Huber, 1975). During this period of reduced intake it has been theorized that the dietary protein content should be increased (Table 1). However, research has not shown benefits from this practice (Schwab et al., 1971; Huber, 1975) probably because of the cows' abilities to use protein reserves to meet a small deficit incurred in early lactation (Coppock, 1968; Paguay et al., 1972). In cows fed .33 kg concentrate per kg milk and offered corn silage ad libitum the dry matter intake per 100 kg BW (range 2.46 to 3.96%) was not related to days postpartum (which ranged 20 to 124 days) (Huber, 1975). However, milk yields were positively correlated with dry matter intakes. Lamb et al. (1974) found that increasing the rate of grain feeding from .25 to .625 kg per kg milk led to decreased hay consumption but increased total DM intake. Milk yields increased with the rate of grain feeding, and could be attributed to the increased consumption

of net energy. In the trials reported by Sparrow et al. (1973), Grieve et al. (1974), and Cressman et al. (1977), dry matter intakes increased with increased CP in the ration. Schwab et al. (1971) reported an increase in the apparent DM digestibility when the ration CP content was increased from 12.8 to 17.7% by the addition of SBM. Moe and Tyrrell (1977) fed cows rations containing 20, 17, and 14% CP and showed that metabolizable energy was equal at 20 and 17% CP but lower for the 14% CP diet.

Based on present knowledge, changes from NRC (1971) in the protein content of rations are not warranted. However, further research is needed where the protein is varied under conditions of constant intake and net energy density of rations.

1.2 Nitrogen sources

To meet the recommended protein requirement in ruminants the selection of natural proteins will rest mainly on the price and availability. There are very few data showing an advantage of one protein source over another in practical dairy rations.

Numerous experiments have shown that nonprotein nitrogen (NPN) can be utilized by rumen bacteria for microbial protein synthesis. The microbial protein is then digested and utilized by the host animal. The synthesis of microbial protein in the forestomachs of ruminants places these species in a unique position in agricultural production, that of being able to upgrade simple nitrogenous compounds to high quality protein.

The concept that rumen bacteria can utilize NPN compounds to form protein was presented as early as 1891 by Zuntz and Hagemann

(ref: Loosli et al., 1949; Chalmers, 1961). Loosli et al. (1949) and Duncan et al. (1953) presented quantitative evidence that ovine and bovine rumen microorganisms can utilize urea as their only source of nitrogen for the synthesis of amino acids. Bryant and Robinson (1962, 1963) showed that 80 to 89% of freshly isolated strains of rumen bacteria prefer to synthesize their cellular constituents from ammonia-N linked with carbon sources other than amino acids. Feeding ^{15}N-ammonium sulfate or ^{15}N-urea lead to the same degree of labeling of amino acids by rumen microorganisms, and the incorporation of label increased with the time of adaptation (Virtanen, 1966). Virtanen (1966) also demonstrated that the amino acids synthesized in the rumen are used for milk protein synthesis. Hungate (1965) hypothesized that the limitation on microbial synthesis imposed by the anaerobic conditions of the rumen limits the productivity of the host, and that increases in the production by the host should be sought in increased synthesis of microbial bodies in the rumen or by shunting of protein directly to the abomasum.

Digestion and metabolism in ruminants has been reviewed extensively (Lewis, 1961; Dougherty, 1965; Hungate, 1966; Phillipson, 1970; McDonald and Walner, 1975; Cole et al., 1976). Their findings will not be recapitulated here. However, from all the studies it becomes clear that the postruminal absorption in ruminants is as in nonruminants except for a more continuous flow of digesta and a larger degree of fermentation in the caecum and colon. The largest difference between ruminants and nonruminants is the modifying effect by microorganisms inhabitating the rumen. The rumen microbes modify the feed protein by catabolism and the production of microbial protein, which

has a fairly uniform amino acid composition, by anabolism. From a cost-benefit point of view it is beneficial to replace natural protein sources by a maximum amount of NPN. Therefore, this review will be centered around the most efficient utilization of dietary nitrogen (N) sources.

1.2.1 Catabolism of nitrogenous compounds in the rumen

Degradation of dietary protein in the rumen depends largely on its rate of passage and solubility (Phillipson, 1972; Miller, 1973; Chalupa, 1975). It is often of no advantage to feed a highly soluble protein, rich in essential amino acids (EAA), because of degradation to ammonia and subsequent use of the nitrogen (N) for bacterial protein and nucleic acid synthesis which is of lower digestibility and protein quality than the original material (Smith, 1969). Rate of passage is affected by feed intake, specific gravity of feed particles, particle size of diet, concentrate to roughage ratio, rate of rumen digestion, and water intake (Chalupa, 1975). Rumen bacterial proteases (exo- and endo-peptidases) are bound at the cell surface, have free access to substrate, and are not inhibited by addition of dietary urea (Chalupa, 1975). Protozoa possess endogenous enzymes for protein catabolism as seen in the digestion of engulfed bacteria (Allison, 1970; Stern et al., 1977a, b). In vitro solubility of proteins increases with pH (Wohlt et al., 1973), and an average 60% of the natural dietary protein is catabolized in the rumen (Smith, 1969; Smith and McAllan, 1970; Al-Rabbat et al., 1971; Phillipson, 1972; Nolan et al., 1973; Chalupa, 1975). The degree of protein degradation varies from about 10% for proteins with low solubility (zein) to 90% for

proteins with a high solubility (casein). In the rumen of cattle the half-life of casein was 5.6 to 21.5 minutes, but it was 175 minutes for ovalbumin (Mangan, 1972). The solubility of albumins and globulins is higher than for proteins composed primarily of prolamines and glutelines (Wohlt et al., 1973).

The amount of dietary protein escaping rumen degradation (rumen bypass) may be increased through a functional esophageal groove or by processing. Rumen bypass of protein was reviewed by Allison (1970), Phillipson (1972), and Chalupa (1975) and is summarized in the following paragraph.

Protection of dietary protein by processing can be accomplished by heat reaction between sugar aldehyde and free amino acid groups which decreases protein solubility. It also destroys proteolytic enzyme inhibitory factors, when present, facilitating digestion in the intestine. Certain chemicals also decrease protein solubility at the pH of the rumen by cross-linkages with amino acid amide groups. These cross-linkages are destroyed by the acidity of the abomasum.

The optimum pH for rumen proteolysis is 6.5 and proteolytic enzyme activity is not inducible (Blackburn, 1965). Amino acids and nucleic acids released from natural protein are deaminated (Blackburn, 1965; Allison, 1970; Mangan, 1972; Chalupa, 1975). Half-lives for free amino acids in the rumen are less than 2 hours (Chalupa, 1975). Ammonia released by amino acid catabolism, feed NPN and salivary urea enters a common rumen ammonia pool (Hutton, 1972; Nolan et al., 1973; Satter and Roffler, 1975).

1.2.2 Protein anabolism in the rumen

Nitrogen Sources.--In reviews by Blackburn (1965), Allison (1965, 1970), Smith (1969), and Chalupa (1975) it was concluded that rumen ammonia probably is the main N source for bacterial growth, whereas many protozoa require amino acids, pyrimidine and purine bases. Oligopeptides have a stimulatory effect on growth of some bacterial strains (Chalmers, 1961; Allison, 1970). Amino acids are synthesized in the presence of exogenous amino acids which usually will not exert a feedback inhibition (Allison, 1970). Supplementary amino acids had no stimulatory effect on microbial growth when rations contained 65% of the total N as NPN (Allison, 1970). However, Maeng et al. (1976a) obtained maximum microbial growth on purified media when 75 and 25% of the total N was supplemented as urea and amino acids, respectively. A mixture of 18 amino acids supported greater growth than the 10 EAA or 8 nonessential amino acids (NEAA). Branched chain amino acids *per se* had no effect on growth. At the optimum mixture of urea and amino acids 53% of the amino acids were incorporated into microbial cells, 14% into carbon dioxide and volatile fatty acids, and 33% remained in the supernatant.

Activation of Ammonia.--The ammonium ion (NH_4^+) is a substrate for a major fixation enzyme (Glutamic dehydrogenase), and is the predominant form of nitrogen in near neutral solutions (Allison, 1970). Moreover, NH_4^+ is not absorbed as well across the rumen wall as is NH_3 (Blackburn, 1965).

The functional biochemical pathways in amino acid biosynthesis by rumen bacteria are relatively unknown (Allison, 1970). Erfle et al.

(1977) found that ammonia activation in mixed rumen bacteria is via glutamine synthetase (Glu + NH_3 + ATP $\rightleftarrows$ GRn + ADP + P_i) and asparagine synthetase (Asp + NH_3 + ATP $\rightleftarrows$ Asn + AMP + PP_i) when the ammonia concentration is low (K_m 1.8 and 4, respectively). At high ammonia concentrations, the activation is via glutamate dehydrogenase (α - KG + NH_4^+ + NAD(P)H $\rightleftarrows$ Glu + $NAD(P)^+$; K_m 33). They concluded that rumen microorganisms have a glutamate synthesizing system which is more efficient than in some nonrumen bacteria, and that the ammonium ion concentration controls amino acid anabolism.

Nutritive Value of Microbial Protein.--Rumen bacteria contain 74 to 80% of their total N in the form of proteins, peptides or free amino acids. Nucleic acids account for about 15% of the total N (Smith, 1969; Allison, 1970; Smith and McAllan, 1970). The diversity of the bacterial population makes it difficult to produce significant changes in the quality of bacterial protein leaving the rumen, even when there are rather large changes in the ration (Chalmers, 1961; Bergen et al., 1968b; Allison, 1970; Fenderson and Bergen, 1972; Chalupa, 1973; Burris et al., 1974). The biological value of the mixture of rumen bacteria and protozoa is not affected by protein source and is only slightly lower than for casein (80 to 85 versus 90) (McNaught et al., 1954; Chalmers, 1961; Bergen et al., 1968a). Net protein utilization by rats for bacteria, protozoa and casein were 75, 87, and 97% (Bergen et al., 1968a). The limiting amino acids in bacterial protein compared with egg protein are methionine and isoleucine (Chalmers, 1961). The true digestibility of bacterial protein was 76% (Hogan and Weston, 1970).

The Importance of Energy and Ammonia Nitrogen for Optional Microbial Growth.--In continuous fermentation systems, the maximum microbial cell yields depend on retention time, production of ATP potentially formed during fermentation, and the ammonia nitrogen available (Balch, 1961; Lewis, 1961; Hungate, 1965; Hogan and Weston, 1970; Al-Rabbat et al., 1971; Hogan, 1975; Satter and Roffler, 1975).

Microbial yield in ruminants is maximized when the cells remain in the fermentation system 2 to 5 hours and is greatly depressed for longer and shorter times (Hogan, 1975). This length of time is sufficient for reproduction and development, and insures removal before microbes enter the lag phase of the logistic population growth curve and start to use substrate for maintenance and autolyses (Hogan, 1975). The microbial yield of dry cells per mole ATP (Y_{ATP}) may change when the dilution rate is increased from .1 h^{-1} to .3 to .5 h^{-1} (Hogan and Weston, 1970). Determining factors in the movement of digesta out of the omasum are feed intake, degree of fermentation, specific gravity of particles in the rumen and rumen motility (Phillipson and Ash, 1965; Balch and Campling, 1965). During an increased rate of passage a decreased digestion of cellulose in the rumen may be compensated for by an increased fermentation in the hind gut (Singleton, 1972).

During anaerobic fermentation energy is usually the primary factor which determines microbial cell yields (Allison, 1965, 1970; Al-Rabbat et al., 1971). Hungate (1965) found a Y_{ATP} yield of 10.5% and suggested it was constant. Hogan and Weston (1970) found that the Y_{ATP} varies with bacterial species, substrate supply and dilution rate. Recent in vivo estimates of microbial N flow to the abomasum

were summarized by Allen and Miller (1976) at approximately 32 g per kg fermentable organic matter.

Rumen microorganisms have a limited capacity for ammonia utilization, but a minimal rumen ammonia concentration is critical for the microbial yield. When the rate of ammonia production exceeds that of utilization by bacteria or when there is excessive NPN in the diet, rumen ammonia accumulates without benefiting the host animal (Smith, 1969; Allison, 1970; Conrad, 1972; Satter and Roffler, 1975).

In pure batch cultures the cell crop is proportional to the ammonia concentration between 0.7 and 5.6 mg per 100 ml of fluid (Allison, 1970). In continuous cultures ammonia is limiting below 5 to 6.4 mg per 100 ml (Allison, 1970; Satter and Slyter, 1972). Bull et al. (1975) obtained maximal protein synthesis at ammonia concentrations in excess of 12.6 mg per 100 ml fluid. Above approximately 5 mg per 100 ml ammonia accumulates linearly with the supplementary level of N, but without effect on the protein content of the fermentor effluent. Rumen ammonia concentrations of 83 mg per 100 ml had no adverse effect on cell yield (Chalupa, 1973; Satter and Slyter, 1972). In sheep, maximum rumen nonammonia nitrogen was obtained at 8.8 mg per 100 ml, but abomasal nonammonia nitrogen was not maximized until rumen ammonia reached 13.3 to 16 mg per 100 ml (Hume et al., 1970; Allen and Miller, 1976).

1.3 Utilization of nonprotein nitrogen in dairy cattle rations

Progress in NPN utilization by dairy cattle has recently been reviewed by Burroughs et al. (1975a, b), Huber (1975), and Satter and Roffler (1975). There is general agreement that NPN is well utilized

in dairy cows producing less than 20 kg milk per day. Cows producing up to 20 kg milk on corn, corn silage, limited hay rations of 12 to 13% CP will maintain milk yields with all the supplementary N as NPN. However, there are major disagreements concerning the use of NPN in rations of cows producing more than 20 kg milk per day.

Burroughs et al. (1975a) and Satter and Roffler (1975) concluded that NPN supplementation of dairy rations is without value for cows producing more than 20 kg milk. Burroughs et al. (1975a, b) calculated an imbalanced supply of absorbable amino acids from rations supplemented with NPN. Satter and Roffler (1975) based their conclusion upon zero utilization of rumen ammonia when the ration CP content exceeds 12 to 13%. Their conclusions were mainly based upon theoretical considerations.

Burroughs et al. (1973, 1974a, b, 1975a, b) termed a urea fermentation potential (UFP) from estimates of metabolizable protein (MP) and metabolizable amino acids (MAA). The above system for calculating requirements was introduced as one which conforms more closely with digestible protein in nonruminants than apparently digestible protein and total protein (N x 6,25) for ruminants. Metabolizable protein and MAA are defined as the quantity of protein digested or amino acids absorbed in the postruminal portion of the digestive tract of cattle and other ruminants. The UFP is defined as the amount of urea that can be useful in any given cattle ration. Calculation of MP, MAA, and UFP are by the equations in (1), (2), and (3):

$$(1)\ MP = (P_1 \times .90) + ([P_2 - 15.0] \times .80)$$

$$(2)\ MAA = (.9\ P_1 \times AA\%P_1)/100 + ([.8\ P_2 - 12.0] \times AA\%P_2)$$

$$(3)\ UFP = (1.04\ TDN - P_3)/2.8$$

where P_1 is grams of undegraded α - amino protein entering the abomasum from 1 kg feed DM; P_2 is grams of abomasal microbial protein limited to grams of rumen degraded protein and 1.04 times the grams of TDN in 1 kg feed DM; P_3 is total grams of rumen degraded protein per kg feed DM whose ammonia contributed to the total rumen pool; 15.0 is abomasal protein required to satisfy the metabolic fecal protein of body origin; .90 is the fraction of undegraded protein truly digested postruminally; and .80 is the fraction of microbial protein truly digested postruminally. The advantage of this system over those previously used is that it recognizes that degradation of natural protein and synthesis of microbial proteins in the rumen are variable. At the same time the system avoids several potentially false assumptions. These are: (a) Absorbable amino acids arise from that portion of the ration protein undergoing ruminal destruction without resynthesis into microbial protein. (b) That all the urea is utilized for protein synthesis in the rumen when the ration has insufficient fermentable energy for transforming the urea into microbial protein. Satter and Roffler (1975) recognized the significant improvements in recommendations and feed evaluations introduced by the MP-system compared to the unqualified use of crude protein (N x 6.26). However, they found it had the disadvantage of being cumbersome and difficult to understand by livestock producers. This led to an alternative method of calculating

metabolizable protein where more accurate information may be readily accommodated as it becomes available. The assumptions of this system are: (a) The amount of nitrogen recycled into the rumen-reticulum is equal to 12% of the total dietary nitrogen intake (DP). (b) Eighty-five percent of the dietary nitrogen intake in typical ruminant rations supplemented with protein is true protein (TP) and the other 15% is NPN from natural sources. (c) Forty percent of the dietary true protein escapes degradation in the rumen and goes to the intestine while all of the dietary NPN and recycled nitrogen passes through the ruminal ammonia pool. (d) Ninety percent of all rumen ammonia N (RAN) produced is incorporated into microbial nitrogen when the ration fed does not exceed the "upper limit" value for crude protein. (e) None of the ruminal ammonia derived from dietary CP fed in excess of the upper limit value for dietary crude protein is incorporated into microbial nitrogen. (f) Eighty percent of microbial nitrogen is true protein and 20% is nonutilizable NPN. (g) Eighty percent of the microbial true protein will be absorbed. (h) Eighty-seven percent of the dietary protein that escapes degradation in the rumen will be absorbed. For rations where the rumen ammonia does not exceed the critical value the calculation is as equation (4):

$$(4)\ MP = [DP \times .85 \times .40 \times .87] + [(DP \times .15) + (NPN) + (TP \times .12) + (DP \times .85 \times .60)] \times .90 \times .80 \times .80$$

When the feed contains CP which will lead to rumen ammonia concentrations in excess of the critical value, these alterations are involved. Metabolizable protein is calculated for CP less than the critical value and the remaining N has to be supplemented as true

protein with a utilization of 30% (0.85 x 0.40 x 0.87). Satter and Roffler (1975) found that expected RAN concentrations depend upon the CP and TDN content in the total DM as in (5):

$$(5)\quad RAN = 38.73 - 3.04\ CP + .171\ CP^2 - .49\ TDN + .0024\ TDN^2$$

By rearrangement of equation (5) the critical CP for zero utilization of RAN may be calculated as in (6) and (7) when the critical RAN value as well as TDN are given.

$$(6)\quad CP = \frac{-(3.04) \pm \sqrt{(3.04)^2 - 4(-.171)(.49TDN - .0024TDN^2 + RAN - 38.73)}}{2 \times (-.171)}$$

The formula in (6) may be reduced to (7):

$$(7)\quad CP = 8.89 \pm \frac{\sqrt{9.24 - (-.684)\ (.49TDN - .0024TDN^2 + RAN - 38.73)}}{(-.342)}$$

The general concepts of the systems are improvements on the system presently in use but some drawbacks for direct implementation are evident. The major drawback to the system described and developed by Burroughs et al. (1973, 1974a, b, 1975a, b) is in establishing the MP value of feeds (Bartley, 1976). Undegraded protein reaching the abomasum is estimated for each feedstuff and is presently based on limited information. Furthermore, the value will depend on the composition of the ration, its physical form, processing, treatment, feeding rate, etc. (Bartley, 1976). Concerning the UFP, no consideration is given to the rate of energy release and whether or not this coincides with the release of ammonia (Bartley, 1976). The most critical assumption in the method by Satter and Roffler (1975) is the point of zero utilization of rumen ammonia. They use the value of 5 mg

per 100 ml established by in vitro methods (Satter and Slyter, 1972). From (7) it may be calculated that a complete ration containing 75% TDN has a critical value of 13.1%. If RAN can still be utilized for microbial growth when the concentration is increased to 8.8 as suggested by Allison (1970) then the critical CP value would be 15.2%. Using the 15.2% value, a ration with 50% of the DM as corn silage and 50% as grain would have TDN (in DM) close to 80%, and could support as high milk production when supplemented with NPN as by true protein. This could be expected if the rate of ammonia release from the NPN were decreased by its addition to silage at ensiling or by use of improved forms of NPN in the grain portion of the feed.

The addition of NPN (urea and ammonia) to corn silage has been investigated by workers at Michigan State University (Huber, 1975). In five trials, where the initial milk yields were 23 to 30 kg per day and one trial where initial milk was 34 kg per day, milk yield persistencies were as high or higher for NPN rations as for rations supplemented with natural protein. In these trials no negative control group was included, but the unsupplemented ration DM would have contained less than 12% CP. Approximately 60% of the supplemental N in the treatment rations came from NPN and the remainder from soybean meal. This indicates utilization of NPN in rations containing more than 12% which was the limit suggested by Burroughs et al. (1975a, b) and Satter and Roffler (1975); or that high yielding cows require less total feed protein than recommended by the National Research Council (1971).

Roffler and Satter (1975) utilized previously published experiments to estimate zero response to added NPN. Most of their

calculations for cows producing over 20 kg milk per day were based upon raw data rather than on milk yields adjusted for pretreatment values. When data were recalculated on the basis of adjusted treatment means, and after deletion of groups where factors other than NPN had a major effect on the response (silage DM > 40%, or an extremely high mineral level in silage which depressed intake) the point of zero response was changed from 12 to 14-14.5% CP (Huber, 1976). Further, Roffler and Satter (1975) did not calculate the response to natural protein supplementation from data collected in the trials which showed a response curve identical to the one obtained for NPN supplemented rations (Figure 1). Based on these results Huber (1975, 1976) concluded that NPN can be used as a N supplement at higher crude protein levels than estimated by Burroughs et al. (1975a, b) and Satter and Roffler (1975). The disagreement may, to some degree, be due to a slower and more synchronous release of ammonia from NPN-treated silages than from urea added to unprocessed grains. The importance of synchronous release of ammonia and energy has not been included in any of the suggested MP systems.

Polan et al. (1976) fed cows corn silage grain-based rations for 140 days starting after a 4 week adaptation period initiated 30 days post-partum. The rations contained 9.4 to 16.2% CP with 0 to 40% of the total N as urea, which was added in the grain portion of the feed. They concluded that urea resulted in less efficient nitrogen utilization than would be expected from equal N supplementation from natural protein. Urea was advantageous at low CP concentrations, but had a negative effect as dietary CP increased. In this trial the initial milk yield was 28.1 kg milk per day and treatment milk was

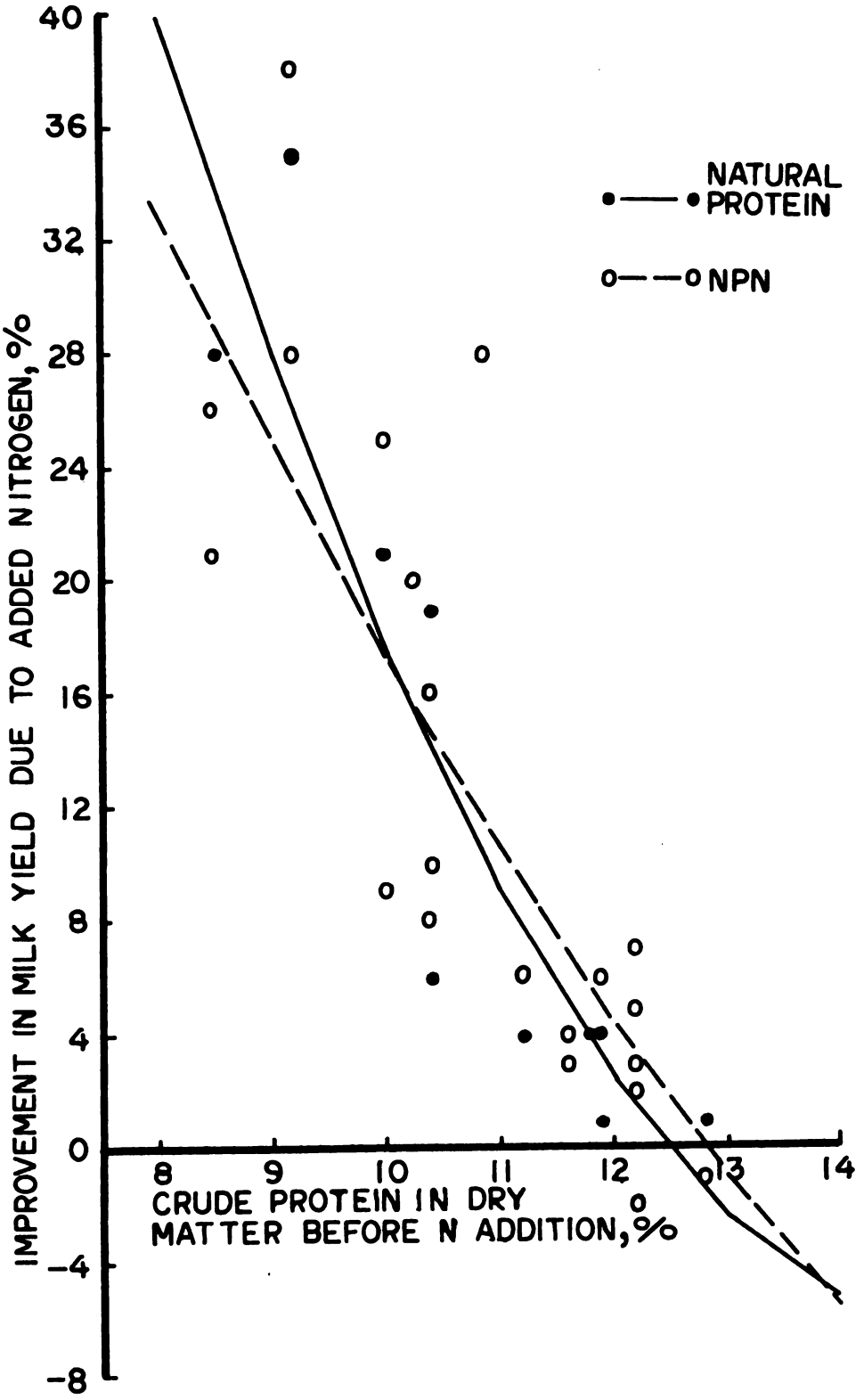

Figure 1. Milk response to added nitrogen from non-protein nitrogen (NPN) or natural protein.

Natural protein: $Y = 203.7435 - 27.7573X + .9166X^2_2$; $R = .91$
NPN: $Y = 126.4486 - 14.5602X + .3668X^2$; $R = .81$.

23.8 kg. The milk yield increased 2.58 kg per unit increase in CP, and decreased .22 kg per unit of urea substituted for CP. Feed intake was highest for 12.8% CP and decreased at lower and higher levels, and by inclusion of urea. In this trial mean rumen ammonia was 27.5 mg per 100 ml at 2 to 3 hours post feeding. This indicates a rapid hydrolysis of the total daily urea intake and may be related to rapid consumption. The negative effects encountered by Polan et al. (1976) when urea was fed in the grain were not observed by Gardner et al. (1975) and Huber et al. (1976) when methods for more synchronous release of ammonia and energy or a decreased rate of ammonia release were implemented. Gardner et al. (1975) found equal production in the first 126 days of lactation when cows, initially producing 30 kg milk, were fed rations containing 15.5% CP from rations to which the following were added to the grain: 1.5% urea + 2% soybean meal; 7.7% heat processed corn urea; or 14.8% soybean meal. Huber et al. (1976) observed equal milk persistencies when cows 130 days post calving, and initially producing 23 kg milk per milk per day, were given soybean meal, urea or fermented ammoniated whey as the only supplemental protein added to grain which was mixed with corn silage. In this trial the highest producers (28.8 kg initial milk) responded as favorably to NPN as to soybean meal supplementation and the persistencies were significantly higher than for negative control cows.

Aitchison et al. (1976) studied the effect of protein solubility on utilization in dairy cows using three latin square experiments. The solubility was varied by adding urea at 0, 8, 16 and 24% of N in grain and the effects were analyzed according to Mertens (1975). They calculated that 81% of insoluble N was utilized, while

utilization of soluble N was 29.3, 30.5, and 56.7% in trials 1, 2, and 3. Apparently soluble N was utilized more efficiently in trial 3 which also had the highest producers. The utilization of N was relatively constant across CP contents (12, 13, and 15%) and is in disagreement with the system proposed by Satter and Roffler (1975). This led Aitchison et al. (1976) to conclude that the utilization of soluble N may depend more upon the wash out effect associated with dry matter and water intake than upon CP content of the ration or production of the cow. Furthermore, they suggested that the solubility of N in the non-urea portion of the ration may be decreased by urea addition.

These recent findings (Gardner et al., 1975, Huber et al., 1976, and Aitchison et al., 1976) indicate NPN utilization at higher CP content and milk yields than suggested by metabolizable protein systems (Satter and Roffler, 1975; Burroughs et al., 1975) or the trial of Polan et al. (1976). The contrasting results may be due to rate of feed consumption or the rate at which ammonia and energy are released.

Huber (1975) reviewed the utilization of improved forms of NPN and the results may be summarized as follows. Silages treated with ammonia and urea NPN have increased lactic acid and water insoluble N, and are more stable when exposed to air. The increased stability is due to an antifungal action of ammonia. The higher lactic acid content is due to buffering by the ammonia. The increased content of water-insoluble N appears related to decreased proteolysis of the original plant protein and increased synthesis of microbial protein. These effects are more pronounced for ammonia than urea treated silages. An additional benefit of using ammonia instead of urea in the treatment

of corn silage is that cows fed ammoniated corn silage can tolerate more NPN in the concentrate (1.4 to 1.5%) without depressing milk yields or feed intake (Huber et al., 1975).

Other methods of increased NPN utilization have been sought by heat expansion of grain in the presence of urea (Starea) and by pelleting urea with alfalfa meal, dicalcium phosphate, sodium sulfate, and sodium propionate (Dehy-100). Both methods have improved urea utilization when compared with unprocessed urea plus grain.

1.4 Summary and conclusion of literature review on nitrogen utilization in dairy cows.

Recent studies of the protein requirement in high yielding dairy cows have not shown conclusive evidence of a need for increases in protein beyond the amounts recommended by the National Research Council (1971). Further research in this area should be conducted under conditions of constant DM intake and net energy density of the feed.

The ability of rumen microbes to utilize ammonia for amino acid and protein biosynthesis and the ruminants ability to utilize microbial protein has been well documented. Much has been learned concerning the metabolism of NPN by the rumen microbes and the host animal. However, the specific pathways of ammonia activation for amino acid biosynthesis and factors controlling rumen ammonia utilization are still in their infancy.

In dairy cattle, calculation of requirements on the basis of total crude protein (N x 6.25) has been used extensively, but neither crude protein nor apparently digestible protein are totally satisfactory,

nor are they as useful as digestible protein in nonruminants. Metabolizable protein, metabolizable amino acids and urea fermentation potential have been introduced as alternative methods for ruminants. These methods (as presently interpreted) allow extensive use of NPN in cows producing up to 20 kg milk per day. However, certain assumptions render them useless in high producing dairy cows.

The data base for these assumptions is very weak. Furthermore, recent studies and reevaluation of these critical assumptions suggest an urgent need to ascertain the usefulness of NPN in high producing dairy cows early in lactation. Limited data on NPN utilization in high yielding dairy cows is due mainly to the initiation of production trials in mid lactation. Only very limited information is available for cows in beginning of lactation when milk yields and dietary needs are highest and intakes are low. Moreover, most studies are of too short duration and the number of cows is too few for objective evaluation of treatment responses.

2. Physiological and biochemical factors in the synthesis of milk lactose and protein and milk volume control

2.1 Composition of normal milk

In dairy cows the milk yield per day (the lactation curve) increases rapidly after calving, peaks 30 to 60 days postpartum, and then decreases at a rather constant rate throughout the remainder of the lactation. The relationship between the concentration (percentage) of the solids and time is the inverse of the lactation curve. The shape and the level of the curves depend on breed and genotype, the

assumed restrictions of age (lactation number), season of the year, feeding, housing, etc. (Rook, 1961; Touchberry, 1974). The averages and standard deviations in Table 2 may be used as a guideline for normal cows, but the possible deviation from the "normal" is large. Several herds produce more than twice these amounts (Touchberry, 1974), and the record for one Holstein cow (milked twice daily) is 25,247 kg milk and 713 kg fat in 365 days (Meadows, 1976). At peak yield of 89 kg milk, the cow consumed 30 kg hay, 30 kg of a grain mixture with 15% crude protein (CP), and 190 liters of water.

Milk fat is synthesized from acetate, β-hydroxy butyrate (BHBA), and plasma free fatty acids. Milk fatty acids shorter than 16 carbon units (C_{16}) and one half of the C_{16} fatty acids are synthesized from acetate and BHBA. The remaining C_{16} fatty acids and those longer than C_{16} have their origin in plasma triglycerides. Further details on the synthesis of milk fat may be obtained from Bickerstaffe et al. (1974), Bauman and Davis (1974), Davis and Bauman (1974), and Jeness (1974).

The ash content of milk is relatively constant. Details on its composition and the content of vitamins and other constituents may be obtained from Jeness (1974).

2.2 Lactose synthesis

Milk lactose is synthesized from glucose absorbed from blood by the mammary gland and is an important determinant of milk volume (Bickerstaffe et al., 1974; Davis and Bauman, 1974; Hardwick et al., 1961; Hartman and Kronfeld, 1973; Lindsay, 1971; Linzell, 1968, 1974; Scott et al., 1976).

Table 2.--Milk yield and content of fat, protein, lactose, ash and solids non-fat for "normal" cows.

Breed		Milk[a], kg		Fat, %		Protein, %		Lacto-Ash		SNF[d]		Reference[e]
		Av.	S.D.	Av.	S.D.	Av.	S.D.	se %	%	Av.	S.C.	
Ayrshire	US[b]	5247	845	3.99	.31	3.34	.24	-	-	8.52	.34	(1)
"	UK[c]	-	-	4.1	-	3.6	-	4.7	.7	9.0	-	(2)
Brown Swiss	US	5800	936	4.16	.30	3.53	.23	-	-	8.99	.30	(1)
	UK	-	-	4.0	-	3.6	-	5.0	.7	9.3	-	(2)
Guernsey	US	4798	832	4.87	.40	3.62	.24	-	-	9.01	.24	(1)
	UK	-	-	5.0	-	3.8	-	4.9	.7	9.4	-	(2)
Holstein	US	7058	1195	3.70	.33	3.11	.20	-	-	8.45	.27	(1)
	UK	-	-	3.5	-	3.1	-	4.9	.7	8.7	-	(2)
Jersey	US	4435	1251	5.13	.46	3.80	.24	-	-	9.21	.30	(1)
	UK	-	-	5.5	-	3.9	-	4.9	.7	9.5	-	(2)
Shorthorn	UK	-	-	3.6	-	3.3	-	4.5	.7	8.5	-	(3)

[a]Mature equivalent in 305 days; [b]United States; [c]United Kingdom; [d]Solids non-fat

[e](1) Touchberry, 1974; (2) Schmidt, 1971; (3) Rook, 1961.

2.2.1 Glucose metabolism by the mammary gland.

Glucose uptake.--Simultaneous measurements of arterial and venous glucose concentrations indicates that the glucose uptake remains approximately constant as percent of the arterial concentration (extraction coefficient) between 30 mg glucose per 100 ml blood in fasting goats and 75 mg% in high yielding dairy cows (Annison et al., 1974; Bickerstaffe et al., 1974; Derring et al., 1974; Hartman and Kronfeld, 1973; Kronfeld et al., 1968; Linzell, 1967a). The mammary blood flow (MBF) varied from 168 to 540 liters per hour. In fasting animals and those treated with insulin the glucose uptake decreased due to changes in the arterial concentration. However, the mammary blood flow was also changed (Linzell, 1967a, 1974), and will be discussed later. Breed differences for glucose uptake have not proven significant (Bickerstaffe et al., 1974), but glucose extraction by the mammary gland is less efficient in late lactation (Linzell, 1974), indicating that something other than substrate availability is involved. The mechanism has not been clarified but progesterone and estrogen have diabetogenic effects and lead to decreased glucose utilization by maternal tissue (Lindsay, 1971). Glucocorticoids (dexamethasone) impair the mammary extraction of glucose even at hyperglycemic conditions and lead to redistribution of glucose to other tissues and a decreased milk production (Hartman and Kronfeld, 1973). Hartman and Kronfeld (1973) found that the decrease in milk yield was linearly related to the pre-treated milk yield, and the cows returned to pre-treatment production levels within five days of injection. Insulin injection or infusion decreased the milk yield in cows and goats

(Linzell, 1967b; Kronfeld et al., 1963), but had no effect when the animals received simultaneous intravenous infusions of glucose. These results suggest that glucocorticoids, and maybe estrogen and progesterone, have a direct effect on glucose extraction by the mammary gland whereas insulin acts through arterial glucose concentrations.

Glucose entry rate into circulation and mammary gland uptake.--The rate of glucose entry into the circulation in cows is of the order of 3.3 to 4.0 kg per day and was highest in cows fed a high starch, low roughage ration (Annison et al., 1974; Bickerstaffe et al., 1974). In comparison, entry rates for acetate and plasma free fatty acids were 1.7 to 2.1 and 0.5 kg per day, respectively (Bickerstaffe et al., 1974). The amount of glucose oxidized equals 4 to 11% of the total carbon dioxide production and was again highest in cows fed a high starch, low roughage ration (Bickerstaffe et al., 1974). Correspondingly, acetate accounted for 32 to 50% of the carbon dioxide production (Annison et al., 1974).

Of the total glucose entering the metabolic pool 60 to 90% is metabolized by the mammary gland in goats and cows (Annison and Linzell, 1964; Lindsay, 1971). The fraction of glucose taken up by the mammary gland in goats and Jersey cows is lower than in Holstein cows (Lindsay, 1971). These values are in good agreement with glucose uptake in cows (Kronfeld et al., 1968; Annison and Linzell, 1964; Hartman and Kronfeld, 1973).

Glucose metabolism within the mammary gland.--Within the mammary gland, absorbed glucose is either phosphorylated to

glucose-6-phosphate or remains as free glucose (Davis and Bauman, 1974). Phosphorylation of glucose to glucose-6-phosphate is by the low K_m hexokinase (Davis and Bauman, 1974). The high K_m glucokinase is not found in mammary tissue suggesting that the mammary gland does not experience high glucose concentrations. Hexose phosphates and free glucose are not in equilibrium due to the low activity or absence of glucose-6-phosphatase. Hence, the chance for glucose-6-phosphate being changed back to glucose is eliminated (Davis and Bauman, 1974).

Glucose-6-phosphate can be metabolized via three routes: (1) conversion to UDP-galactose via glucose-1-phosphate and then combine with free glucose and form lactose, (2) generate triose phosphates via fructose-6-phosphate and the glycolytic pathway, and (3) oxidation via 6-phosphogluconate and the pentose-phosphate pathway with generation of NADPH for fatty acid synthesis (Bauman and Davis, 1974; Davis and Bauman, 1974; Lindsay, 1971; Smith, 1971).

Smith (1971) hypothesized that glucose oxidation is limited to NADPH generation via the pentose-phosphate pathway and that the only other use of glucose, except for lactose synthesis, is to replace intermediates removed from the tricarboxylic acid cycle by NEAA synthesis.

Some of the galactose moity in lactose may be derived from sources other than glucose (Lindsay, 1971). One is triose phosphates which appear to recombine by the action of fructose-1, 6-diphosphatase and pyruvate carboxykinase (Davis and Bauman, 1974). Also, some glycerol was incorporated into lactose by in vitro incubation of cow mammary gland; but other gluconeogenic substrates

(alanine, glutamate, lactate, pyruvate) were not used at either optimal or suboptimal glucose levels (Scott et al., 1976). In goats, some gluconeogenesis from glutamate carbon took place within the mammary gland (Mepham and Linzell, 1974). Incorporation of gluconeogenic substrates into glucose, regardless of the glucose concentration, is consistent with the absence of glucose-6-phosphatase activity and the lack of excess gluconeogenic substrate in the mammary gland (Scott et al., 1976). The activities of pyruvate carboxylase, phosphoenol carboxykinase and fructose-1, 6-diphosphatese are lower in the mammary gland than in the liver (Scott et al., 1976).

Glucose does not contribute carbon units to fatty acid synthesis in the bovine mammary gland due to low activities or the absence of ATP, citrate, lyase and malate dehydrogenase (Smith, 1971).

Lactose biosynthesis accounted for 60 to 75%, oxidation for 20 to 30%, glycerol formation for 5%, and NEAA synthesis for 5% of the absorbed glucose (Armstrong and Prescott, 1971; Annison and Linzell, 1964; Davis and Bauman, 1974; Lindsay, 1971; Linzell, 1974; Smith, 1971). Further, glucose accounts for 50% of the glycerol and citrate, respectively (Linzell, 1974), and of the glucose entering the glucose-6-phosphate pool 50 to 60% is used for lactose synthesis (Davis and Bauman, 1974). In the goat the amount of glucose and acetate remaining after oxidation can account for all the lactose, all the glycerol, and 17 to 45% of the fatty acids in milk fat (Annison and Linzell, 1964). The NAD^+:NADH ratio has been suggested as a major factor regulating the metabolism of glucose via the glycolytic pathway (Davis and Bauman, 1974). Mannose will not substitute for glucose in

lactose biosynthesis, but does stimulate oxygen consumption and fatty acid synthesis from acetate to about the same extent as glucose (Davis and Bauman, 1974).

2.2.2 Lactose synthetase

The substrates for lactose biosynthesis are free glucose and UDP-galactose, and the reaction is catalyzed by lactose synthetase. The action of lactose synthetase has been reviewed by Ebner and Schanbacker (1974), and Whitney et al. (1976), and is summarized below.

Lactose synthetase is a combination of the enzyme galactosyltransferase (A-protein) and the protein α - lactalbumin (α-LA; B-protein). Galactosyltransferase is a nonspecific enzyme located in the wall of the Golgi apparatus and is involved in sequential addition of carbohydrates to an acceptor. It requires Mn^{2+}, but is not affected by Mg^{2+} and Ca^{2+}. The intracellular location of α-LA is less well known, but it is probably synthesized on the ribosomes under strict hormonal control. In the absence of α-LA, galactosyltransferase ads galactose residues to the side chain of glycoproteins, and glucose is not used as a galactosyl acceptor due to a high K_m (1.4 M) for glucose. During lactation α-LA is believed to pass into the tubules and vesicles of the Golgi apparatus and form a short lived kinetic complex with galactosyltransferase. The complex formation lowers the K_m for glucose (to 5 mM), and UDP-galactose is transferred to glucose instead of N-acetylglucosamine. The dissociation of α-LA from galactosyltransferase occurs prior to product release. It is expected that a single molecule of α-LA can interact with several molecules of galactosyltransferase as it passes through the Golgi apparatus and that control

of lactose biosynthesis is directly related to the concentration and rate of flow of α-LA through the Golgi apparatus. The function of the system assumes that the substrates, UDP-galactose and glucose, as well as the UDP are permeable to the Golgi membrane whereas lactose is not.

Lactose synthetase activity in the mammary gland of dairy cows is negligible 30 days pre-partum, increases to measurable levels by 7 days pre-partum and reaches an activity 2.5 to 4 times higher than at 7 days pre-partum between 7 days pre- and 40 days post-partum (Mellenberger et al., 1973). The change in lactose synthetase activity may now be related to α-LA synthesis. Even though this mechanism appears to be wasteful, it is very effective and regulated via protein synthesis suggested to be under normal hormonal control.

2.3 Protein synthesis

2.3.1 Protein fractions

Crude protein (Kjeldahl N x 6.38) in milk consists of approximately 95% true protein and 5% non-protein nitrogen (Larson and Gillespie, 1957; Yousef et al., 1970). The major part of NPN in milk is as free amino acids. True protein in milk consists of α-,κ-,β-, and δ-casein (casein fraction) and the whey proteins; β-lactoglobulin (β-LG), α-lactalbumin (α-LA), serum albumin (SA), immunoglobulins (Ig) and proteosepentones (PP) (Thompson and Farrell, 1974; Whitney et al., 1976). The distribution of proteins in the various fractions is given in Table 3.

Table 3.--The proteins of cow's milk and their percentage distribution in raw skim milk protein.

	Caseins				Whey Proteins					Minor protein	NPN	Reference
	α_s-CA	κ-CA	β-CA	δ-CA	SA	β-LG	α-LA	Ig	PP.			
Minimum	45	8	25	3	.7	7	2	1.9	.2	--	--	Whitney et al., 1976
Maximum	55	15	35	7	1.3	12	5	3.3	.3	--	--	Whitney et al., 1976
Average	55.6	--	23.3	4.2	.8	8.4	3.4	2.0	--	2.3	5.9	Larson & Gillespie, 1957

Caseins (CA) are phosphoproteins precipitated from raw skim milk by acidification to pH 4.6 at 20 C. Whey proteins are those remaining after CA precipitation (Whitney et al., 1976).

The caseins, β-LG and α-LA are homogeneous proteins synthesized in the mammary gland from a pool of free amino acids in equilibrium with the pool of free amino acids in the blood stream, and comprise over 90% of the milk proteins (Larson and Gillespie, 1957; Linzell, 1968; Larson and Jorgensen, 1974; Davis and Bauman, 1974). Their amino acid sequence has been determined and compositions are given in Table 4. Genetic variants exist for all of these proteins. Their frequency in Holstein cows, and related changes in the amino acid sequences are given in Tables 5 and 6. Whether δ-CA is synthesized in the mammary gland is still unknow, but the evidence suggests that it is derived from β-CA by proteolysis (Jeness, 1974; Whitney et al., 1976; Thompson and Farrell, 1974). Serum albumin, Ig and PP are heterogeneous proteins and originate from the blood (Larson and Jorgensen, 1974).

The evidence suggests that the amino acid composition of milk protein remains constant, except for the presence of genetic variants and variation in individual protein fractions. In cows fed rations containing 9 to 18% protected protein the amino acid composition of milk protein was unaltered (Broderick et al., 1974).

2.3.2 Amino acid metabolism by the mammary gland

The precursors of synthesized proteins are from a pool of free amino acids in balance with the pool of free amino acids in blood, as previously described. In dry cows the arterio-venous differences (A-V)

Table 4.--Amino acid composition of bovine milk protein (after Whitney et al., 1976).

	Casein (78-85%)						Whey Proteins[d]				
				δ (3-7%)			15-22%				
	α_s	κ	β	δ_1	δ_2	δ_3	SA	β-LG	d-LA	Ig	PP
Genetic variants.	A,D, B,C.	A,B	A^1; A^2; A^3; B, B D, E, C.	A^1; A^2; A^3; B.	A^2; A^3; B	A,B.		A B,C, D,Dr^2	A,B.		
Minor fractions	α_{s0} α_{s2} α_{s3} α_{s4} α_{s5}			β-CA 29-209	β-CA 106-209	β-CA 108-209					
% of skim milk prot.	45-55	8-15	25-35		3-7%		.7 1.3	7-12	2-5	1.9-3.3	.2-.3
Primary structure-genetic variance	α_{si}-B	B	A[c]					A	B		
Arg	6[a]	5	4	2	2	2	Identical to blood serum albumin	3	1		
His	5	3	5	5	4	3		2	3		
Ile	11	13	10	7	3	3		10	8		
Leu	17	8	22	19	14	14		22	13		
Lys	14	9	11	1	4	3		15	12		
Met	5	2	6	6	4	4		4	1		
Phe	8	4	9	9	5	5		4	4		
Thr	5	14	9	8	4	4		8	7		
Trp	2	1	1	1	1	1		2	4		
Val	11	11	19	17	10	10		10	6		
Ala	9	15	5	5	2	2		14	3		
Asn	8	7	5	3	1	1		5	12		
Asp	7	4	4	4	2	2		11	9		
Cys (1/2)	--	2	--	--	--	--		5	8		
Gln	14	14[b]	22	22	11	11		9	5		
Glu	25	13	17	10	4	4		16	8		
Gly	9	2	5	4	2	2		3	6		
Pro	17	20	35	34	21	21		8	2		
Ser + SerP	8+8	12+1	11+5	10+1	7+0	7+0		7+0	7+0		
Tyr	10	9	4	4	3	3		4	4		
Total AA-mol	199	169	209	181	104	102		162	123		
MW: calculated	23,613	19,005	23,980					18,362	14,174		

[a]Number of molecules per protein molecule.

[b]hereof 1 Pyroglu.

[c]Not point mutation

[d]SA is serum albumins, LG is lactoglobulin, LA is lactalbumin, Ig is immunoglobulin, and PP is proteones-pentones.

Table 5.--Gene frequency of milk protein variants in Holstein cows[a] (after Thompson & Farrell, 1974).

Variant	Casein							
	α_{si}	κ	β	δ	α-LA	β-LG	SA	Ig
A	.08	+	.98	+	+	+	-	-
B	.87	+	.02	+	+	+	-	-
C	.05	-	0	-	-	+	-	-
D	+	-	0	-	-	+	-	-
Dr	-	-	+	-	-	+	-	-

[a]Abbreviations: α-LA = α-Lactalbumin, β-LG = β-Lactoglobulin, SA = Bovine serum albumin, Ig = Immunoglobulin, + = found, - = not found in dairy cattle.

Table 6.--Amino acid changes due to genetic variants (after Whitney et al., 1976).

Protein	Genetic Variant	Residue Change	
		Change	Amino Acid Number
α_s - CA	B → A	13AA missing	14 to 26
	C	Glu → Gly	192
	D	Ala → Thr-P	53
κ - CA	B → A	Lle → Thr	136
		Ala → Asp	148
β - CA	A^2_2 → A^1_3	Pro → His	67
	A^2_2 → A^3	His → Glu	106
	A^2 → B	Pro → His	67
		Ser → Arg	122
	A^2 → C	Pro → His	67
		Glu → Lys	37
		Ser-P → Ser	35
	A^2_2 → E	Glu → Lys	36
	A^2 → B	-Not available.	Found in Bos Indicus only.
	D	-Not available.	Found in Bos Indicus only.
β - LG	A → B	Val → Ala	118
		Asp → Gly	64
	A D	Glu → Gly	51
		Asp → Gly	64
		Val → Ala	118
	A → Dr.	AA composition as A + carbohydrate moieties attached	ratio
		N-acetylneuraminie acid	1
		glucosamine	3.4
		galactosamine	.9
		mannose	1.9
		galactose	.8
α - LA	B → A	Arg → Gln	10

for amino acids are negligible (Verbeke and Peeters, 1965), but in the lactating cow, goat and sow the total uptake of N as amino acids is sufficient to provide for all of the N of milk proteins synthesized by the mammary gland (Mepham and Linzell, 1966; Linzell, 1968, 1974; Bickerstaffe et al., 1974; Davis and Bauman, 1974; Yousef et al., 1970; Verbeke and Peeters, 1965).

Uptake of amino acids.--In fasted goats the A-V for α-amino acid N decreases as a percent of the arterial concentration, when compared to fed goats (Linzell, 1967a). This led to a decrease in actual uptake, which was further exaggerated by a decrease in MBF. In goats given arterial infusions of amino acids the uptake was increased as were milk and protein yields (Linzell and Mepham, 1974). Milk protein yields were higher in cows fed a high concentrate, low roughage ratio compared to a normal ration, but the A-V for α-amino acid N were unchanged (Yousef et al., 1970).

Considerable differences exist for the uptake of individual amino acids (Clark et al., 1974; Derring et al., 1974; Bickerstaffe et al., 1974; Verbeke and Peeters, 1965; Mepham and Linzell, 1966; Linzell and Mepham, 1974), and the uptake as a percentage of the arterial concentration do not appear to be related (Figure 2). Some of the reported differences may be related to the stage of lactation. Chandler and Polan (1972) estimated that the extraction coefficients decrease with decreasing milk yield when the MBF to milk ratio was considered constant.

The uptake of EAA is in good agreement with the output of individual EAA in milk and the extraction coefficients are high

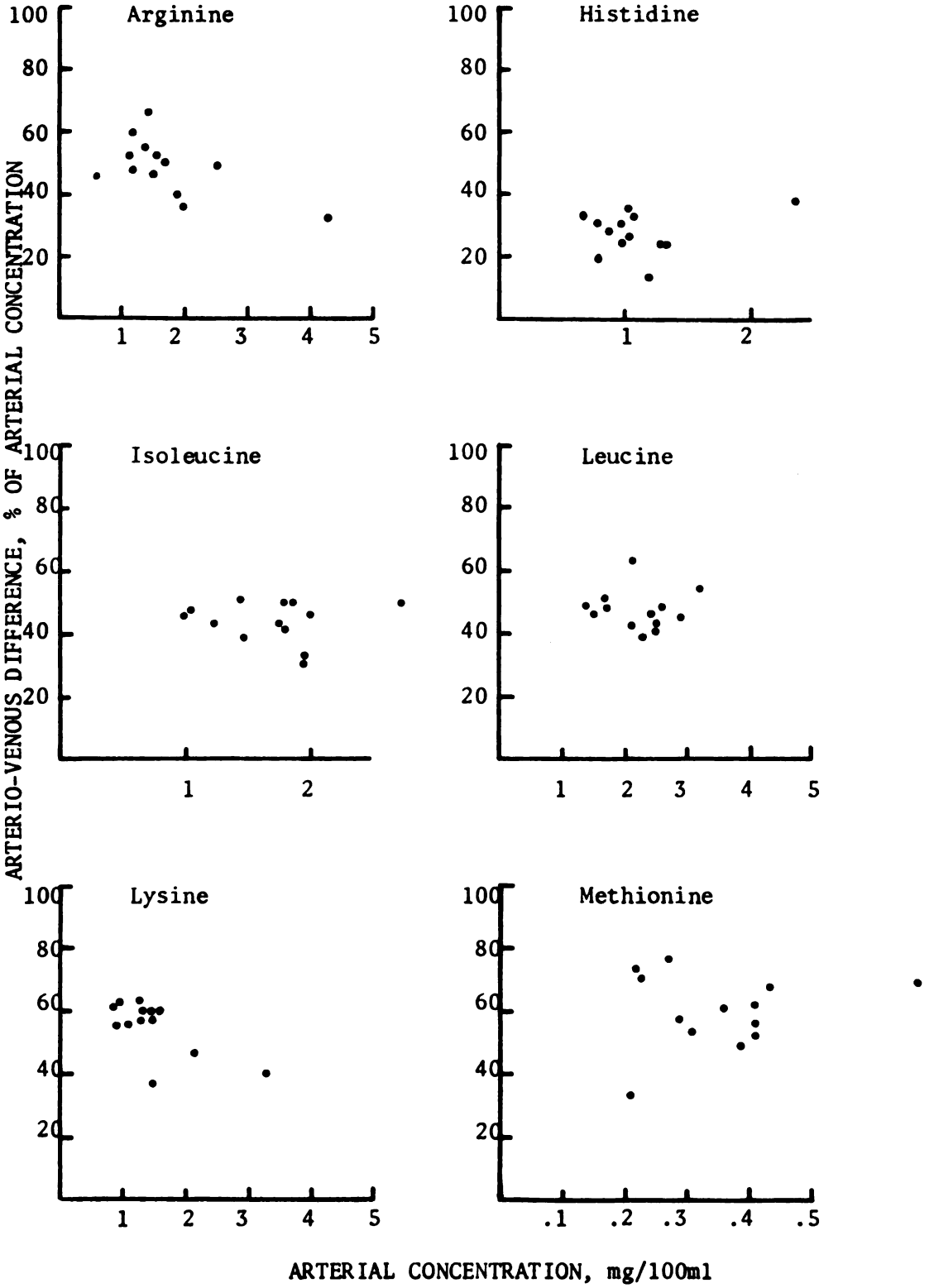

Figure 2. Arteriovenous differences for amino acids as a percentage of the arterial concentration (adapted from Clark et al. 1972; Derring et al. 1974; Bickerstaffe et al. 1974; Verbeke and Peeters 1965; Linzell and Mepham 1974a; Mepham and Linzell 1966).

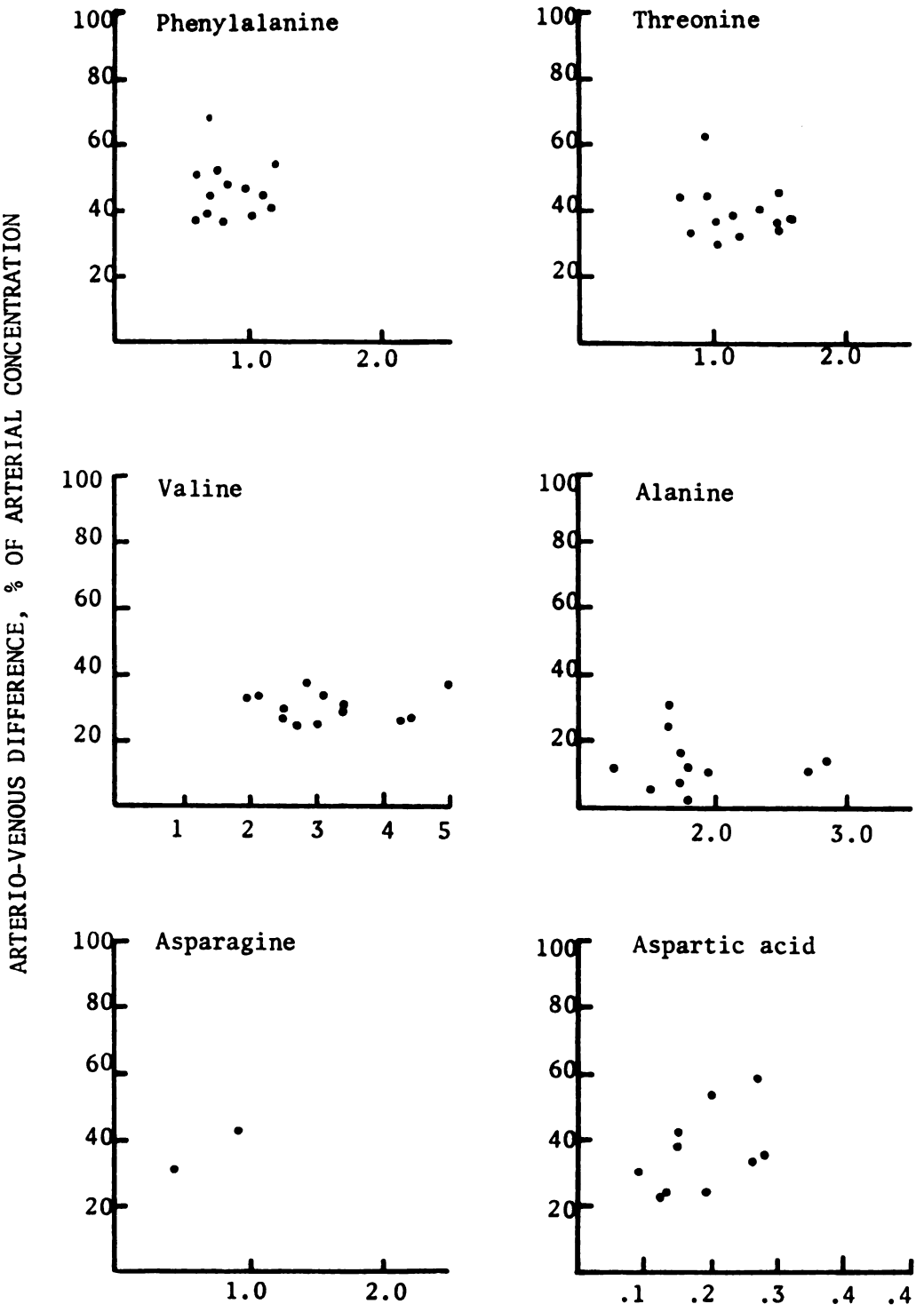

Figure 2. (cont.)

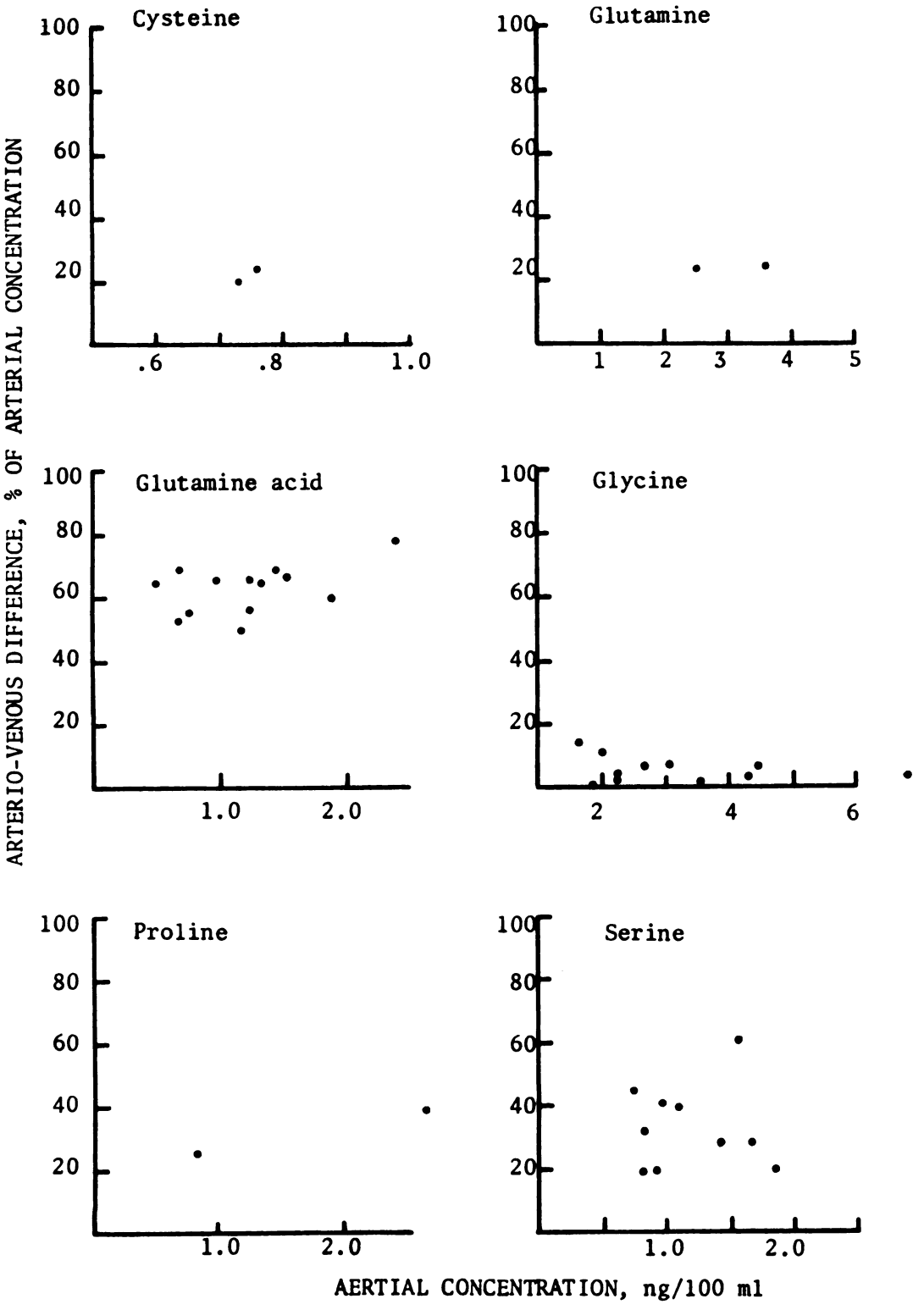

Figure 2 (cont.).

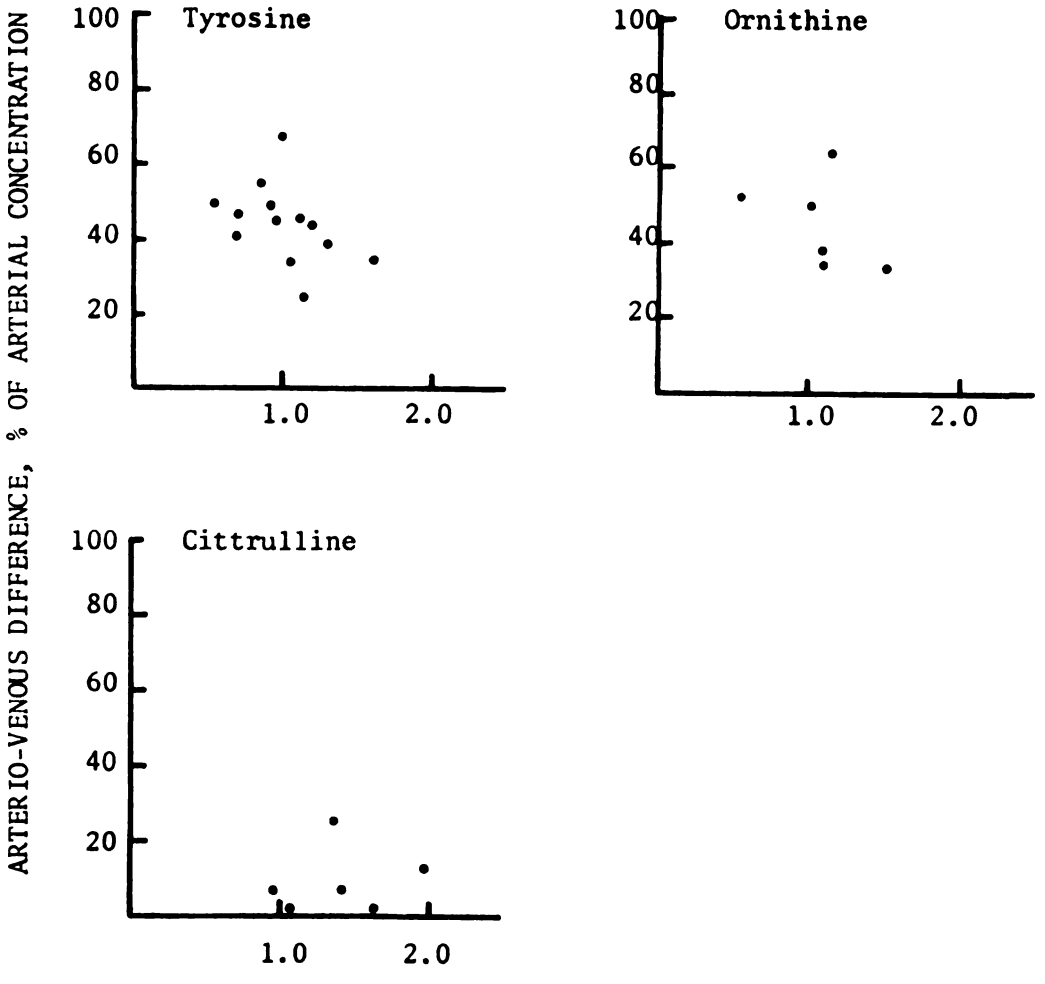

Figure 2. (cont.).

(Mepham and Linzell, 1966; Bickerstaffe et al., 1974; Derring et al., 1974). Exceptions are arginine and valine whose uptake exceed the output in milk. Ornithine and citrulline are extracted by the mammary gland even though they are not found in milk protein (Mephan and Linzell, 1966; Bickerstaffe et al., 1974; Verbeke and Peeters, 1965; Derring et al., 1974; Clark et al., 1975).

The uptake of most NEAA cannot account for the respective concentrations in milk protein (Verbeke and Peeters, 1965; Mephan and Linzell, 1966; Linzell, 1968; Bickerstaffe et al., 1974; Derring et al., 1974; Clark et al., 1975). Data suggest that the mammary gland has a capacity for NEAA anabolism (Verbeke and Peeters, 1965).

Amino Acid Catabolism and Anabolism by the Mammary Gland.--Arginine extracted in excess of the output in milk protein is converted to ornithine and urea, and the ornithine produced along with that absorbed from the blood form a portion of the proline pool (Mephan and Linzell, 1967; Linzell, 1968; Verbeke et al., 1968; Derring et al., 1973; Clark et al., 1975; Davis and Bauman, 1974). Arginine and ornithine also add to the pools of aspartate, glutamate, urea and citrulline, but arginine is not formed from ornithine. It was suggested that proline and glutamate are formed from ornithine via an incomplete urea cycle (no argininosuccinate synthetase activity). Verbeke et al. (1968) found that 13 to 9% of the protein in casein had its origin in plasma ornithine and arginine, respectively. Mepham and Linzell (1967) suggested that 14% of the carbon in arginine is transferred to urea.

Leucine was to a large extent incorporated in milk protein in perfusion studies with DL[1-^{14}C]-leucine. Catabolism of leucine was equal to 1% of the recovered carbon dioxide (Verbeke et al., 1959).

Increased plasma levels of methionine could not overcome the mammary glands requirement for cysteine (Davis and Bauman, 1974). The low activity or absence of enzymes for the conversion of methionine to cysteine is in agreement with the uptake: output ratios for cysteine and methionine, which are high and low, respectively.

An active phenylalanine hydroxylase in the mammary gland converts phenylalanine to tyrosine, and the rate of conversion was increased 30 fold when the plasma phenylalanine concentration was increased 4 times (Verbeke et al., 1972; Davis and Bauman, 1974). Phynylalanine infusion did not lead to any production of CO_2, lactose and citric acid. This indicates that phenylalanine is not catabolized by the mammary gland (Verbeke et al., 1972).

Threonine aldolase activity is found in the mammary gland and threonine can add to the pools of glycine, glutamate and aspartate. An amount equal to 0.4% of the expired CO_2 was catabolized (Verbeke et al., 1972).

Excess valine is converted to alanine, aspartate, glutamate and glycine. This suggests it is metabolized via succinyl-coenzyme A (Derring et al., 1973).

Alanine, asparagine, aspartate, glutamine, glutamate, glycine and serine appear to be formed by transamination and amination from glucose carbon with excesses of other amino acids as the nonspecific nitrogen source (Linzell, 1968; Linzell and Mepham, 1968; Davis and

Bauman, 1974). In the goat mammary gland acetate was also a carbon source, but its contribution to NEAA was insignificant (Linzell, 1968). Isovalerate can contribute to the pools of glutamate and asparatate (Verbeke et al., 1959). Smith (1971) concluded that milk protein synthesis requires 4% of the absorbed glucose via the tricarboxylic acid cycle, if allowance is made for NEAA synthesis from excess arginine and ornithine.

Net synthesis of amino acids within the mammary gland has not been established (Davis and Bauman, 1974). The rate of transmission and amination of precursors appears to be affected by precursor-uptake in relation to the output in milk; also by the degree to which the precursor is used for systhesis of other amino acids (Davis and Bauman, 1974).

This discussion of the amino acid metabolism suggests that the catabolism of amino acids and the anabolism of NEAA follow known pathways for amino acid metabolism in eucaryotic cells, except for the incompleteness of the urea cycle and the apparent lack of a pathway to convert methionine to cysteine.

2.3.3 Protein synthesis

The overall mechanism of protein synthesis in the mammary gland is similar to the general pathways believed to exist in cells in all organisms (Larson and Jorgensen, 1974; Keenan et al., 1974). The site of polypeptide chain synthesis appears to be the ribosomes of the rough endoplasmic reticulum (RER), with subsequent transport through the passages of the RER to the Golgi region, and concentration in the vacuoles (Larson and Jorgensen, 1974; Saache and Heald, 1974;

Topper and Oka, 1974). The addition of prostetic groups (phosphate groups of CA and sugar moities of certain proteins) and the previously described action of α-LA in lactose synthesis take place in the Golgi region prior to their incorporation into vacuoles (Larson and Jorgensen, 1974; Keenan et al., 1974).

Milk protein synthesis is under genetic, hormonal and specific subcellular pathway control. Inherited genes control the sequence of amino acid additions as seen in genetic variants in milk proteins (see Tables 4 to 6) (Larson and Jorgensen, 1974; Thompson and Farrell, 1974; Whitney et al., 1976).

Hormonal control of protein synthesis is seen in mammary cell development, induction of lactation, and maintenance of lactation (Larson and Jorgensen, 1974). Tucker (1974) hypothesized that lactation is initiated when progesterone secretion is low, and the secretion of prolactin and adrenal corticoids are elevated. In the bovine, growth hormone and thyroxine appear to have a synergistic effect on the latter hormones. Tucker (1974) also described the sequential events in hormonal changes in cattle prior to or around parturition. Progesterone levels are elevated during gestation and appear to block the induction of α-LA and lactose synthesis. Estrogen stimulates prolactin and ACTH secretion but its effects are inhibited by progesterone. Shortly before parturition the circulating progesterone declines markedly. Concurrently, estrogen levels increase 10 fold and achieve a maximum two days pre-partum, then decline precipitously during the last two days of pregnancy. Beginning about two days before calving prolactin increases sharply, reaches a maximum one day before

parturition and then declines to basal levels by parturition. Adrenal corticoids are released about 12 hours before parturition, peak at parturition, and return to baseline levels 12 hours after calving. Starting at calving growth hormone is released and remains elevated for 36 hours. Luteothropic hormone concentrations do not vary significantly during this interval. The importance of these hormone spurts above basal levels is unclear (Tucker, 1974) but the sequence of events is in good agreement with those leading to lactogenesis in virgin mice mammary tissue in vitro (Topper and Oka, 1974; Keenanet al., 1974). Prolactin renders secretory cells sensitive to insulin. Upon insulin treatment such cells undergo division with an accompanying increase in the synthesis of deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). Following insulin treatment glucocorticoids and prolactin increase RER throughout the cytoplasm, translocate RER and the Golgi apparatus, and secretory vesicles appear. These events lead to a marked increase in CA synthesis and later α-LA emerges (Topper and Oka, 1974).

Maintenance of milk synthesis is closely associated with milk removal, and requires prolactin, growth hormone, ACTH (or glucocorticoids), TSH (or thyroxine), and parathyroid hormone (Tucker, 1974). In cows, prolactin appears to have little effect on milk yields, but changes in its blood levels parallel changes in milk yield with advancing lactation. Prolactin release can be elicited by washing the udder without milk removal, simulated venipuncture, washing the brisket, feeding and TRH injection. Further, prolactin levels are higher at night than during the day, and are higher during the summer than during the winter (Tucker, 1974).

ACTH and gluco- and mineralocorticoids cause a temporary decrease in milk production due to their effects on electrolyte, protein and carbohydrate metabolism (Tucker, 1974). ACTH is released by milking and the ability to discharge it is not lost with progressing lactation (Tucker, 1974). Adrenocorticosterone release is closely related to the nucleic acid content of the mammary gland during lactation (Tucker, 1974).

Thyroxine stimulates milk secretion if the feed intake is increased concurrently, but the stimulatory effect is lost after two to four months (Tucker, 1974). Thyroxine also stimulates the production of corticosteroidbinding globulin and the release of prolactin (Tucker, 1974).

In cows, insulin decreases milk yields when the blood glucose concentration is not maintained (Kronfeld et al., 1963). Milking causes insulin release (Tucker, 1974).

Estrogen inhibits lactation and its effects are enhanced by progesterone (Tucker, 1974). Pregnancy causes reduction in milk yield in late lactation but whether the cause is due to estrogen and progesterone or an increased nutrient demand of the fetus is not known (Tucker, 1974).

The described hormonal events during lactogenesis and maintenance of lactation suggests that prolactin, insulin and ACTH are required for initiation of protein synthesis. These hormones are also released at each milking and the release of prolactin parallels changes in milk yield with advancing lactation. Therefore it may be hypothesized that the hormones which initiate mammary synthesis and secretion are also required for the maintenance of the synthesizing

and secretory activity concurrently with milk removal. A concurrent pregnancy inhibits the effects of prolactin due to an increasing estrogen and progesterone secretion.

At the cellular level, hormones, as well as cellular cycles, product feedback inhibition, and variations in the intraalveolar pressures must control protein synthesis (Larson and Jorgensen, 1974; Saache and Health, 1974). Saache and Health (1974) suggested that variations in the intraalveolar pressure, along with the residual milk causing this pressure, are the most important factors governing alveolar activity. This may be related to effects on the RNA content and the number of secretory cells, since both of these factors are reduced by omission of several milkings (Tucker, 1974). Subcellular pathway control is seen before parturition where absence of protein synthesis is due to a low concentration of tRNA with the proper tri-nucleotide combination for amino-acyl-tRNA complex formation, or because the ribosomes are deficient in one or more of their components and are unable to bind activated amino-acyl-tRNA complexes (Larson and Jorgensen, 1974).

2.4 Milk secretion

The secretion of milk consists of the secretion of solids and their dilution with water until it is isotonic with blood.

2.4.1 Protein and lactose secretion

Protein synthesized in the ribosomes is transferred to the Golgi region and concentrated in vacuoles as previously discussed. During its passage α-LA leads to lactose synthesis. The rate of

lactose biosynthesis is suggested to be directly related to the concentration and rate of flow of α-LA through the Golgi apparatus. This is substantiated somewhat by the positive correlation between the content of lactose and α-LA in milk (Yousef et al., 1970; Brew, 1969). Lactose and certain minerals are concentrated in the vacuoles along with the proteins (Keenan et al., 1974). The vacuoles are pinched off, migrate to the apical region of the cell where their membranes fuse with the plasma membrane, and contents are discharged (Keenan et al., 1974). This process supplies a continuous source of new plasma membrane to restore that lost with the fat droplets, which are also released from the cell; but are surrounded with plasma membrane and pieces of cell contents (Larson and Jorgensen, 1974; Keenan et al., 1974).

2.4.2 Milk volume

Lactose is an osmoregulator and draws water from the cell before or after its release into the alveolar lumen (Saache and Heald, 1974; Linzell, 1974; Davis and Bauman, 1974).

In goats, milk secretion is not stimulated by acetate, small amounts of glucose, fumarate or amino acids (Hardwick et al., 1961; Linzell, 1968). The milk yield in fasting goats decreased to 90% within 8 hours, decreased further to 56% after 24 hours of fasting, and was then maintained at that level for 10 to 12 hours (Linzell, 1967a, a, 1971; Lindsay, 1971). Similar results were obtained within two hours when goats and cows were treated with insulin (Linzell, 1967b; Kronfeld et al., 1963). Likewise, when glucose was omitted from the perfusate in perfusion studies with mammary glands the milk volume was very small and very rich in fat and protein (Linzell, 1974;

Smith, 1971; Lindsay, 1971). In these studies lactose reappeared and the milk volume was restored when fasted animals were refed or received IV infusions of glucose; and when glucose was added to the perfusate in perfusion studies.

The evidence presented suggests that secretion of protein, lactose, fat and water are interrelated. Protein synthesis, under hormonal control, is directly involved in the control of lactose biosynthesis and replenishment of the cell membrane after fat secretion. Lactose, in turn, regulates the milk volume due to its osmotic effects.

2.5 Mammary blood flow.

In the mammary gland, secretory tissue is equal to 80 to 90% of the total udder tissue (Linzell, 1974). In dry cows the blood flow is similar in skin, teat capsule, fat and secretory tissue, but during lactation the secretory tissue receives 2 to 3.5 times as much blood as the other tissues (Linzell, 1974).

The mammary gland receives about 10% of the cardiac output, which itself may increase 45% at parturition (Linzell, 1974). The mammary blood flow (MBF) in goats and cows has been measured by the thermodilution (Linzell, 1960b, 1971; Bickerstaffe et al., 1974; Annison et al., 1974; Hartman and Kronfeld, 1973) and the antipyrine methods (Kronfeld et al., 1968). By the antipyrine method the MBF to milk ratio was 750:1 which is probably an overestimation (Linzell, 1974). By the themodilution method the ratio is approximately 500:1. Under normal conditions in established lactations the MBF was not affected by age, lactation number and stage of lactation when subjected to analysis of variance (Linzell, 1974). The day to day and

hour to hour variation in MBF is of the order of 10% of the mean and occasionally as high as 30%, but within the secretory tissue the blood flow varies from 70 to 200% of the mean (Linzell, 1974). Even when considering the variation in MBF within the secretory tissue a high positive relationship exists between the overall MBF and the milk yield in the above mentioned studies. The peak MBF coincides with peak milk yield and declines steadily thereafter, but at a rate slower than the rate of decline in milk yield (Linzell, 1974), and suggests that MBF is a major determinant of quantitative differences in the mammary metabolism. This is supported by the absence of a relationship between A-V for glucose, acetate, BHBA and triglycerides and milk yield (Bickerstaffe et al., 1974). The unsynchronized decrease in milk yield and blood flow after peak yield leads to a highly inflated MBF to milk ratio in late lactation (1000:1) (Linzell, 1974). The inflated MBF to milk ratio and a decreased milk yield in late lactation may be related to the previously discussed hormonal effects.

The lymph flow from the mammary gland is linearly related to the milk secretion but is less than 1/1000 of the MBF and can be neglected in A-V studies (Linzell, 1974).

2.5.1 Control of mammary blood flow

The control of MBF can only be studied in conscious animals (Linzell, 1974). The MBF is affected by feeding and tissue metabolite concentrations and hormones, but the mechanisms involved (cause and effect) are largely unknown (Linzell, 1974).

Effect of Feeding.--The MBF in goats was decreased to one-half by fasting (Linzell, 1967a). In fasted cows MBF was also decreased, but was similar in normal and spontaneous ketotic cows (Kronfeld et al., 1968). The MBF was increased in cows fed a high starch, low roughage diet (Annison et al., 1974).

Hormonal Effects on Mammary Blood Flow.--Two to three days before parturition the MBF begins to increase, but the effects of hormones are generally unknown. Potent vasoconstrictors and vasodilators were discussed by Linzell (1974) and their effects on MBF are summarized below.

Potential vasoconstrictors in MBF control are adrenalin, noradrenalin, antidiarrhetic hormone (ADH), oxytocin, serotonin, prostaglandins, partial CO_2 pressure (pCO_2) and blood temperature. Adrenalin and noradrenalin have no effect on the general blood pressure in anesthesized dogs given one to 10 mg IV, but the MBF is reduced, and leaves these hormones as serious contenders in MBF regulation. The adrenalin released in insulin hypoglycemia in the goat causes a serious reduction in MBF and could lower the milk yield without altering the A-V for glucose. The quantities of ADH relase for water balance regulation are below effective levels (10 mu/min) for decreased MBF in cows and goats. Oxytocin in doses 10 times larger than the quantities released in response to milking resulted in a negligible reduction of MBF and small doses even caused an increase. Serotonin is a mammary vasoconstrictor released from platelets as they come in contact with foreign surfaces. It is active in vitro but the in vivo effect is unknown. Prostaglandin ($PGF_{2\alpha}$) is also a potent

vasoconstrictor. In women the MBF is lowest during menstruation and in goats the MBF was markedly reduced by infusion of 28 mg or more per minute of PGF_2 into the mammary artery.

The normal pCO_2 in goats is approximately 50 mm Hg. In a hot humid climate the pCO_2 may decrease, due to hyperventilation, and lead to a decreased MBF. A high pCO_2 will also decrease the MBF, but is unlikely under physiological conditions.

Mammary blood temperatures below 25 C lead to a marked but reversible decrease in the MBF. However, milk yields are unaltered by varying mammary blood temperatures between 34 and 39 c.

Potent vasodilators are acetylcholine, histamine, adenosine, and bradykinin. Acetylcholine and histamine at 0.1 to 1.0 g levels lead to vasodilation, but there is no evidence of both parasympathetic and sypathetic innervation of the mammary gland. Adenosine has been proposed to be involved in the control of microcirculation and to have the same effects as adenosine phosphates and NaH_2PO_4. Bradykinin causes vasodilation in vivo and is produced during zero flow. It also caused vasoconstriction under in vitro conditions.

3. Effects of postruminal administration of energy (glucose) and protein (casein and amino acids) on milk production.

The discussion of milk synthesis and secretion suggests that MBF and arterial metabolite concentrations are major determinants of milk production. However, saturation experiments for maximum responses, as used in non-ruminants, cannot be performed under normal conditions due to the complicating factor of rumen fermentation and the lack of adequate methods for rumen bypass. Hence, abomasal or intra arterial

and/or IV infusions must be used. Accumulated data obtained by these methods will give the requirement at the abomasal level, or at the level of the mammary gland. In combination with data on the maximal abomasal flow of nutrients under various conditions of rumen fermentation, the quantity of rumen bypass for maximum milk production may be calculated. Only abomasal and IV infusion studies will be considered in this review.

The effects of abomal and IV infusions of glucose, casein, and amino acids on the milk production in dairy cows have been studied by Yousef et al. (1969, 1970), Broderick et al. (1970), Hale and Jacobson (1972), Hale et al. (1972), Derring et al. (1972, 1974), Tyrrell et al. (1972), Vik-Mo et al. (1972, 1974a, b), Spires et al. (1973), Schwab and Satter (1973, 1974), Schwab et al. (1975, 1976), Fisher (1969, 1972), Fisher and Erfle (1974), Clark et al. (1973), and Teichman et al. (1969), and have been reviewed by Chalupa and Chandler (1972) and Clark (1975).

3.1 Effect on milk protein

When Yousef et al. (1969) intravenously infused an enzymatic hydrolysate of casein (50 g/day), the milk protein content increased 9 and 28% in cows fed normal and high grain rations, respectively. However, the infusate led to high fever and a decreased milk yield, and resulted in net decrease in milk protein synthesis. An acid hydrolysate of casein (50 g/day) increased the protein content 14%. Inclusion of glucose at a 1:1 ratio increased both milk yield and the protein content, and fever was prevented. They concluded that both amino acids and energy limit milk protein synthesis, and that extra

grain in the diet of lactating cows should usually increase milk protein synthesis.

Abomasal infusion of 200 to 1400 grams casein per day have increased the milk protein yield when compared with saline controls (Broderick et al., 1970; Tyrrell et al., 1972; Vik-Mo et al., 1972, 1974a; Schwab and Satter, 1974; Schwab et al., 1975, 1976), abomasal infusion of isonitrogenous, isocaloric urea-glucose solutions (Spires et al., 1973; Clark et al., 1973), urea treated corn silage per se (Hale et al., 1972; Vik-Mo et al., 1974a), or ruminal infusion of casein (Derring et al., 1972, 1974). The daily protein yield increased from -2.9 to + 18.4% and was due to an additive increase in milk yield and the protein content. The increased protein synthesis is usually seen immediately after infusion commences (Broderick et al., 1970; Vik-Mo et al., 1974a). Such may be expected because of the high amino acid turnover in lactating cows.

For cows fed above the NRC-standards (NRC 1971) for energy and protein the response to abomasally infused casein was positively related to both the protein yield in the control period and the amount infused (Vik-Mo et al., 1974a). They concluded that the magnitude of the two variables might depend on common causative factors such as the protein synthesizing apparatus in the mammary gland, and the hormonal status of the cows. Both IV and abomasal infusions of amino acids led to elevated growth hormone and prolactin levels. Prolactin apparently has an all or none effect in stimulating RNA and protein synthesis (Vik-Mo et al., 1974a). Vik-Mo et al. (1974a) did not find any interaction between feed NPN and infused casein in cows fed above the

NRC-standard for energy and protein. Abomasally infused glucose and a 1:1 mixture of glucose and casein also stimulate milk protein production (from 1.7 to 17.1%). Responses to abomasal infusions of casein, a casein-glucose mixture and glucose decreased in that order (Vik-Mo et al., 1974a).

Productive N from abomasally infused casein was diverted with 84 and 16% towards milk protein and N-retention, respectively (Derring et al., 1972). However, the increase in milk protein yield amount to less than 20% of the infused casein (Broderick et al., 1970; Vik-Mo et al., 1974a). After abomasal infusion of 433 to 860 grams of casein per day, 12 to 24% of the infused casein was recovered as increased milk protein (Tyrrell et al., 1972). The remainder of the infused casein caused 20 to 30% increases in fecal, urinary, and retained N. The amount of infused casein available for protein synthesis is slightly higher than the observed increases and may be attributed to differences among experiments and a "requirement of the protein synthesizing apparatus." Of the energy in abomasally infused glucose (3.6 Mcal/day) 48% was excreted in the feces (Tyrell et al., 1972).

Casein and glucose infusions increased true protein in milk and to a lesser extent its NPN content (Broderick et al., 1970; Vik-Mo et al., 1974a). Milk NPN was positively correlated with feed NPN (Vik-Mo et al., 1974a), and may be due to an upper limit for the cows ability of concentrate urea in urine (Vik-Mo et al. 1974a) with a consequent spillover in milk.

Rook and Line (1961) and Yousef et al. (1970) found that the increase in protein by infusion led to an increased content of CA, β-LG and α-LA. This suggests an increased protein synthesis within the mammary gland and is in agreement with in vitro studies by Park et al. (1974) and Park and Chandler (1976). They found that the rate of β-LG and CA synthesis increased linearly when the EAA complement was increased five-fold. However, the increase in α-LA synthesis was linear only from levels one to three. This may suggest uneven changes in the synthesis of proteins form the various fractions, and is in agreement with characteristic changes in the amino acid composition of wool from sheep receiving abomasal infusions of sulfur amino acids (Gillespie et al., 1969). Effects of non-synchronous changes in the protein fractions in milk on the amino acid composition are doubtful partly because α-LA is only a small fraction of the total protein, and partly because the amino acid composition of milk protein was the same in cows fed rations containing 9 and 18% protected protein (Broderick et al., 1974). The previously discussed additive increase in milk yield and protein content by abomasally infused nutrients may be due to such a non synchronous change in the protein fractions. Yousef et al. (1970) found a positive linear relationship between α-LA and milk yield.

The increased synthesis of true protein by casein and glucose infusion suggest an increased availability of amino acids. The plasma concentrations of individual EAA was either unchanged or increased by abomasally infused casein (Spires et al., 1973; Hale et al., 1972; Broderick et al., 1970; Derring et al., 1974; Vik-Mo et al., 1974b).

The response in NEAA was more variable but usually the total essential to total non-essential amino acid ratios were increased. Casein infusion had no effect on plasma glucose and decreased the blood urea nitrogen content in cows fed according to the NRC-standards (Derring et al., 1972). In cows fed above these standards casein infusion increased plasma glucose and blood urea nitrogen (Vik-Mo et al., 1974b). In the latter cows, glucose infusion decreased the blood urea nitrogen. Effects similar to those for glucose have been obtained in cows receiving rumen infusions of proprionate, but not by acetate and butyrate (Rook and Balch, 1961; Rook et al., 1965; Armstrong, 1968). These results suggest that extra protein (casein) increases availability of plasma amino acids for milk synthesis and leads to increased gluconeogenesis from amino acids in the liver. The increased availability of amino acids may lead to an increased protein synthesis and decrease the demand for glucose for NEAA anabolism in the mammary gland. This, along with an increased availability of glucose by gluconeogenesis from amino acids in the liver may increase lactose synthesis. On the other hand, extra glucose (or propionate) decreases the demand for gluconeogenesis from amino acids in the liver, which in turn may increase the availability of amino acids for protein synthesis. The latter may explain the protein-sparing effect of glucose and propionate as discussed by Yousef et al. (1970) and Huber and Boman (1966b). The changes in blood (plasma) metabolite concentrations might not be detectable by ordinary methods but even statistically non-significant changes may be highly significant biologically, due to the large multiplication factor from MBF. Whether a change in the plasma

glucose concentration affects the MBF per se in well fed and healthy animals is unknown, but the MBF was decreased to one half in fasted goats (Linzell, 1967a). Linzell (1974) discussed the possible importance of epinephrine as a vasoconstrictor in insulin hypoglycemia. Whether or not an increased nutrient availability has a positive effect on the integrity of the cells which synthesize milk is also unknown.

3.2 Lactose and milk yield

In the previously describe experiments, abomasal infusion of casein and glucose in dairy cows increased milk yield an average of 4.4% (range -5.5 to +13.1%). The response to abomasal infusion of casein tended to be larger in cows with high production fed below or at the NRC-standards for energy and protein. In cows fed above these standards the response is less and more variable (Vik-Mo et al., 1974a). Low producing cows (5 to 6 kg milk per day) fed a urea containing diet did not respond to abomasal infusions of casein, gelatin, partially delactosed whey, zein or soybean meal. Milk yields were increased in cows fed above the NRC-standards for energy and protein and abomasally infused with 270 to 540 grams glucose per day or a 1:1 mixture of glucose and casein, and increased milk was positively related to the amount of casein infused (Vik-Mo et al., 1974a). When Derring et al. (1974) infused casein into the abomasum, the milk lactose content was unchanged but the lactose yield was increased 3.9% compared to controls receiving rumen infusions of casein. This experiment is the only one where milk lactose was determined, but the evidence suggests that increases in the solids-non-fat (SNF) content

may be attributed to an increased protein content, as suggested by Rook and Line (1961) and Armstrong (1968).

The described effects of casein and glucose infusions on milk lactose and milk volume are in agreement with the hypothesis that a greater lactose biosynthesis increases milk secretion (Linzell, 1974). An improved availability of amino acids increases α-LA (protein) synthesis in the mammary gland which increases lactose biosynthesis from the infused glucose or casein. The greater amount of lactose increases milk volume, but lactose content remains relatively constant.

3.3 Intravenous infusion of graded levels of amino acids

Intravenous infusion of graded levels of amino acids have been studied by Fisher (1969, 1972), Teichman et al. (1969), and Fisher and Erfle (1974). Fisher (1969) infused 13 and 26 g DL-methionine or 26 g methionine plus 52 g L-lysine-HCL per day. The treatments had no effect on milk yield, milk protein or feed intake. Fisher (1972) also compared zero, low and high levels of methionine (0, 11.2, 24.8 g/d), lysine (0, 21.2, 50.3 g/d) and histidine (0, 33.5, and 65.9 g/d) in cows fed at 70 to 90% of the NRC-standard for CP. Milk yields were not significantly affected by any of the treatments. Cows receiving low methionine produced more protein than those receiving none or the high level. At zero histidine, milk protein content was higher than for the low and high groups. Low level lysine infusion tended to increase the milk protein yield, but no further increase was obtained at the high level due to a decrease in milk yield. For both methionine and lysine the protein content tended to increase linearly with the level of infused amino acid. Treatment had

no effect on the milk lactose content. Fisher and Erfle (1974) intravenously infused lysine (15g/d), lysine plus methionine (15 and 10g/d, respectively), carnitine (30 g/d) or saline to cows fed 67% of the NRC-standards for CP. The treatments had no significant effect on milk yield and milk composition. Likewise IV infusion of methionine at 5, 10, or 20% of its expected secretion rate did not affect milk yields or composition (Teichman et al., 1969).

3.4 Effect of abomasal infusion on feed intake, dry matter digestibility, and N-utilization

Feed Intake.--Mugerwa et al. (1969) hypothesized that an amino acid imbalance might limit feed intake and N-utilization in cows. When Broderick et al. (1970) infused 800 g casein plus 24 g methionine per day, grain intake was decreased 10%. Vik-Mo et al. (1974a, trial II and III) found that the DM intakes decreased when more than 500 grams casein per day were infused, but it was unaffected in experiments where 200 to 500 g casein were infused per day (Spires et al., 1973; Mugerwa et al., 1969; Derring et al., 1972, 1974; Hale and Jacobson, 1972a; Vik-Mo et al., 1974a). In low producing cows, 200 g casein per day tended to increase the DM intake (Hale et al., 1972; Hale and Jacobson, 1972a). Vik-Mo et al. (1974a) suggested that a high postruminal protein load caused the decrease in feed intake at high infusion rates.

Dry Matter Digestibility.--In cows fed concentrate and alfalfa hay the DM digestibilities were increased significantly by abomasal

infusion of casein compared with ruminal infusion (Derring et al., 1972, 1974). However, Spires et al. (1973) found no difference in the DM digestibility when casein or a urea-glucose solution were infused into the abomasum.

Nitrogen Utilization.--Cuthbertson and Chalmers (1950) hypothesized that entry of high quality protein into the rumen may not be as beneficial to the host as entry into the abomasum. This has been confirmed by Mugerwa et al. (1969), Spires et al. (1973) and Derring et al. (1972, 1974). They found that the N-utilization in cows was improved by casein infusion into the abomasum compared to abomasal infusion of gelatin or urea, or ruminal infusion of casein, gelatin, or urea. When casein was abomasally infused, fecal and urinary N was decreased. The resulting increase in productive N led to an increase in both milk N and N-retention (Derring et al., 1972, 1974).

3.5 Factors affecting blood metabolites in dairy cows

Diurnal Variation.--No consistent diurnal change in plasma amino acid (PAA) concentrations was apparent in cows offered feed twice daily at milking (Halfpenny et al., 1969; Vik-Mo et al., 1974b), but blood urea nitrogen tended to increase and blood glucose decreased with time after feeding (Vik-Mo et al., 1974b).

Stage of lactation.--A marked decrease in PAA concentrations was noted between pregnancy and the sixth week of lactation (Halfpenny and Rook, 1968; Halfpenny et al., 1969b). However, cows which were further advanced in lactation increased in PAA concentrations between the

fourth and the ninth week of an experiment regardless of protein level or source (natural protein negative and positive controls, urea and ammonia corn silages). In this experiment total EAA concentrations in plasma were consistently lower in high than low producing cows at the fourth week of experimentation but differences had diminished by the ninth week, as had also differences in milk yields. (Lichtenwalner et al., 1971).

Energy.--It was hypothesized that increased energy or ruminal infusion of propionate lead to an increase in the plasma concentrations of glucogenic NEAA, and a resultant decrease or no change in EAA (Halfpenny et al., 1969a, b; Huber and Boman, 1966a, b; Rook and Line, 1961). Halfpenny et al. (1969a, b) theorized that an increased output of amino acids in milk proteins results in a depression in the plasma concentrations of the EAA and certain NEAA. Huber and Boman (1966b) suggested that propionate had a controlling effect on the amino acid metabolism in the liver.

Protein Source and Level.--Intravenous infusion of various amino acids (methionine, lysine, histidine) increased the plasma concentration of that particular amino acid (Fisher, 1969, 1972). Infusion of 26 g DL-methionine per day increased plasma methionine and cysteine, but inclusion of 52 g L-lysine-HCL per day had no effect on plasma lysine. The infusions led to a decrease in blood ammonia but had no significant effect on blood urea, citrulline and arginine. When Fisher (1972) infused zero, low and high levels of methionine, plasma methionine was higher for the low than for the zero and the

high levels of infusion. Histidine infusions resulted in a two-phase response in plasma histidine. Plasma lysine was increased when only lysine was infused; but the plasma concentrations were not given for the zero group, so the response curve could not be established.

Broderick et al. (1974) fed rations containing 11.2, 13.5, 15.7 and 18% CP in the DM to dairy cows where formaldehyde-treated sodium caseinate, (0.8 w/w), was the sole protein supplement to a basal ration of oat straw, concentrate and corn silage. The plasma concentration of glycine decreased, total EAA increased slightly, total NEAA was not affected and urea increased linearly with the level of protein in the diet. Plasma concentrations of methionine, lysine and valine also increased with the dietary protein level, but no consistent changes were seen for phenylalanine, leucine, isoleucine, tryptophan, threonine and histidine.

Up to 423 g urea per day had no effect on PAA profiles compared to controls (81 g urea/day) (Van Horn et al., 1969), and NPN did not affect total PAA and total NEAA in plasma compared to a positive control group. (Lichtenwalner et al., 1971). However, Polan and Miller (1975) reported that CP (0 to 20% in DM) and urea (0 to 40% of CP) accounted for zero to 71% of the variation in PAA concentrations.

4. Limiting amino acid(s) for milk protein synthesis

Abomasal infusion of the 10 EAA in the same ratio as in casein led to as high increases in milk and protein yields as casein infusion (Schwab and Satter, 1973; Schwab et al., 1976). The limiting

amino acid for milk protein production in cows fed corn, corn silage and alfalfa grass hay was then explored by successive delations form the complete EAA mixture (Schwab and Satter, 1973, 1974; Schwab et al., 1975, 1976). They concluded that lysine and methionine were the first and second limiting or co-limiting amino acids for milk protein synthesis. Under varying conditions and by various methods without direct determination, the following amino acids have been termed the first limiting: methionine (Chandler, 1970; Chandler and Polan, 1972; Brown, 1969; Fisher, 1972; Broderick et al., 1974; Chalupa and Chandler, 1972; Dingley et al., 1975; Mepham and Linzell, 1974), phenylalanine (Derring et al., 1974; Vik-Mo et al., 1974b; Spires et al., 1973; Clark et al., 1974), lysine (Jacobson et al., 1969; Schwab et al., 1976), throenine (Derring et al., 1974; Broderick et al., 1970), and histidine (Spires et al., 1973) (Table 7). Halfpenny et al. (1969b) suggested that the supply of glutamate and proline may limit the protein synthesis. However, this may be considered disproved since IV infusion of NEAA had no effect on the milk protein yield in goats (Mepham and Linzell, 1974). Schwab et al. (1976) obtained a response in protein synthesis only from abomasal infusion of casein and a mixture of EAA with the same ratios as casein.

5. Summary of interactive review on metabolic effects on milk synthesis and production

The synthesis of milk protein and lactose and milk volume are functions of the mammary blood flow, the uptake of glucose and amino acids by the mammary gland, and the in situ metabolism of the absorbed metabolites. The rate of synthesis appears to be under an overall

Table 7.--Limiting amino acids in milk protein production.

Source	Amino Acid									
	Arg	His	Ile	Leu	Lys	Met	Phe	Thr	Trp	Val
Mepham & Linzell 1974 a. Goat						1			1	
Jacobson et al., 1969. Low[b]		4			1	2		3		
Jacobson et al., 1969. Supplemented[b]		3			1	2		4		
Chandler 1970[c]			3		5	1			4	2
Chandler and Polan, 1972					2	1	3	5		
Virtanen, 1969		2				1				
Derring et al., 1974[d]	4	6	7	8	5	3	2	1		9
Broderick et al., 1970[d]	2	7	6	8	5	4	3	1		9
Derring et al., 1974[e]	9	7	6	5	3	2	1	4		8
Derring et al., 1974[f]	9	2	7	6	4	3	1	5		8
Vik-Mo et al., 1974b				3	1		1			
Halfpenny et al., 1969	supply of glu & pro may limit protein synthesis									
Fisher, 1972			2	2		1				
Broderick et al., 1974					2	1				
Chalupa & Chandler, 1972[g]			3		5	1			4	2
Spires et al., 1973. Casein		3			4	2	1			
Spires et al., 1973. Urea		1			4	3	2	5		
Schwab et al., 1976					1	2				
Dingley et al., 1975		3			4	1	2			
Clark et al., 1974					3	2	1	4		

[a]Within a row amino acids numbered one through nine are first to ninth limiting.
[b]Dietary sulfur.
[c]Calculated from the amino acid composition of microbial protein.
[d]From increase in plasma level by casein infusion; basal increase most limiting.
[e]From lowest theoretical protein output by rumen.
[f]From lowest theoretical protein output by abomasum.
[g]Review.

hormonal control. The control of mammary blood flow is generally unknown but the mammary blood flow to milk ratio remain constant at approximately 500:1 except for a relatively sharp increase in late lactation because of a decrease in milk flow rate.

The uptake of glucose and amino acids appear to remain constant as a percentage of arterial blood concentration; but the mechanisms of extraction have not been established. The extraction coefficient for glucose is impaired by glucocorticoids, whereas insulin appears to have an indirect effect on blood glucose concentrations. It is speculated that hormonal vasoconstrictors decrease blood glucose concentration by reducing mammary blood flow. Overall the uptake of amino acids can account for the synthesized protein, but large variations exist for individual amino acids. Extraction coefficients for EAA are large and the uptake equals the output in milk. The uptake of arginine and valine is in escess of the output in milk, and ornithine and citrulline are extracted even though they are not found in milk. The uptake of NEAA is less than the output in milk which suggests NEAA anabolism in the mammary gland.

Amino acid metabolism in the mammary gland conforms with known pathways, except for an incomplete urea cycle (no arginino-succinase activity), and an apparent lack of the enzymes for cysteine generation from methionine. Some NEAA are generated from glucose with excess amino acids as the non specific N source. The rates of amino acid transamination and amination appear to depend on substrate concentrations and little is oxidized. Absorbed glucose is apparently partitioned with relatively constant percentages towards lactose, oxidation, glycerol and NEAA anabolism. It is unknown whether an

increased uptake of glycerol, NEAA, etc. affect the amount of glucose utilized by the respective pathways. Little gluconeogenesis takes place within the mammary gland, except for triose phosphate salvage.

Milk protein consists of caseins, the whey proteins (α-LA, β-LG, SA, Ig, and PP). Serum albumin, Ig and PP are transferred from blood. The caseins, α-LA and β-LG are synthesized from absorbed amino acids via the general pathways believed to exist in all cells in all species. This synthesis is under an overall hormonal control. The cause-effect relationships and control mechanism governing milk protein synthesis are generally unknown. It was hypothesized that the same hormones (prolactin, insulin, and ACTH) are responsible for lactogenesis as are involved in maintaining the integrity of the protein synthesizing apparatus. The hormonal stimulation relating to the maintenance function is dependent upon a regular removal of milk (milking). With progressing lactation, the maintenance function is impaired by an increased release of estrogen and progesterone, which decreases milk production. Lactose is synthesized from UDP-galactose and a pool of free glucose. Its biosynthesis is controlled by the concentration and rate of flow of α-LA through the Golgi apparatus.

Milk protein, lactose and minerals are secreted via secretory vesicles formed from the Golgi apparatus. The vesicle membrane replenishes that lost with fat droplets. Water is added to the solids mainly by the osmotic effects of lactose. This suggests interrelated processes in biosynthesis and secretion, but how strict the relationships are, have not been determined.

The substrate availability in mammary blood affects the production of protein and lactose and the milk volume. In dairy cows the substrate concentrations of amino acids and glucose in mammary blood have been increased by abomasal and intravenous infusions. This led to an increased yield of protein, lactose, and milk and may be explained by current knowledge of milk synthesis and secretion. An increased protein intake (by abomasal infusion of casein or amino acids) increases the availability of amino acids and increases the protein synthesis. The increased protein intake also increases the availability of glucose, by greater gluconeogenesis from amino acids in the liver. It is unknown whether the synthesis of CA, α-LA and β-LG increase to the same extent during increased protein synthesis, but an increased α-LA production may increase the lactose synthesis when more glucose is available. An increased energy intake (by infusion of glucose) increases the availability of glucose for lactose biosynthesis and leads to an increased milk yield. However, the protein yield is also increased, and may be explained by an amino acid sparing effect of glucose in the liver, and/or less NEAA anabolism from glucose in the mammary gland.

Several amino acids have been suggested by various investigators to limit milk protein synthesis, but there has not been a consensus on the one which is most limiting. However, by different workers methionine, lysine, phenylalanine and threonine have been mentioned as those in shortest supply. This lack of consensus may be due to experimental differences, but are also the result of the methods used for

calculating the order of limitation of amino acids. Except for two experiments, the limiting amino acid was determined by back calculations instead of actual determination.

6. Research proposal and objective

The limited information on the feasibility of NPN substitution for natural protein in feed rations for high yielding dairy cows suggests the need for further research. Hence, this investigation will study the utilization of NPN in rations of dairy cows in early lactation when milk yields and nutrient demands are highest relative to nutrient intake.

The objective of the proposed research will be: (1) Test protein requirements and the feasibility of substituting two sources of NPN for preformed protein in rations for high yielding dairy cows early in lactation, and (2) Measure the effect of protein source on critical rumen and blood metabolites.

MATERIALS AND METHODS

Sixty-eight lactating Holstein cows from the dairy herd at Michigan State University were employed in an experiment to test protein requirements and the feasibility of nonprotein nitrogen (NPN) substitution for proformed protein in dairy cattle rations in early lactation.

Experimental.--The experimental variables and ration compositions are given in Table 8. The only difference between the negative control (NC) and the positive control (PC) treatments was the total crude protein (CP) content in the dry matter (DM). Treatment NC received 12 to 13% and treatment PC received 15 to 16% CP in the DM. For both treatments the planned CP contents were obtained by varying soybean meal and corn grain in the concentrate. In the two remaining treatments the negative control ration was supplemented with either urea (treatment U) or ammonia plus urea (treatment AU) to equal the CP content of the positive control group. Treatment U cows received corn silage treated with urea at ensiling and the NC concentrate supplemented with 1.25% urea. Treatment AU cows were fed corn silage treated with ammonia at ensiling plus the same grain used for treatment U. The detailed composition of the concentrates used for treatment NC, PC, U, and AU are given in Table 9.

Table 8.--Ration components and percent crude protein in the different treatments.

Treatment	Concentrate no.[a]	Corn silage			CP (%) of total DM[c]
		No treatment	Urea[b]	Ammonia[b]	
NC	D-199	+			12-13
PC	D-200	+			15-16
U	D-201		+		15-16
AU	D-201			+	15-16

[a]Concentrate composition is given in Table 9.

[b]NPN treatment at ensiling.

[c]CP is crude protein (N * 6.25) and DM is dry matter.

Corn Silage.--The urea and ammonia treated corn silages were stored in 3.1 x 12.2 m concrete stave silos. Untreated silage for the NC and PC groups was stored in a similar silo in 1975 and a 4.3 x 12.2 m silo in 1976. Additional untreated corn silage was obtained from a 6.1 x 18.3 m concrete stave silo and a 11.0 x 24.1 x 4.0 m bunker silo. The fresh corn plant was harvested with a 2-row conventional forage harvester, cut to an average length of 1.0 to 1.5 cm, and delivered into wagons with side unloaders. Each load was weighed and urea and ammonia were added at 0.65% and .40% of the fresh weight, respectively. The urea was applied in a uniform layer across the top of each load before unloading. Ammonia was applied at the silo blower by the cold-flow method, and amounts were controlled by flow meters. In 1975 the quantity added was ascertained by weight differences of the supply

Table 9.--Ingredient composition of the concentrates used in this study[e], %.

Ingredient	Treatment		
	NC D-199[a]	PC D-200[a]	U + AU D-201[a]
Corn[b]	54.00	43.50	53.25
Oats[c]	26.50	21.25	26.00
Soybean meal (50% CP)	11.50	27.25	11.50
Urea	--	--	1.25
Molasses	5.00	5.00	5.00
Dicalciumphosphate	1.50	1.50	1.50
Limestone	.50	.50	.50
TMS[d]	1.00	1.00	1.00

[a]Herd concentrate number.

[b]Coarsely ground shell corn.

[c]Rolled.

[d]Trace mineralized salt contain a guaranteed minimum of: .35% Zn, .12% Fe, .15% Mg, .03% Cu, .005% Co, .007% 1, and 96.00% NaCl.

[e]To all concentrates was added 4400 I.U. Vitamin A and 400 I.U. Vitamin D per kg.

tank, while in 1976 a flowmeter was connected to a small nursing tank placed on a scale. The amount of corn ensiled, nutrient content at ensiling, and level of added NPN are given in Table A1.

Cows.--The 68 lactating Holstein cows employed in this experiment were from best, control and worst genetic groups (24, 16, and 28, respectively). Within each genetic group cows were blocked in groups of 4 based on age and expected date of calving. Cows within blocks were randomly assigned to treatments. The milk production in the previous lactation was not considered in the blocking of cows because only a limited number of cows within each genetic group were available at any given time. The cows' date of birth, record in the previous lactation (age at calving, milk production and calving interval), actual date of calving and assignment to blocks within genetic group are given in Table A2.

Cows were housed in a stanchion barn and allowed to exercise for about 5 hours each morning in a dry lot. The time spent exercising was shortened to about 2 hours on cold and rainy days. The cows were milked twice daily at 4 A.M. and 3 P.M. in a double eight herringbone milking parlor.

From 28 days before calving and until 15 days after calving, all cows were fed similarly. From April 15 to October 15, 1976 dry cows grazed pasture and were also fed up to 23 kg/day of urea or ammonia treated corn silage. During this period cows were moved to indoor maternity pens about one week before expected calving. Dry cows held indoors (for winter calvings) were fed up to 23 kg/day corn silage to which 0.5% urea was added at feeding, 1.8 kg. concentrate

(D-201 in Table 9), and 2.3 kg alfalfa hay per day until 7 days before the expected date of calving, at which time concentrate and the hay were increased to 2.7 and 4.5 kg per day, respectively. After calving, the amount of concentrate was gradually increased to 7.3 kg per day, and then adjusted according to milk yield on a weekly basis. Concentrate was fed at 1 kg per 2.5 and 3 kg milk from day 8 to 70 and from 71 to 140, respectively. On day 15 post-partum cows were switched to treatment rations as outlined in Table 8. Hay was fed at 4.5 kg per day until day 15 and then reduced to 2.3 kg per day for the remainder of the feeding trial. Corn silage was fed ad libitum throughout treatment at an amount frequently adjusted to insure 10% weigh back. Corn silage and hay were fed once daily at approximately 0800 and 1300 hours, respectively. Concentrate was fed in two equal portions by pouring on top of the silage at about 0900 and 1400 hours. Unconsumed feed was weighed back the following morning between 0500 and 0700 hours. Refusals of concentrate, corn silage, and hay were separated as well as possible and weighed separately. Daily weights were recorded for components fed and weighed back and for milk.

Sampling.--Cows were weighed 14 and 28 days before the expected calving date and biweekly, thereafter, starting the second week after calving. Corn silage samples were taken Monday, Wednesday, and Friday of each week and stored at 3C until processed. Concentrates and hay were sampled biweekly. Beginning in the first week after calving, composite milk samples of the afternoon and morning milking was taken biweekly.

Rumen fluid and blood samples were collected 2 weeks before calving, and 3, 10, and 18 weeks after calving. An additional blood sample was taken 6 weeks after calving. Rumen and blood samples were collected on Tuesdays of every week at 2 to 3 hours after the A.M. concentrate feeding. Sampling of blood was attempted to approximate days -14, 21, 42, 70, and 126 after calving. Rumen contents were sampled by stomach tube and strained immediately through 4 layers of cheesecloth prior to placement in an icebath. At the time of sampling ruman fluid was also prepared for ammonia determination according to Kulasek (1976).

Blood samples were collected from the tail vein using single draw needles[1] with 15 ml vacutainer tubes.[2] Each tube contained 30 mg potassium exalate and 37 mg sodium fluoride as anticoagulant and glycolytic pathway inhibitor, respectively. After drawing blood, the tube was inverted gently several times and placed in an ice bath until processed.

Preparation of Samples and Analyses.--Corn silages, concentrates, and hay were composited on a biweekly, bimonthly and monthly basis, respectively. To uniformly mix concentrates and hay were ground through a 40 mesh screen and corn silages in a model 84142 Hobbart Silage Chopper.[3] A portion of the corn silage composites

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[1]Single Draw Vacutainer Needle 20G. Becton-Dickinson, Rutherford, New Jersey 07070.

[2]Vacutainer Evacuated Glass Tube. Becton-Dickinson, Rutherford, New Jersey 07070.

[3]The Hobbart Manufacturing Company, Troy, Ohio.

was diluted with water (1:10) homogenized[4] for 3 minutes in an icebath, and filtered through four layers of cheesecloth. Readings for pH were made from the filtered homogenate, which was then centrifuged at 27,000 x g for 10 minutes (at 0 to 5 C). The supernatent was frozen at -20 C until analysis. All feed samples were analyzed for DM, total nitrogen, acid detergent fiber (ADF), and acid detergent nitrogen (ADN). The water extracts of silages were analyzed for soluble nitrogen (SOLN), ammonia, urea, lactic acid, ethanol, and volatile fatty acids.

Dry matter was determined by drying in a forced air oven at 100 C for 48 hours. Total nitrogen and SOLN were determined by Kjedahl (AOAC 1965) as modified by Wall and Gehrke (1975). Some of the nitrogen readings were by ammonium electrode (Brenner and Tabatabai, 1972). Acid detergent fiber and ADN were by the Van Soest method (Goering and Van Soest, 1970). Samples for these analyses were dried at 60°C for 48 hours in a forced air oven and then ground through a 40-mesh screen. Urea and ammonia were analyzed by the method of Okuda et al. (1965), modified by Kulasek (1972, 1976) for rumen ammonia and plasma urea. The method of Barker and Summerson (1941) was used for lactic acid analysis.

Volatile fatty acids (acetate, propionate and butyrate) and ethanol in the water extract of silages were measured using a Hewlett-Packard gas liquid chromatograph model 5730A with flame ionization detector.[5] A glass colum (6 ft x 2 mm ID) was packed with 3% Carbowax

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[4]Servall Omni mixer. Ivan Servall, Inc., Norwalk, Connecticut.

[5]Hewlett-Packard, Avondale, Pennsylvania 19311.

20 M, 0.5% H_3PO_4 on 60/80 Carbopack B.[6] Nitrogen was the carrier gas at a flow rate of 60 ml/min. The temperature program used was two minutes beginning at 140 C with a temperature increase of 4°C/min until 180 C. The final temperature was maintained for 8 to 12 minutes. Prior to injection, the samples (approximately 1.5 ml) were acidified with one drop 9 N H_2SO_4. Injection volume was 3 micro liters. Volatile fatty acids was calculated relating the areas under the peaks for the standards with the areas under the peaks of samples.

Fat, protein, and total solids in milk samples were determined within 48 hours after sampling. Milk fat was determined by The Michigan Dairy Herd Improvement Association Central Laboratory using the Babcock method; milk protein by the Udy dye method (Udy, 1971); and total solids by drying 3ml at 90 C for 3 hours in a forced-air oven.

Upon arriving in the laboratory about one hour after sampling rumen fluid samples were measured for pH; and then centrifuged at 27,000 x g (0-5 C) for 15 minutes. The supernatant was stored at -20 C until analyses for content of volatile fatty acids and ammonia nitrogen. Rumen volatile fatty acids (acetic, propionic, iso-bytyric, butyric, 2-methylbutyric, iso-valeric, and valeric) were determined by gas liquid chromatography as described for water soluble extracts of silages. Rumen ammonia nitrogen was analyzed by the method of Okuda (1965) modified by Kulasek (1976). Rumen ammonia nitrogen was determined in all frozen samples and approximately 50% of the fresh samples. Fresh samples were analyzed within 24 hours of sampling.

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[6]Supleco Inc., Bellefonte, Pennsylvania 16823.

Two hours after sampling whole blood was centrifuged at 6800 x g (0-5 C) for 10 minutes. One-half of the plasma was stored at -20 C until analysis for urea ammonia and glucose. The remainder was prepared for amino acid analysis by adding on the day of sampling .1 ml (1 micromolar) nor-Leucine-PEC internal standard and .1 ml 50% sulfosalisylic acid per milliliter plasma. The percipitated protein was removed by centrifugation at 34,800 x g (0-5 C) for 20 minutes and supernatant was frozen at -20 C until analysis. Amino acids were determined on individual samples by ion exchange chromatography.[7] Plasma urea nitrogen and plasma ammonia nitrogen were determined as described by Okuda (1965) and Kulasek (1972, 1976). Plasma glucose content was determined using the coupled system of glucose oxidase and peroxidase.[8]

Statistics.--In each of the two years of harvest three types of corn silage were placed in two or more silos. Nutrient contents were determined on biweekly composites of samples taken at feeding. The analysis of variance for each of the nutrients in corn silage was as a 2 x 3 factorial experiment with repeat measurements according to the model in (8):

$$(8) \quad Y_{ijkl} = \mu + A_i + F_j + (AF)_{ij} + S_{(ij)k}$$

where Y_{ijkl} is the observed value in the ith year, jth feed, kth silo within year and feed, and lth measurement within silo;

μ is the overall mean;

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[7]Technicon TSM System, Technicon Instruments Corporation, Tarrytown, New York.

[8]Worthington Biochemical Corporation, Freehold, New Jersey.

A_i is the effect of the ith year; i = 1,2;

F_j is the effect of the jth feed; j = 1,2,3;

$(AF)_{ij}$ is the year by feed interaction;

$S_{(ij)k}$ is the effect of the kth silo nested within year and feed. S is error for A and F.

The four treatments were tested in 68 cows. Cows within three breeding groups (best, control, worst) were blocked according to expected date of calving and age. The number of blocks within best, control and worst breeding groups were 6, 4 and 7, respectively. Feed intake, milk yield and yield of milk components were calculated as average per day and week from calving. Rumen and blood were sampled and assayed for each cow at 14 days pre-partum, and 21, 70, and 126 days post-partum. Blood was also sampled at 42 days. The analysis of variance for each dependent variable was a double-split plot design with respect to space (blocks within breeding groups) and time from calving, and was performed according to the model in (9):

$$(9)\quad Y_{ijklm} = \mu + B_i + D_{(i)j} + T_k + BT_{ik} + DT_{(i)jk} + C_l + BC_{il} + TC_{kl} + E_{(ijkl)m}$$

where Y_{ijklm} is the observed value of mth cow at the lth time on kth treatment, and jth block within ith breeding group;

μ is the overall mean;

B_i is the effect of the ith breeding group, i = 1,2,3;

$D_{(i)j}$ is the effect of the jth block within the ith breeding group, j = 1,2. . .6 for i = 1; j = 1,2. . .4 for i = 2; j = 1,2. . .7 for i = 3. D is error for B;

T_k is the effect of the kth treatment, k = 1,2. . .4;

BT_{ik} is the interaction between breeding group and treatment;

$DT_{(i)jk}$ is the interaction between block within breeding groups and treatments. DT is error for T and BT;

C_l is the effect of the lth time, l = 1,2. . .20;

BC_{il} is the interaction between breeding group and time;

TC_{kl} is the interaction between treatment and time;

$E_{(ijkl)m}$ is the residual error.

Milk yields and yields of milk components were tested by covariate analysis and the data were adjusted for significant covariates before testing by the model in (9). The covariate analysis was according to the model in (10):

$$(10) \quad Y_{ijk} = \mu + Cov + B_i + D_{(i)j} + T_k + BT_{ik} + DT_{(i)jk}$$

where Y_{ijk} is the average response for weeks 3 through 20 to the kth treatment, jth block and ith breeding group;

μ is the overall mean;

Cov is the covariate and is equal to the daily response in week two after calving; and

B_i, $D_{(i)j}$, T_k, BT_{ik} and $DT_{(i)jk}$ are as described in equation (9).

Adjustment for significant covariates were according to (11):

$$(11) \quad Y_{ADJ} = Y - (Y_{Cov} - \bar{Y})b$$

where Y_{ADJ} is the adjusted value.

Y is the observed value.

Y_{Cov} is the average for the second week after calving.

$\bar{Y}$ is the overall mean.

b is a constant.

The number of observations for time (C_1) in (9) was 18 when milk data were adjusted for covariates. For rumen and plasma parameters the number of observations with time were 4 and 5, respectively, except for plasma amino acids 42 days post-partum, which were analyzed statistically according to the model in (10) after removal of the covariate. The sources of variation for the various models and the degrees of freedom per source are summarized in Table 10.

An initial experiment on factors affecting plasma amino acid concentrations was studied using 5 treatments in cows at two levels of milk production. Cows were bled at three sampling hours at two stages of the experiment. The analysis of variance for individual amino acids was according to the model in (12) for a double split plot factorial design with respect to time.

$$(12)\quad Y_{ijkl} = \mu + T_i + L_j + (TL)_{ij} + P_k + (TP)_{ik} + (LP)_{jk} + (TLP)_{ijk} + H_l + (TH)_{il} + (LH)_{jl} + (TLP)_{ijl} + (PH)_{kl} + (TPH)_{ikl} + (LPH)_{jkl} + (TLPH)_{ijkl}$$

where Y_{ijkl} is the observed value at the lth hour, kth period, jth level of milk production and ith treatment;

μ is the overall mean

T_i is the effect of the ith treatment, i = 1,2. . .5;

Lj is the effect of the jth level of milk production, j = 1,2;

$(TL)_{ij}$ is the interaction between treatment and level of milk production. TL is error for T and L;

P_k is the effect of the kth period of sampling, k = 1,2;

Table 10.--Analysis of variances used and degrees of freedom per Source of Variation.

Sources of variation	Degrees of Freedom						
	Model (9)[a]					Model (10)[g]	Model (10)[h]
	Feed[b]	Milk[c]	Rumen[d]	Plasma[e]	PAA[f]		
Breeding group (B)	2	2	2	2	2	2	2
Blocks within B (D)	14	14	14	14	14	14	14
Treatment (T)	3	3	3	3	3	3	3
B x T	6	6	6	6	6	6	6
D x T	42	42	42	42	12	41	42
Time (C)	19	17	3	4	2	--	--
B x C	38	34	6	8	4	--	--
T x C	57	51	9	12	6	--	--
Residual error	1178	1054	184	298	39	--	--

[a]Model for analysis of variance.

[b]Dependent variables not adjusted for significant covariate.

[c]Dependent variables adjusted for significant covariate.

[d]Rumen pH, volatile fatty acids and ammonia nitrogen.

[e]Plasma glucose, urea and ammonia nitrogen.

[f]Plasma amino acids.

[g]Covariate analysis for yield of milk components.

[h]Plasma amino acids at 42 days post partum.

$(TP)_{lk}$ is the interaction between treatment and period;

$(LP)_{jk}$ is the interaction between level of production and period;

$(TLP)ijk$ is the interaction between treatment, level of production and period. TLP is error for P, TP and LP;

H_l is the effect of the lth hour of sampling, l = 1,2,3;

$(TH)_l$ is the interaction between treatment and hour of sampling;

$(LH)_{jl}$ is the interaction between level of production and hour of sampling;

$(TLP)_{ijl}$ is the interaction between treatment, level of production and period. TLP is error for H, TH and LH;

$(PH)_{kl}$ is the interaction between period and hour of sampling;

$(TPH)_{ikl}$ is the interaction between treatment, period and hour of sampling;

$(LPH)_{jkl}$ is the interaction between level of production, period and hour of sampling;

$(TLPH)_{ijkl}$ is the interaction between treatment, level of production, period and hour of sampling. TLPH is error for PH, TPH, and LPH.

Diurnal changes in PUN and PAN were studied in two cows selected from each treatment. Each cow was bled at 8 sampling hours on two occasions and the analysis of variance was according to the model in (13):

$$(13)\quad Y_{ijkl} = \mu + T_i + C_{(i)j} + P_k + (TP)_{ik} + (TCP)_{(i)jk} + H_l + (TH)_{il} + (TCH)_{(i)jl} + (PH)_{kl} + (TPH)_{ikl} + E_{(ijkl)}$$

where Y_{ijkl} is the observed value for the jth cow within the ith treatment, at the kth time from calving, and the lth hour of sampling;

μ is the overall mean;

T_i is the effect of the ith treatment. i = 1,2,3,4;

$C_{(i)j}$ is the effect of the jth cow within the ith treatment, j = 1,2. C is error for T;

P_k is the effect of the kth time from calving, k = 1,2;

$(TP)_{ik}$ is the interaction between treatment and period;

$(TCP)_{(i)jk}$ is the interaction between treatment, cow and time. TCP is error for P and TP;

H_l is the effect of the lth hour of sampling, l = 1,2. . .8;

$(TH)_{il}$ is the interaction between treatment and hour of sampling;

$(TCH)_{(i)jl}$ is the interaction between treatment, cow and hour of sampling. TCH is error for H and TH;

$(PH)_{kl}$ is the interaction between days from calving and hour of sampling;

$(TPH)_{ikl}$ is the interaction between treatment, days from calving and hour of sampling;

$E_{(ijkl)}$ is the residual error.

Differences between treatment means were only tested for significant sources of variation. Main effects with an equal number of observations per group were tested by orthogonal contrasts (group NC vs. PC, U, AU; group PC vs. U, AU; group U vs. AU). Bonferroni's t-test was used when additional contrasts were tested.

Calculations.--Nutrient intakes, milk yields, yields of milk components, and estimated nutrient balances (percent of NRC (1971) requirement consumed) per day and week from calving for individual cows were estimated utilizing a computer program which matched feed analysis arranged by week of the year with intakes and yields arranged by days from calving. Intakes were calculated on a daily basis and then averaged per 7 days from calving. Analysis of

variances, other statistical analysis and plots of responses utilized standard programs (Nie et al., 1975; Cohen and Burns, 1976; Tuccy, 1976; STAT Systems Group, 1974) and were performed on the Control Data Corporation 6500 Computer, Computer Laboratory, Michigan State University.

RESULTS

Main emphasis has been placed on the effects of treatments and time. However, variables affected by breeding groups and its interactions with treatments and time are reported at the end of each section.

1. Nutrient content in feeds

Concentrates.--The concentrates were prepared as described in Table 9 and analysis are given in Table 11. Dry matter (DM), acid detergent nitrogen (ADN), calculated net energy for lactation (NE_1), and acid detergent fiber (ADF) in DM were essentially equal in the three concentrates. Total nitrogen (TN) and crude protein (N * 6.25; CP) in DM was increased from 2.52 and 15.73% in mixture D-199 to 3.66 and 22.89% in D-200 by replacement of corn and oats (2:1) for soybean oil meal. The nitrogen content of D-201 was elevated to 3.11% by replacement of corn and oats for urea. In D-201 urea nitrogen amounted to 19.0% of the total nitrogen.

Corn silage.--The nutrient content in corn silage is also summarized in Table 11. Differences between feeds and years was analyzed statistically according to the model in (8).

Table 11.--The dry matter content and the content of nutrients in the dry matter of concentrate, corn silage and hay, %.[13]

		No.	Dry Matter	Nitrogen Fractions							Crude Protein	ADF[10]	NE_l[11]
				Total	Water Soluble				Water Insol.				
					Total	Urea	NH_3	Undt.[7]	ADN[8]	Undt.[9]			
Concentrate:													
D-199[3]	Av[1]		87.81[a]	2.52[a]					.22[a]	2.30	15.73[a]	6.39[a]	2.14
	s.e.[2]	7	.69	.07					.01	--	.41	.39	--
D-200[4]	Av		87.63[a]	3.66[b]					.23[a]	3.43	22.89[b]	6.35[a]	2.11
	s.e.	7	.45	.08					.01	--	.49	.28	--
D-201[5,6]	Av		87.30[a]	3.11[c]	.25[12]	.25	ND	0	.21[a]	2.65	19.45[c]	6.12[a]	2.11
	s.e.	7	.43	.05	--	--	--	--	.01	--	.30	.30	--
Corn Silage:													
Control[3,4]	Av		34.46[a]	1.36[a]	.67[a]	ND	.16	.51	.16[a]	.53	8.50[a]	26.03[a]	1.70
	s.e.	28	.85	.03	.03	--	.05	--	<.01	--	.17	.42	--
Urea[5]	Av		34.21[a]	2.10[b]	1.41[b]	.33	.39	.69	.18[b]	.51	13.06[b]	26.09[a]	1.70
	s.e.	28	.97	.05	.06	.04	.03	--	.01	--	.28	.62	--
Ammonia[6]	Av		33.87[a]	1.81[c]	.96[c]	ND	.49	.47	.22[c]	.63	11.31[c]	27.68[a]	1.70
	s.e.	34	.83	.05	.04	--	.03	--	.01	--	.30	.40	--

Table 11.--Continued.

		No.	Dry Matter	Nitrogen Fractions						Crude Protein	ADF[10]	NE_1[11]
				Total	Water Soluble				Water Insol.				
					Total	Urea	NH_3	Undt.[7]	ADN[8]	Undt.[9]			
Hay:													
Hay[3,4,5,6]	Av		88.28	2.17					.26	1.91	13.59	37.24	1.19
	s.e.		1.02	.13					.01	--	.81	.79	--

[1]Average

[2]Standard error of the mean

[3]Treatment NC

[4]Treatment PC

[5]Treatment U

[6]Treatment AU

[7]Undetermined Soluble N = Total N - (urea + NH_3)

[8]Acid detergent nitrogen

[9]Undetermined = Total N - (Water soluble N + ADN)

[10]Acid detergent fiber

[11]Calculated net energy for lactation (NRC. 1971)

[12]Urea added to the concentrat

[13]Columns within feed without a common superscript are significant ($P<.001$)

The overall DM content in the corn silage was 34.15% and tended to be lower ($P<.10$) in silage harvested in 1975 than in 1976 (30.83 vs. 36.75%).

The TN content of silages was increased by NPN treatments ($P<.001$), and was higher for the urea than the ammonia treated silage ($P<.001$). The lower content of TN in the ammonia treated silage was in part due to a lower content in the fresh untreated material (1.31 vs. 1.42 in DM), but mainly to a higher loss as vapors during ensiling and great difficulties in adjusting the rate of ammonia application which was by the cold flow method (Kjelgard et al., 1973).

The amounts of total NPN and ammonia nitrogen in water soluble fractions (SOLN) were determined and the amount of urea nitrogen was calculated by difference between the two fractions (Table 11). Urea nitrogen was set at zero when total NPN was equal to or less than ammonia nitrogen. Urea nitrogen in dry matter averaged .33% for the urea treated silage and was equal to 48.3 of the added which is similar to previous reports (e.g., Huber et al., 1973). Urea was not detected in the control nor the ammonia treated silages.

The content of ADN in silages did not differ among years but was higher in NPN treated than untreated silages ($P<.05$). Also, the ADN content was higher in the ammonia than the urea treated corn silage ($P<.05$).

The ADF content did not differ among years nor treatments at ensiling.

The change in TN and the nitrogen fractions between harvest and feeding of silages is given in Table 12. In control silage the total nitrogen content decreased 7.5% with a redistribution from the

Table 12.--Changes in total nitrogen and nitrogen fractions between harvest feeding in untreated and NPN treated corn silage.

Nitrogen Fraction	Treatment at Harvest								
	None			Urea			Ammonia		
	% in DM		Change[a]	% in DM		Change	% in DM		Change
	Harvest (A)	Feeding (B)	% (C)	Harvest (A)	Feeding (B)	% (C)	Harvest (A)	Feeding (B)	% (C)
Total Nitrogen	1.47	1.36	-7.5	1.42	2.10	+47.9	1.31	1.81	+38.2
Water Soluble	.44	.67	+52.3	.41	1.41	+243.9	.36	.96	+166.7
Urea	ND[b]	ND	0	ND	.33	∞	ND	ND	0
Ammonia	.06	.16	+166.7	.29	.39	+34.5	.26	.49	+88.5
Undetermined	.38	.51	+34.2	.12	.69	+475.0	.10	.47	+370.0
Water Insoluble	1.03	.69	-33.0	1.01	.69	-31.7	.95	.85	-10.5
Acid Detergent	.14	.16	+14.3	.23	.18	-21.7	.21	.22	+4.8

[a](C) = (B - A) * 100/(A).

[b]Not detectable.

water insoluble to the water soluble fractions. Insoluble proteins are hydrolyzed and to a large degree deaminated during silage fermentation. The decrease in protein nitrogen during fermentation was inhibited some by urea treatment but more by ammonia. Ammonia treatment also increased the ADN content during fermentation.

Quality measurements on the corn silages included ethanol, acetate, propionate, iso-butyrate, lactate, and pH (Table 13). None of the measurements were significantly affected by treatment at ensiling, and only the ethanol ($P<.01$) and propionate ($P<.05$) content differed among years. The obtained values are all characteristic of high quality silage.

Table 13.--Content of ethanol, acetate, propionate, iso-butyrate, butyrate, and lactate as well as pH in corn silage, % in DM (89 observations per item).

Item	Average	Standard Error
Ethanol	.260	.037
Acetate	1.734	.118
Propionate	.050	.005
Iso-butyrate	.007	.001
Butyrate	.071	.024
Lactate	4.862	.230
pH	4.510	.090

The simple and significant correlations among nutrient and quality measurements are given in Table A3. All the correlations, except between TN and SOLN were low and explained less than 20% of the variation of each other. A stepwise multiple regression was used to estimate the dependence of a quality measurement on the nutrient content. The results are given by partial and multiple correlations in Table A4. Thirty-five percent of the variation in the ethanol content was explained by the DM content and the concentrations of SOLN and ADF in DM. The content of acetate in DM was only related to the ADF content which in turn only explained 5% of the variation. Propionate, iso-butyrate, and lactate were not related to any of the nutrient content measurements. The content of butyrate was negatively related to DM and ADF in DM and positively related to ADN in DM. The three variables explained 33% of the variation in the butyrate content in silage DM.

2. Feed intake

Concentrate, corn silage, hay, and total dry matter.--The average intake per day by week from calving of concentrates, corn silage and total dry matter is given in Figures 3 to 5 and for periods (weeks 3 through 6, 7 through 10, 11 through 15, and 16 through 20) in Table 14. Intake of the various feed components did not differ between treatments.

The intake of concentrate increased throughout the first six weeks post-partum and then remained constant through week 10 (Figure 3 and Table 14). The sharp decrease in concentrate intake 10 weeks after calving is due to a change in the rate of concentrate feeding from

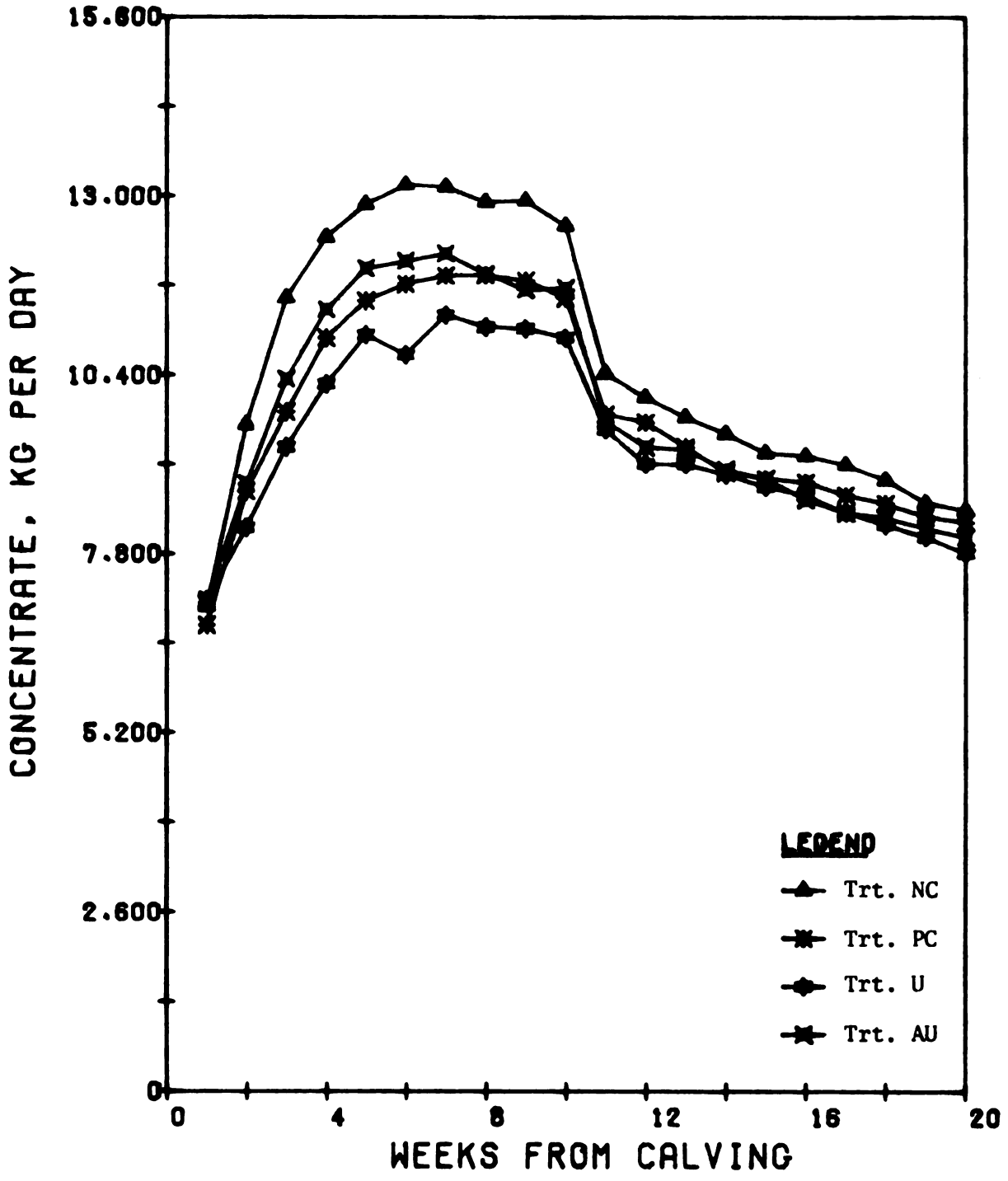

Figure 3. Daily intake of concentrate per treatment and week from calving (kg/d; standard error ±.07).

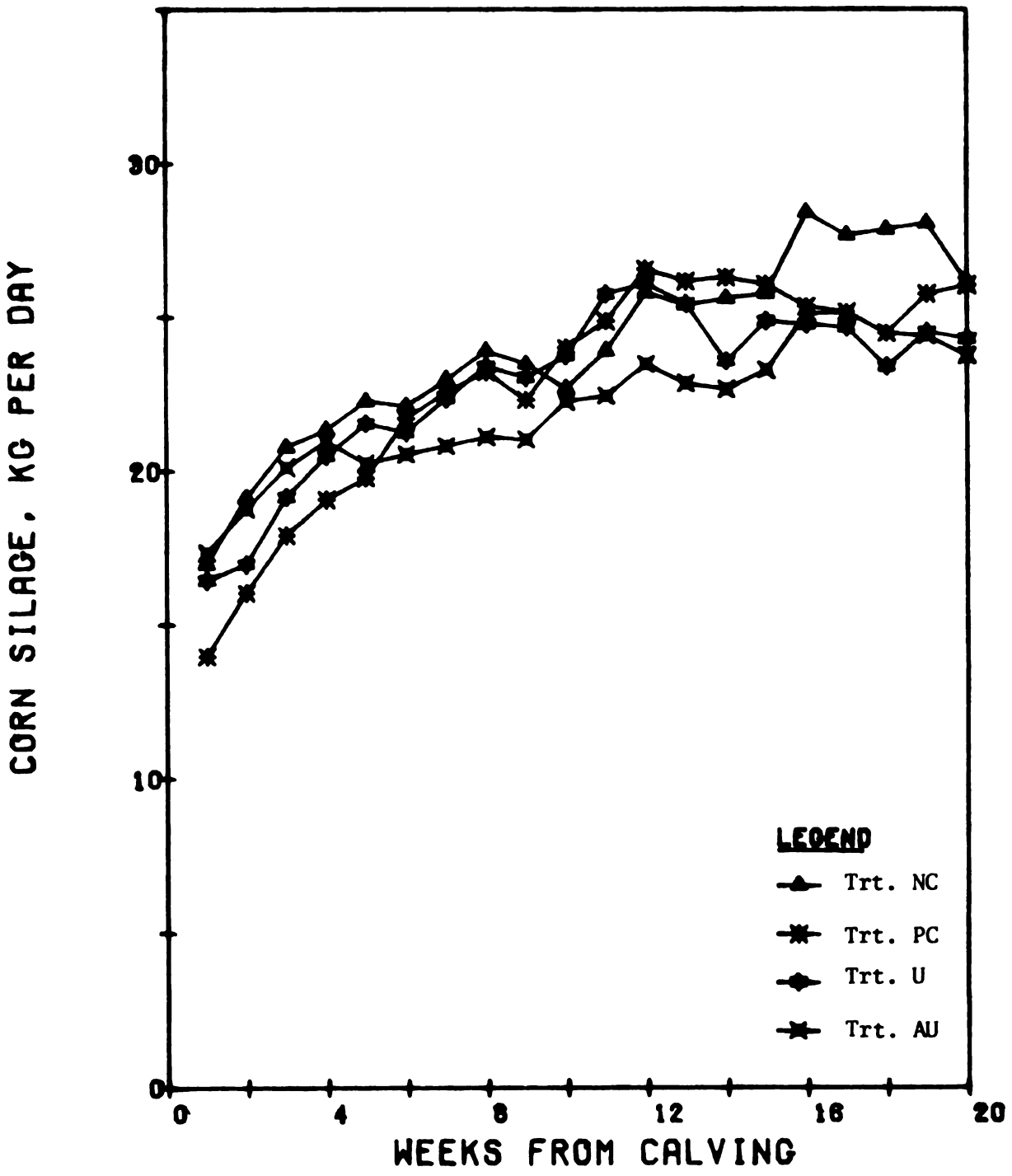

Figure 4. Daily intake of corn silage per treatment and week from calving (kg/d; standard error ±1.02).

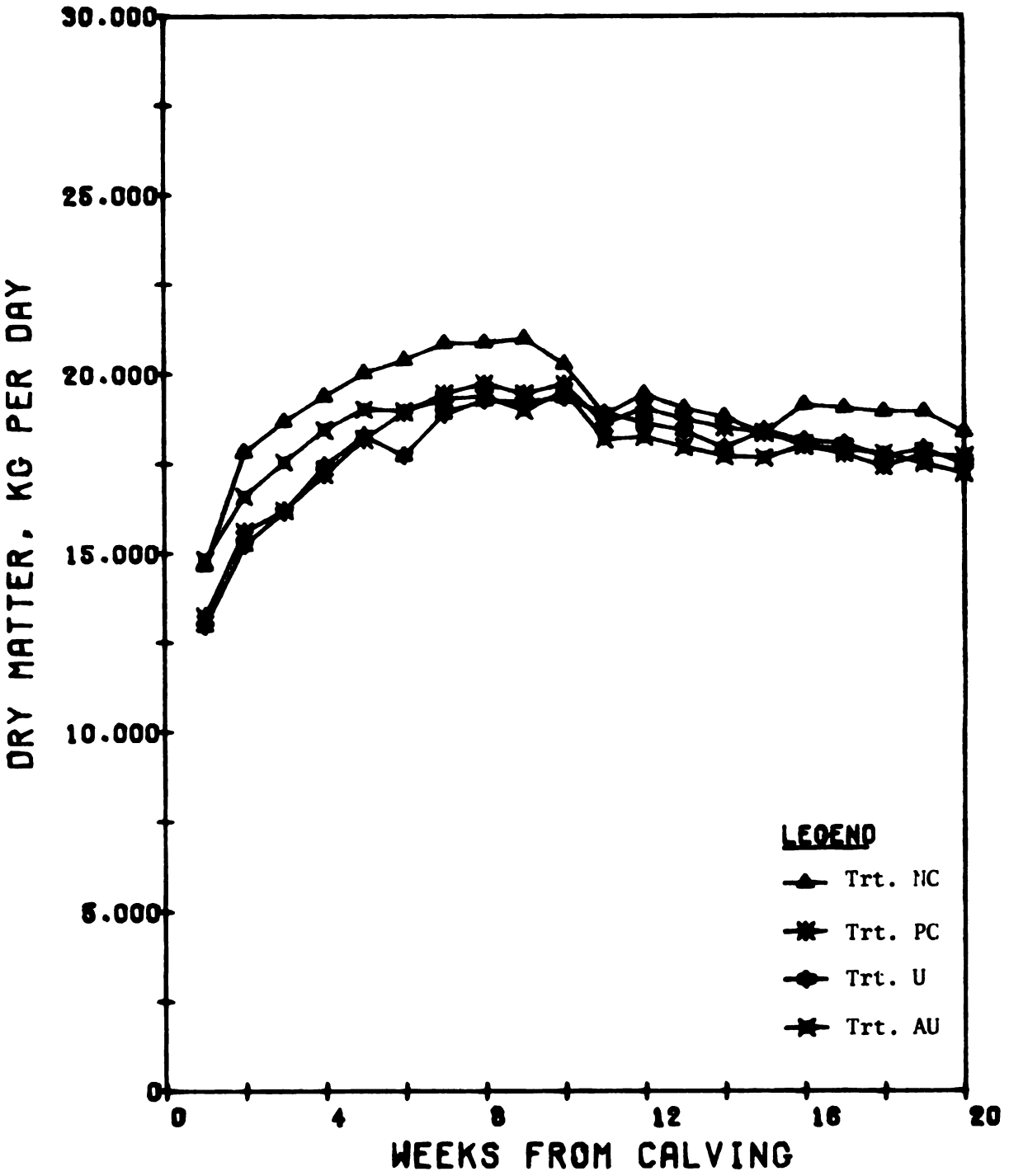

Figure 5. Daily intake of total dry matter per treatment and week from calving (kg/d; standard error ±.42).

Table 14.--Intake of concentrate, corn silage, hay, total dry matter and net energy for the different periods after calving.[b]

Period	Treatment				s.e.[a]
	NC	PC	U	AU	
Concentrate[c];kg/d:					
Weeks 3 through 6	12.49	11.00	10.32	11.42	.62
" 7 " 10	12.88	11.74	11.09	11.83	.70
" 11 " 15	9.81	9.35	9.10	9.26	.56
" 16 " 20	8.83	8.52	8.22	8.30	.54
Corn silage[c]; kg/d:					
Weeks 3 through 6	21.62	19.62	20.61	20.46	1.48
" 7 " 10	23.26	23.03	23.13	21.29	1.57
" 11 " 15	25.28	25.99	25.14	22.94	1.62
" 16 " 20	27.61	25.34	24.32	24.57	1.36
Hay[c]; kg/d:					
Weeks 3 through 6	2.21	2.18	2.19	2.29	.14
" 7 " 10	2.18	2.12	2.18	2.19	.09
" 11 " 15	2.20	2.12	2.18	2.17	.05
" 16 " 20	2.17	2.09	2.15	2.10	.06
Dry Matter[c];kg/d;					
Weeks 3 through 6	19.75	17.78	17.52	18.55	.79
" 7 " 10	20.86	19.72	19.31	19.28	.83
" 11 " 15	18.93	18.68	18.56	17.93	.77
" 16 " 20	18.93	17.74	18.00	17.70	.75
Net energy; Mcal/d:					
Weeks 3 through 6[d]	37.35	33.18	31.74	34.57	1.54
" 7 " 10	39.40	36.75	35.13	36.01	1.65
" 11 " 15	34.96	34.15	33.67	32.80	1.48
" 16 " 20	34.61	32.29	32.58	32.12	1.42

[a]Standard error.

[b]17 cows per mean.

[c]None of the differences between means within a row are significant.

[d]By orthogonal contrast: Treatment NC different than treatments PC, U, and AU. No other difference within a row was significant ($P<.05$).

1 kg per 2.5 kg milk to 1 kg per 3 kg milk. The corn silage intake increased throughout the experiment ($P<.001$) and the rate of increase was approximately constant (Figure 4 and Table 14). Hay was fed at a constant rate of 2.3 kg per day after the second week post-partum. The dry matter intake increased from calving to six weeks post-partum, and remained constant until weeks 10 to 11. After the change in the rate of concentrate feeding, the intake of DM decreased slightly, but remained approximately constant throughout the last 10 weeks of the experiment (Figure 5 and Table 14).

For concentrate intake there was a significant interaction between treatment and time ($P<.05$). However, this interaction was not significant for corn silage and it only approached significance for total dry matter ($P<.25$). The significant interaction between treatment and time for concentrate but not for total DM may be due to a difference in change of milk yields between treatments. Hence, the fixed ratio between milk and concentrate would lead to the significant interaction observed. Also cows on reduced amounts of concentrate compensate by increased intake of corn silage. The total dry matter is the sum of concentrate, corn silage and hay eaten and any tendency towards compensatory intake of feed components fed ad libitum, when restricted ingredients are reduced, would decrease differences due to time.

Dry matter intake averaged 3.05% of body weight during the third week after calving, increased to a peak of 3.5% during the seventh week, and remained at that level until week 11. When concentrate was reduced at the end of the tenth week, intakes dropped to 3.3% and then declined at approximately .03% per week to 3.0% at

20 weeks post-partum. Only the changes with time were significant ($P<.001$), but cows on treatment NC tended to eat more dry matter per unit body weight than those on the other three treatments, particularly from weeks 2 to 10 and 16 to 20.

Breeding groups.--The average intake of concentrate, corn silage, hay and total DM in breeding groups is given in Table A5, but none of the differences were significant. However, the interaction between breeding group and time was significant ($P<.001$) for concentrate, corn silage and hay, and approached significance for total DM ($P<.10$). The significant interaction for individual feed components but not for total DM may be due to compensatory intake of corn silage when the concentrate was decreased.

3. Nutrient intake

Energy.--The intake of NE_l was estimated utilizing standard values from NRC (1971). The averages per day by week and periods from calving are shown in Figure 6 and 14, respectively. The NE_l intake was not significantly affected by treatments but did change with time ($P<.001$), and the interaction between treatment and time was significant ($P<.025$). The time trend and the interactions with time may be explained by the previously described changes in the intake of individual feed components and a compensatory increase in corn silage when the amount of concentrate was reduced. Furthermore, the time by treatment effect is enhanced by crossover for treatment PC and AU six weeks after calving, and the decrease in intake for treatment U during weeks 6 and 7.

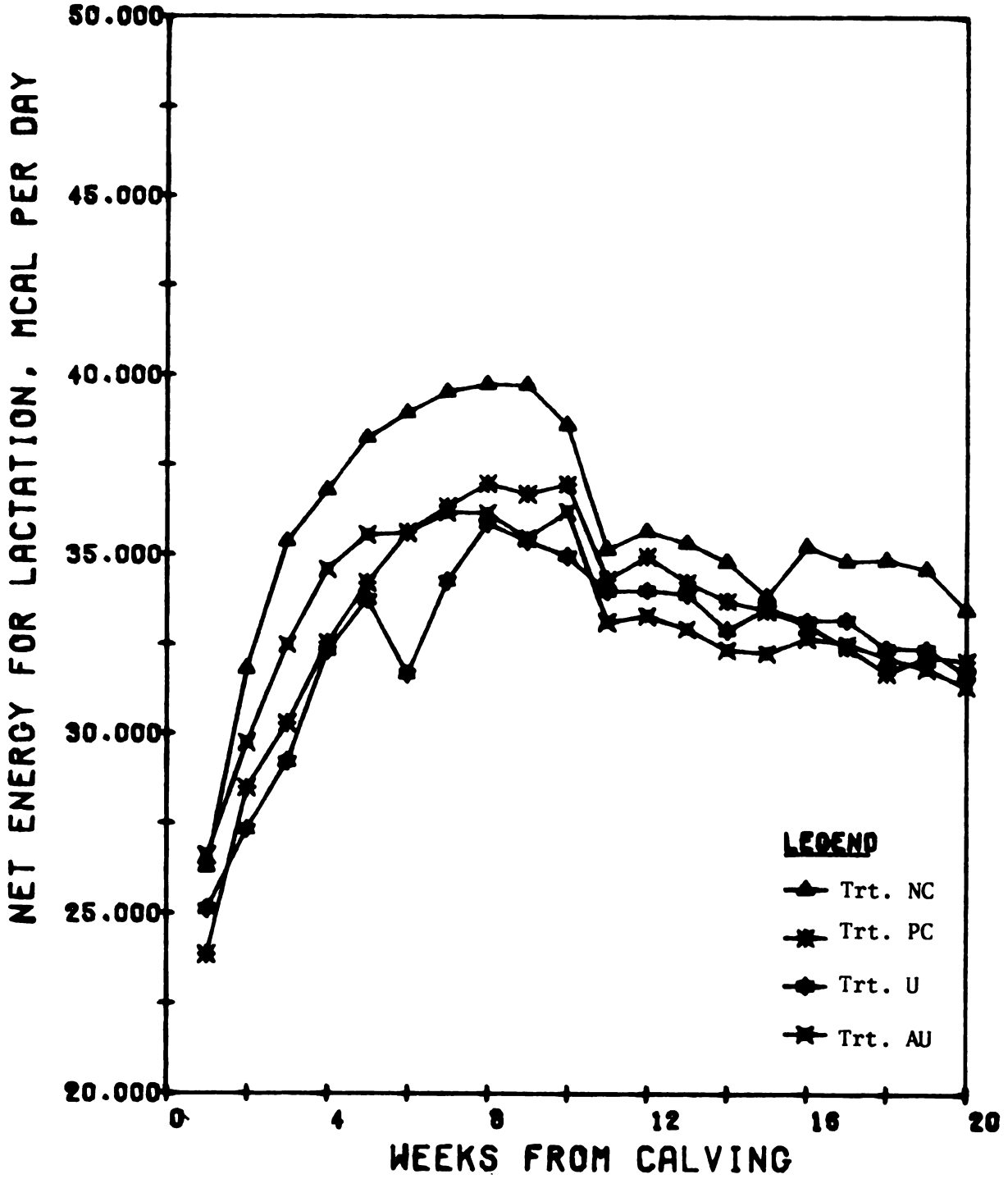

Figure 6. Daily intake of net energy for lactation per treatment and week from calving (Mcal/d; standard error ±.812).

Crude protein and nitrogen fractions.--The daily intake of total CP by week from calving and the averages for periods are given in Figure 7 and Table 15, respectively. The only difference between treatments was the lower ($P<.001$) intake for group NC than for other groups. The trend for the CP intake with time was significant ($P<.001$), and may be attributed to the trends in total DM intake and changes in intake of the different feed components. The interaction between treatment and time was also significant ($P<.001$). This is probably in part due to the initial decrease for treatment NC when the cows were changed to treatment diets. Furthermore, the crossover between treatment PC and U at 11 and 13 weeks as well as the uneven increases for treatment AU during weeks 5 through 10 attributed to the interactions.

The CP content in DM remained approximately constant at 12.5% for treatment NC, and at 15 to 17% for PC, U and AU (Figure 8 and Table 15); and was affected by treatment ($P<.001$), time ($P<.001$) and the interaction between treatment and time ($P<.001$). The CP content was higher for U than AU. The significance of the interactions between treatments and time may be attributed to none parallel changes and crossovers. For group NC the CP content in DM remained relatively constant at 12.5%. For PC the initial content was approximately 17.5% and decreased with time to 15%. The CP content remained constant between 15.5 and 16% for U, whereas it decreased from 16 to 15% for AU. The larger changes with time for treatments PC and AU than for U may be attributed to a larger difference in CP between the concentrate and corn silage for these two treatments.

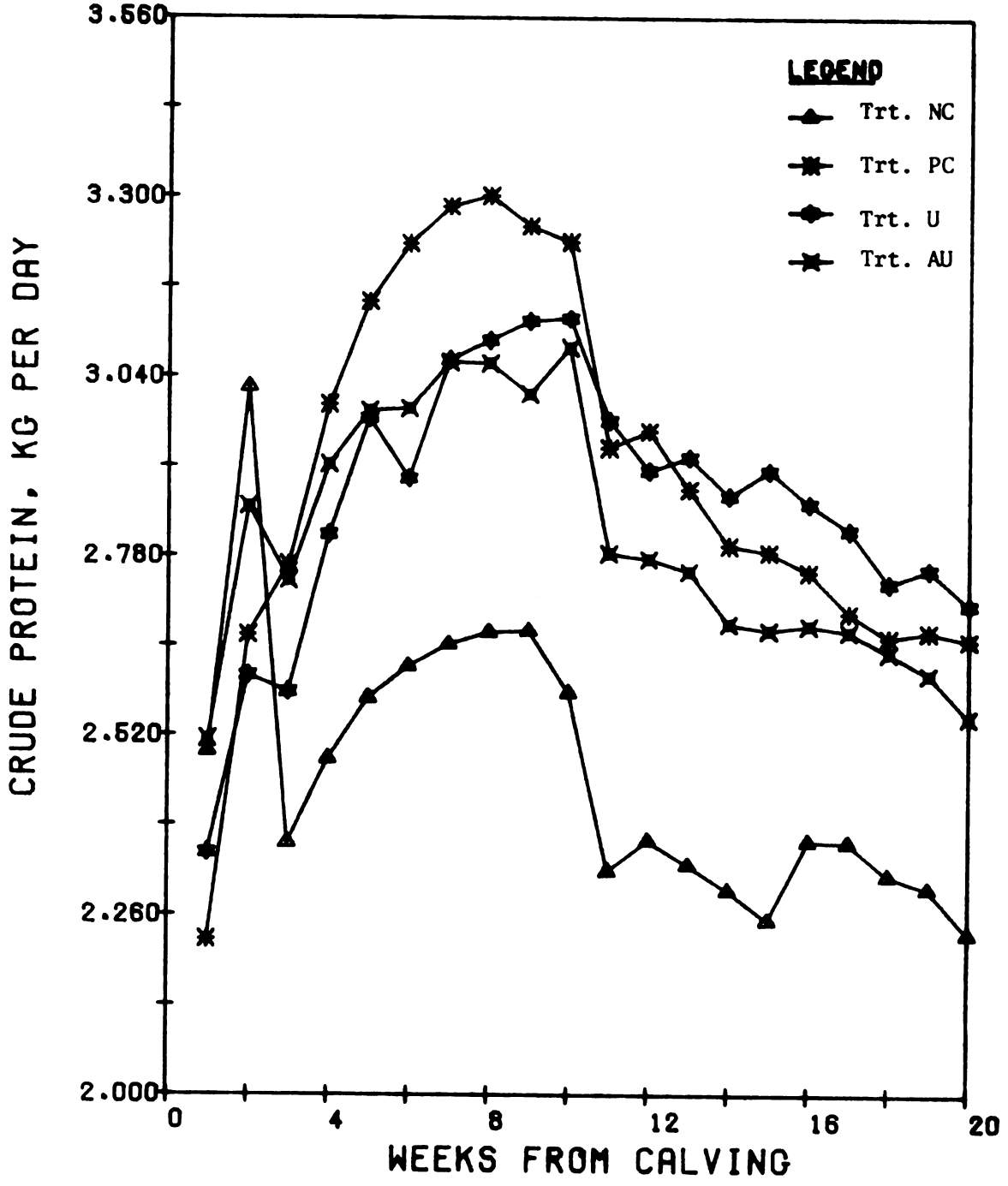

Figure 7. Daily intake of total crude protein per treatment and week from calving (kg/d; standard error ±.06).

Table 15.--Intake of crude protein, water soluble nitrogen, acid detergent nitrogen (ADN), true protein, and percent crude protein during treatment periods.

Period	Treatment					Significance[c]		
	NC	PC	U	AU	s.e.[a]	(A)	(B)	(C)
Crude protein, kg/d:								
Weeks 3 through 6	2.511	3.037	2.817	2.910	.138	**	--	--
" 7 " 10	2.644	3.270	3.100	3.054	.150	**	--	--
" 11 " 15	2.315	2.872	2.915	2.735	.122	***	--	--
" 16 " 20	2.317	2.690	2.777	2.630	.117	**	--	--
Water soluble nitrogen, kg/d:								
Weeks 3 through 6	.047	.043	.123	.092	.005	***	***	***
" 7 " 10	.053	.052	.136	.098	.006	***	***	***
" 11 " 15	.055	.055	.136	.096	.005	***	***	***
" 16 " 20	.058	.053	.134	.098	.005	***	***	***
ADN, kg/d:								
Weeks 3 through 6	.041	.038	.035	.039	.002	*	--	--
" 7 " 10	.042	.042	.039	.043	.002	--	--	--
" 11 " 15	.038	.038	.038	.039	.002	--	--	--
" 16 " 20	.037	.036	.036	.037	.002	--	--	--
True protein, kg/d:								
Weeks 3 through 6	1.961	2.531	1.830	2.091				
" 7 " 10	2.050	2.683	2.006	2.173				
" 11 " 15	1.734	2.291	1.828	1.891				
" 16 " 20	1.723	2.134	1.715	1.786				

Table 15.--Continued.

Period	Treatment				s.e.[a]	Significance[c]		
	NC	PC	U	AU		(A)	(B)	(C)
Crude protein in dry matter, %:								
Weeks 3 through 6	12.620	16.990	16.110	15.670	.230	***	**	*
" 7 " 10	12.610	16.470	16.070	15.820	.240	***	e	e
" 11 " 15	12.180	15.310	15.720	15.250	.190	***	--	*
" 16 " 20	12.190	15.130	15.440	14.850	.200	***	--	*

[a]17 cows per mean.

[b]Standard error.

[c]Significance of orthogonal contrasts: (A) NC vs. PC, U, AU; (B) PC vs. U, AU; (C) U vs. Au, e, P<.25; *, P<.05; **, P<.01; ***, P<.001.

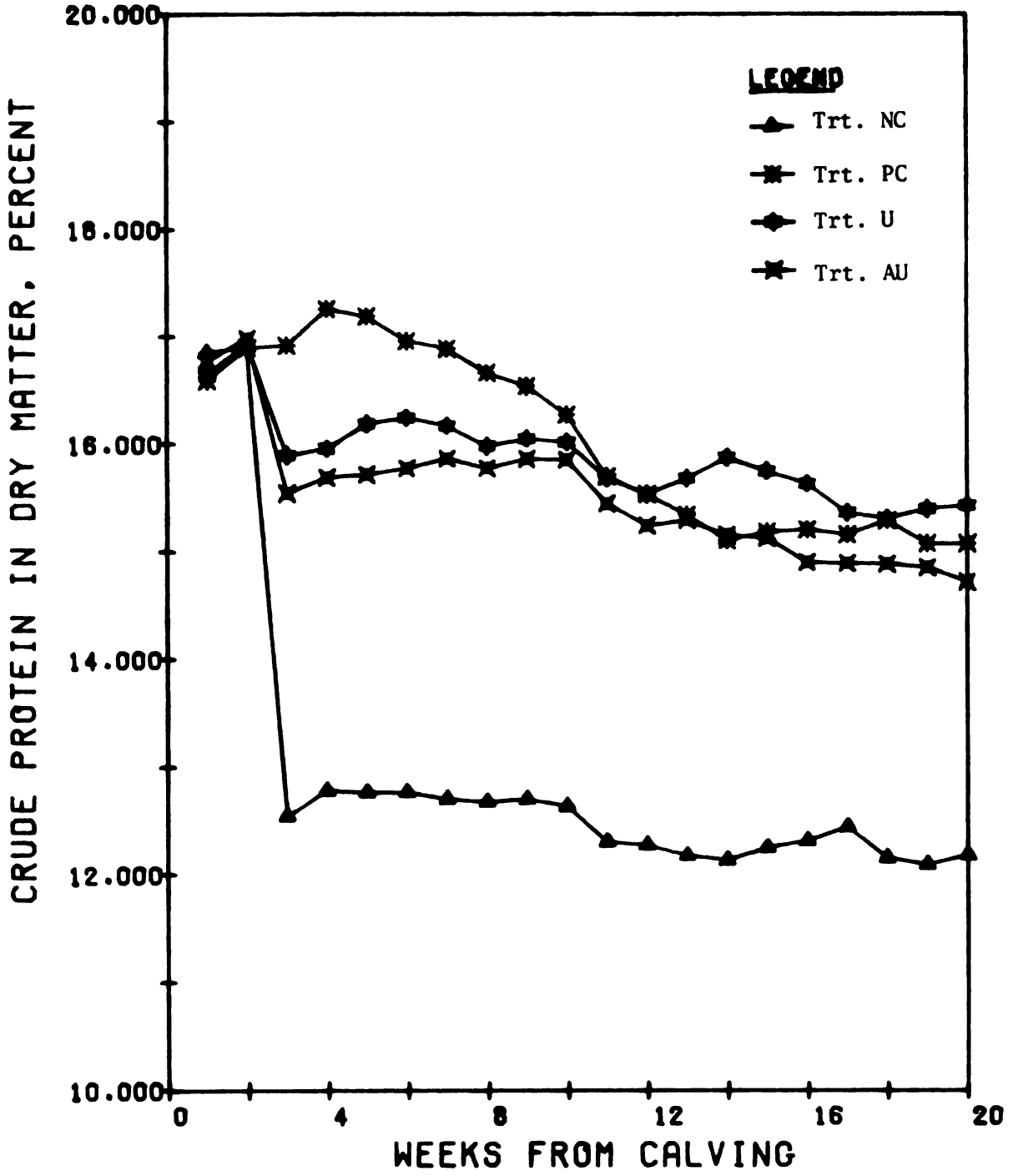

Figure 8. Crude protein in dry matter per treatment and week from calving (%, standard error ±.168).

The intake of SOLN was calculated as the sum of urea in concentrate and SOLN in the corn silage. The results are shown in Figure 9 and Table 15, and the effects of treatments and time were significant (P<.001). The intake of SOLN was the same for NC and PC, but less than for U and AU. Also, cows on AU consumed less than on U.

The intake of ADN was 35 to 45 grams per day for all four treatments (Table 15) and was only affected by time from calving (P<.001).

The intake of "true protein" was calculated as the difference between CP and SOLN plus ADN and is shown in Table 15. "True protein" in periods was equal for NC and U, and slightly less than for AU. Cows on PC consumed more "true protein" than the other groups.

The intake of ADF increased with time (P<.001), from 2.5 to 3.8 kg per day. The treatment by time interaction approached significance (P<.10), and may be explained by changes in the intake of feed components as described previously.

The intake of acetate and lactate increased (P<.001) as the intake of corn silage increased. Acetate intake ranged from 95 to 168 g per day, and lactate from 249 to 515 g. Treatments approached significance for acetate (P<.10), with NC and PC consuming less than U and AU. Treatment effects for lactic acid were not significant.

Breeding groups.--The averages for daily intake of nutrients and CP in DM are given in Table A5. Differences between breeding groups only approached significance for CP in DM. However, all variables reported showed significant interactions between breeding group and time, and may be attributed to significant interactions for individual components.

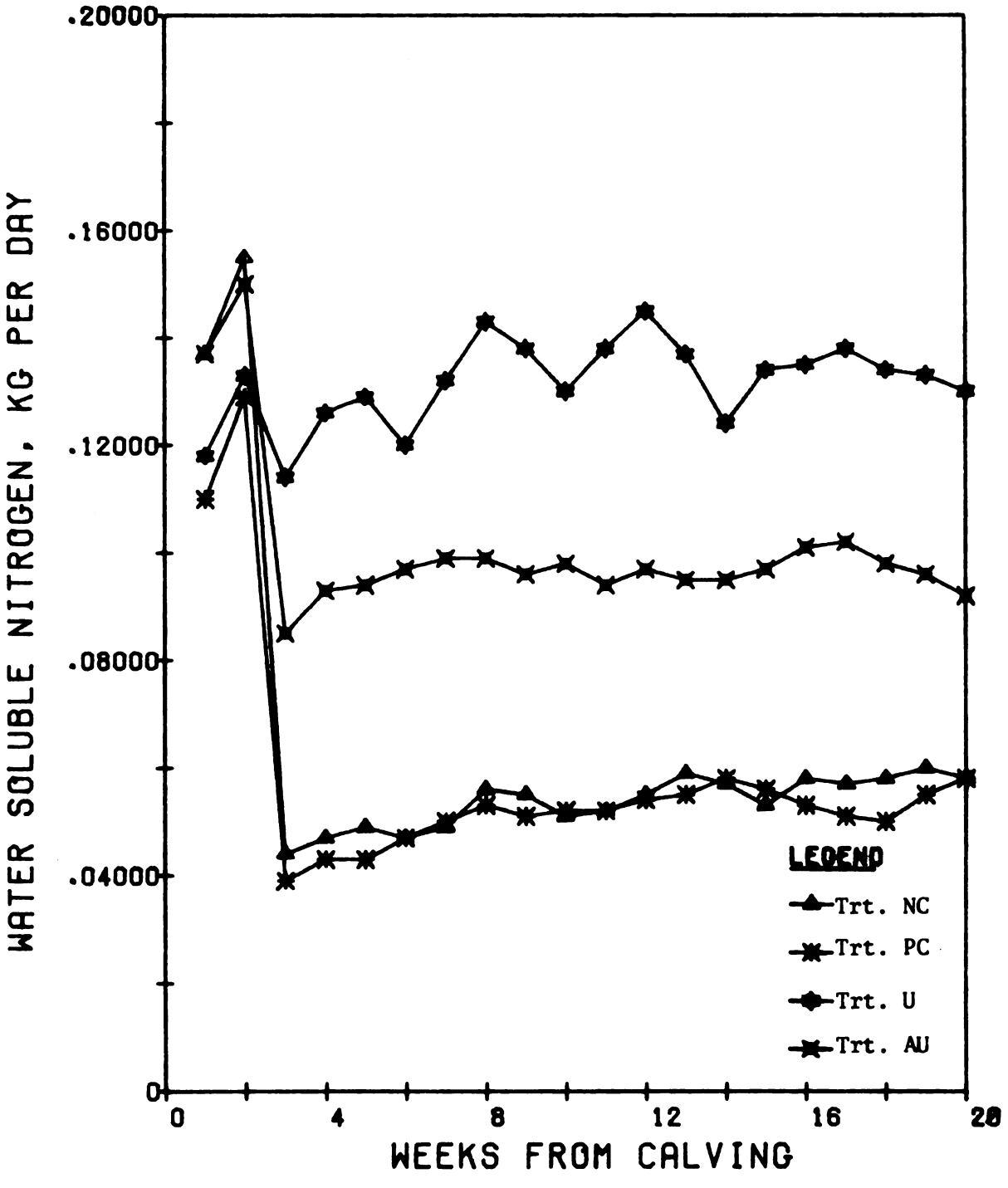

Figure 9. Daily intake of water soluble nitrogen per treatment and week from calving (kg/d; standard error ±.009).

Table 16.--Overall means and b-values for significant covariates.

Item	Overall Mean	b-value	Significance of b[a]
Milk, kg/d.	27.837	.6851	***
Protein, %	3.260	.2424	**
Fat, kg/day	.975	.1572	*
Protein, kg/day	.898	.4556	***
Total solids, kg/day	3.397	.5698	***
Solids non fat, kg/day	2.426	.4849	***
Fat corrected milk, kg/day	25.766	.3607	***
Solids corrected milk, kg/day	25.819	.4788	***
Body weight, kg.	574.598	.8477	***

[a]Significance levels: *, P<.05; **, P<.01; ***, P<.001.

4. Milk yield and composition

For the yields of milk and milk components reported in this section the analysis of variance was carried out as follows. An average was calculated for weeks 3 through 20. The averages were subjected to covariate analysis according to the model in (10), utilizing week two as the covariate. The data was adjusted for significant covariates according to (11), and analyzed statistically according to the full model in (9). The overall mean and the values for significant covariates are given in Table 16.

Milk yield.--The average unadjusted milk yield per week and per period are shown in Figure 10 and Table 17. The analysis of variance for adjusted data showed that only changes with time were

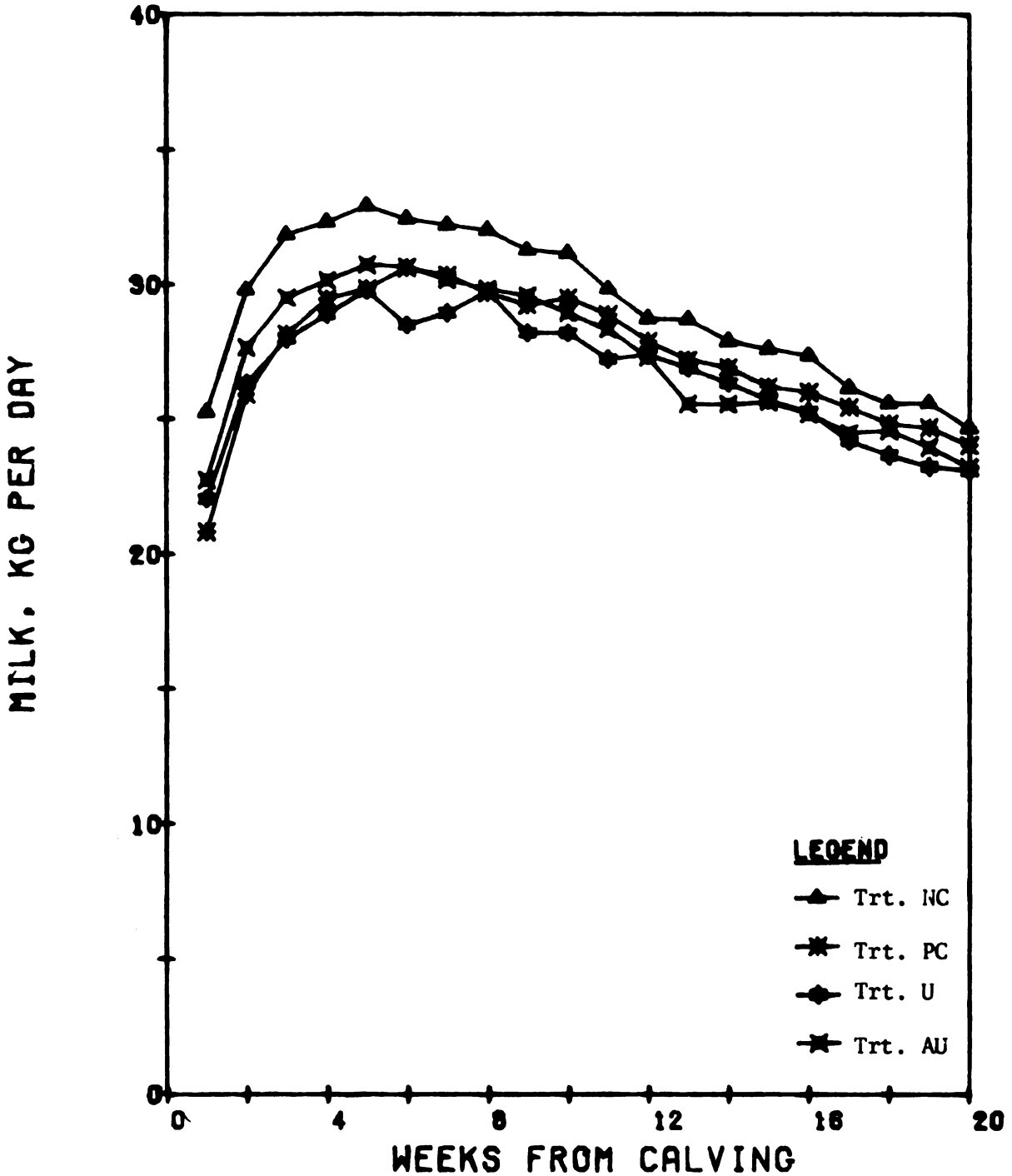

Figure 10. Daily actual milk yields per treatment and week from calving (kg/d; standard error ±1.16).

Table 17.--Actual and adjusted milk, actual components yields, and milk yield persistencies for the different periods after calving.[f]

Period	Treatment				s.e.[a]
	NC	PC	U	AU	
Actual milk, kg/d.					
Week 2	29.80	25.92	26.35	27.66	1.59
Weeks 3 through 6	32.39	29.53	28.79	30.28	1.84
" 7 " 10	31.68	29.70	28.77	29.64	1.81
" 11 " 15	28.55	27.42	26.72	26.49	1.67
" 16 " 20	28.87	25.00	23.90	24.29	1.54
Adjusted milk, kg/d.					
Weeks 3 through 6	31.05	30.85	29.81	30.40	1.84
" 7 " 10	30.33	31.02	29.80	29.76	1.81
" 11 " 15	27.21	28.76	27.94	26.60	1.67
" 16 " 20	24.53	26.31	24.92	24.41	1.54
Fat, kg/d.[b]					
Week 2	1.47	1.30	1.40	1.36	.12
Weeks 3 through 6	1.20	1.11	1.06	1.08	.09
" 7 " 10	1.08	1.06	1.00	.96	.08
" 11 " 15	.96	.95	.97	.88	.06
" 16 " 20	.90	.87	.86	.83	.05
Protein, kg/d.[b]					
Week 2	1.06	.98	.96	.98	.06
Weeks 3 through 6	.99	.92	.88	.96	.06
" 7 " 10	.99	.93	.90	.95	.05
" 11 " 15	.91	.91	.87	.87	.05
" 16 " 20	.86	.85	.82	.82	.05
Total solids, kg/d.[b]					
Week 2	4.11	3.58	3.72	3.73	.24
Weeks 3 through 6	3.97	3.60	3.56	3.65	.23
" 7 " 10	3.80	3.57	3.49	3.54	.22
" 11 " 15	3.44	3.35	3.35	3.20	.19
" 16 " 20	3.15	3.10	3.03	2.96	.18
Solids non fat, kg/d.[b]					
Week 2	2.64	2.28	2.33	2.37	.16
Weeks 3 through 6	2.78	2.50	2.50	2.58	.16
" 7 " 10	2.72	2.52	2.50	2.59	.16
" 11 " 15	2.49	2.40	2.38	2.32	.14
" 16 " 20	2.25	2.24	2.17	2.14	.14

Table 17.--Continued.

Period	Treatment				s.e.[a]
	NC	PC	U	AU	
Persistency of milk yields[c]					
Weeks 3 through 6	1.08	1.13	1.11	1.10	.03
" 7 " 10	1.06	1.14	1.12	1.09	.04
" 11 " 15	.96	1.06	1.04	.97	.04
" 16 " 20	.88	.97	.94	.89	.04

[a]Standard error.

[b]None of the differences within a row are significant.

[c]Persistency = Milk per day in treatment period/Milk in week two.

[d]Within rows P<.25 for the difference between treatments PC, U and AU (added nitrogen). Treatment PC vs. U, Au, and U vs. Au were not significant.

[e]Treatment differences within periods were not significant.

[f]17 cows per mean.

significant (P<.001). Persistencies for milk production, also given in Table 17, tended to be lower for treatment NC than for PC, U and AU, but did not differ between treatment PC and the NPN groups nor between the NPN groups. The apparent lower persistencies for NC and AU are largely due to higher milk yields during the second week after calving when cows were fed similar rations. The validity of using only week two of lactation as a standardization period is questionable and will be discussed later. Treatment differences for milk yields in week two approached significance (P<.10). The average age at calving for the four treatments was 38.5±6, 44.6±1.2, 39.1±.7, and 38.5±.7 months.

The literature review indicated that protein levels and protein sources are most critical in cows producing more than 25 kg milk per day. Therefore, cows producing more than 25 kg milk per day during the second week after calving were compared after covariate analysis. The crude protein content in dry matter for these cows was slightly higher than average values. For treatments NC, U and AU the difference was less than .3% and it decreased from period one to four. For treatment PC the higher producers received .66, .78, .52, and .32% more CP than the treatment average. The increased crude protein content may be attributed to more grain fed high yielding cows. The covariate was significant for all 4 periods (P<.05), but only in period one did differences between treatments approach significant (P<.10) (Table 18). The low protein group (NC) did not differ significantly from those of high protein (treatment PC, U, AU), but PC cows produced more than U, (P<.05) and the difference between the NPN groups was not significant. The overall adjusted milk yields for periods 2 through 4 were 37.23±.68, 30.59±.75, and 25.46±.63, respectively.

Table 18.--Adjusted yields of milk and milk components for the different periods in cows with more than 25 kg milk per day in week two.

Period	Treatment				s.e.[a]	Sig. of trt[b]
	NC	PC	U	AU		
No. of Cows	13	10	9	9		
Milk, kg/d:						
Week 3 through 6	38.88[cd]	40.44[c]	37.78[d]	39.08[cd]	.70	P<.10
" 7 " 10	36.92	39.25	35.56	37.09	1.38	NS
" 11 " 15	29.88	32.69	30.11	29.75	1.53	NS
" 16 " 20	24.65	26.94	25.00	25.44	1.27	NS
Fat, kg/d:						
Week 3 through 6	1.25[cd]	1.31[c]	1.15[d]	1.19[d]	.06	P<.10
" 7 " 10	.98	1.13	.96	.96	.08	NS
" 11 " 15	1.02	1.10	1.11	.98	.06	NS
" 16 " 20	.96	.96	.99	.90	.04	NS
Protein, kg/d:						
Week 3 through 6	1.06[cd]	1.06[cd]	.99[c]	1.09[d]	.04	P<.10
" 7 " 10	1.06	1.06	.98	1.09	.04	P<.25
" 11 " 15	.93	1.00	.92	.94	.04	NS
" 16 " 20	.82	.86	.82	.84	.04	NS
Total solids, kg/d:						
Week 3 through 6	4.29[c]	4.38[c]	4.02[d]	4.31[c]	.12	P<.25
" 7 " 10	3.84	4.06	3.63	3.96	.17	NS
" 11 " 15	3.30	3.59	3.40	3.36	.17	NS
" 16 " 20	2.85	3.14	2.99	2.99	.15	NS
Solids non fat, kg/d:						
Week 3 through 6	3.26	3.07	2.87	3.12	--	--
" 7 " 10	2.86	2.93	2.67	3.00	--	--
" 11 " 15	2.28	2.49	2.29	2.38	--	--
" 16 " 20	1.89	2.18	2.00	2.15	--	--

[a]Standard error for 10 cows per group.

[b]Significance of treatments.

[cd]Means within a row without a common superscript are different (P<.05).

Fat, protein, total solids and solids non fat production.--The covariates for the production of milk fat, protein, total solids and solids non fat were significant ($P<.001$). Only the differences in time were significant ($P<.001$) when the adjusted data were subjected to analysis of variance. The average production of the four milk components is shown in Table 17. The production of the four components was highest in period one and decreased with time.

The production of milk fat, protein and total solids in periods in high yielding cows was analyzed by covariate analysis. The covariate was significant for all variables and periods, except for milk fat in periods 3 and 4. Table 18 gives component yields according to treatment and period, but only significant differences will be discussed. Production of milk fat was greater ($P<.05$) for treatment PC than U and AU in period one. In periods one and two cows on AU produced more milk protein ($P<.05$) than those on U with NC and PC intermediate. In period one total solids production was less ($P<.05$) for cows on U than other groups.

The contents of fat, protein, total solids and solids non fat in periods are given in Table 19. Time significantly affected concentrations of all milk components ($P<.01$), but treatment differences were not significant. Interactions between treatments and time for the SNF percentage approached significance ($P<.25$) and was due to frequent crossovers among treatments (Figure 11).

Fat corrected and solids corrected milk yields.--The production of fat corrected (FCM) and solids corrected (SCM) milk was significantly correlated with the initial production (Table 16). For both estimates adjusted yields were significantly affected by time ($P<.001$).

Table 19.--The influence of protein source on milk composition in periods from calving.[bc]

Period	Treatment				s.e.[a]	Overall Mean
	NC	PC	U	AU		
Fat, %						
Weeks 3 through 6	3.62	3.66	3.67	3.66	.17	3.65
" 7 " 10	3.46	3.56	3.41	3.29	.17	3.43
" 11 " 15	3.39	3.54	3.63	3.37	.13	3.48
" 16 " 20	3.51	3.56	3.60	3.50	.13	3.54
Protein, %:						
Weeks 3 through 6	3.04	3.14	3.05	3.21	.08	3.11
" 7 " 10	3.14	3.17	3.16	3.25	.07	3.18
" 11 " 15	3.21	3.36	3.28	3.32	.08	3.29
" 16 " 20	3.33	3.42	3.45	3.44	.08	3.41
Total Solids, %:						
Weeks 3 through 6	12.20	12.12	12.40	12.19	.18	12.23
" 7 " 10	12.00	12.10	12.10	12.05	.18	12.06
" 11 " 15	12.11	12.31	12.55	12.20	.18	12.29
" 16 " 20	12.23	12.50	12.71	12.32	.18	12.44
Solids non fat, %:						
Weeks 3 through 6	8.58	8.46	8.73	8.53	.14	8.58
" 7 " 10	8.53	8.54	8.69	8.76	.13	8.63
" 11 " 15	8.71	8.77	8.92	8.83	.09	8.81
" 16 " 20	8.72	8.94	9.11	8.82	.12	8.90

[a]Standard error.

[b]Treatment differences within periods were not significant.

[c]17 cows per mean.

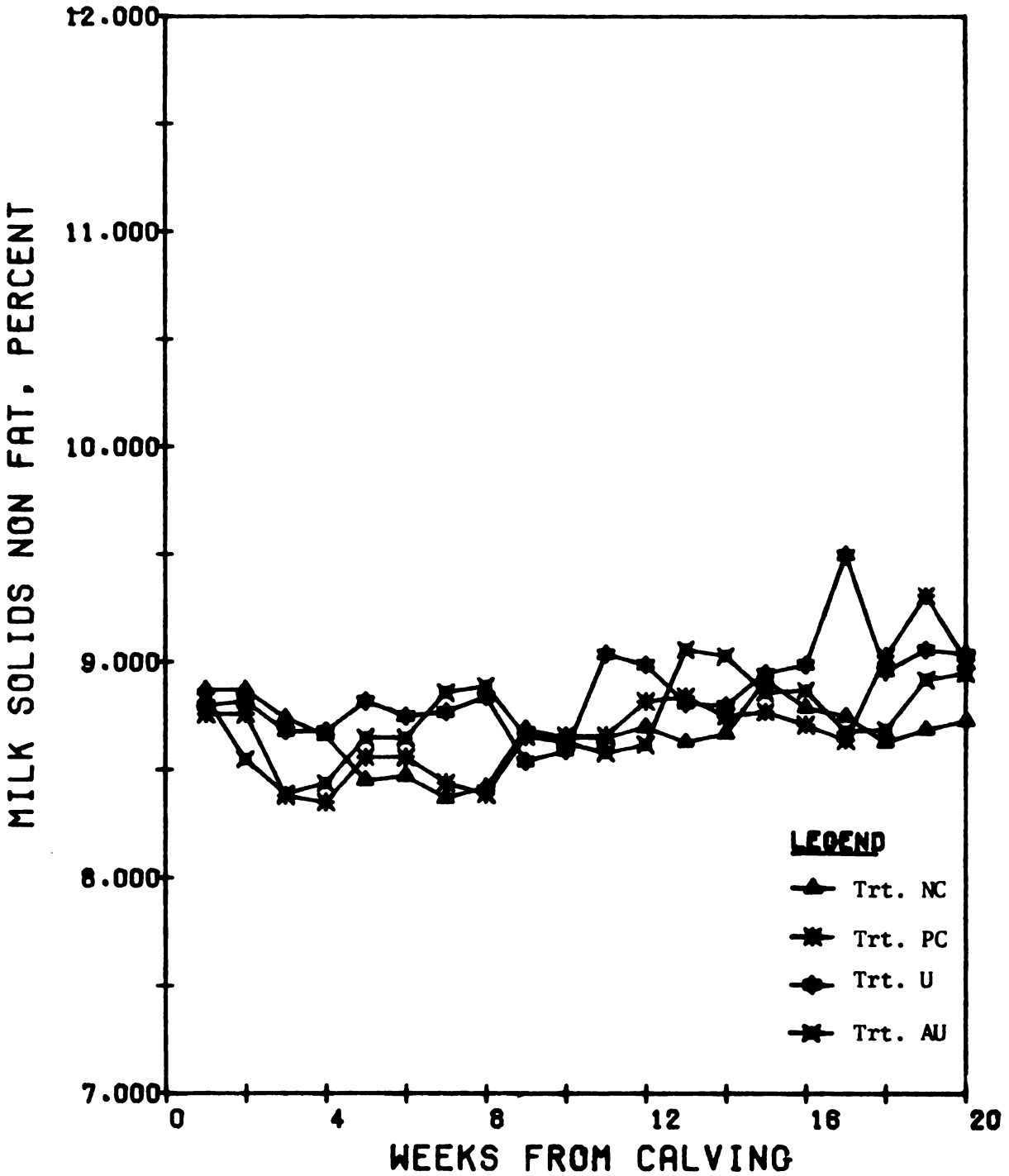

Figure 11. Content of solids non fat in milk per week from calving for treatments NC, PC, U and AU. (%; standard error ±.16.)

The overall production of FCM and SCM was 28.78 and 28.39 in period 1, 27.33 and 27.27 in period 2, 24.87 and 24.24 in period 3, and 22.86 and 23.20 kg per day in period 4.

In periods one and two the production of FCM and SCM by high yielding cows was correlated with the initial yields ($P<.01$), but for periods three and four the correlation only approached significance ($P<.10$). Adjusted treatment means in periods are given in Table 20. Treatment differences in FCM in period one were significant ($P<.05$), and approached significance ($P<.25$) in period two. Differences in SCM approached significance in period one ($P<.10$).

Breeding group.--Milk yields and milk composition for breeding groups are given in Table A5, but only the protein percent was significantly affected ($P<.01$). The interaction between breeding groups and time was significant for the SNF percentage and is shown in Table A6. The interaction between breeding group and time was significant for the persistency of milk yield ($P<.001$), protein percent ($P<.05$; Figure B1), and the SNF percent ($P<.05$; Figure B2); and approached significance for the yields of total solids ($P<.25$) and FCM ($P<.25$).

The effect of breeding groups on yields of milk and milk components by high yielding cows in periods are given in Table A5. Milk yields were not different, but differences in the yields of fat, total solids and FCM approached significance in periods one ($P<.25$), two ($P<.25$) and three ($P<.10$), and were significant in period four ($P<.05$). Differences in protein yields approached significance in period two ($P<.25$). The differences in SCM yields approached significance in periods one and two ($P<.25$) and were significant in periods three

Table 20.--Adjusted yields of fat corrected and solids corrected milk in periods from calving for cows with more than 25 kg milk per day in week two.

Period	Treatment				s.e.[a]	sig of trt.[b]
	NC	PC	U	AU		
No. of cows	13	10	9	9		
Fat corrected milk, kg/d:						
Weeks 3 through 6	32.67[c]	33.90[c]	30.42[d]	32.30[c]	1.06	P<.05
" 7 " 10	27.58	30.44	26.41	27.78	1.48	P<.25
" 11 " 15	27.66	29.24	28.72	27.32	1.50	NS
" 16 " 20	25.50	25.89	25.86	25.19	1.02	NS
Solids corrected milk, kg/d:						
Weeks 3 through 6	32.23[c]	33.05[c]	29.99[d]	32.03[c]	1.00	P<.10
" 7 " 10	27.41	29.54	25.96	28.18	1.36	NS
" 11 " 15	27.87	29.47	28.98	27.56	1.24	NS
" 16 " 20	25.48	26.70	26.50	25.61	1.10	NS

[a]Standard errors for 10 cows per treatment.

[b]Significance of treatments.

[cd]Means within a row without a common superscript are different (P<.05).

(P<.01) and four (P<.05). The interaction between breeding groups and treatments approached significance (P<.10) for the yield of milk fat in period one.

5. Body weights and average daily gains

The average body weight for all cows during the experiment was 576 kg and treatment means are shown in Figure 12. Changes with time from calving were significant (P<.001) but neither the differences between treatments nor the treatment by time interaction was significant.

Average daily gains were also subjected to analysis of variance and only the time effect was significant (P<.001). Table 21 shows the number of weeks from calving to minimum BW and daily gains before and after minimum was attained. Group U attained minimum BW in week eight whereas this occurred in week 4 for the other groups. Weight losses were least rapid for group U and most for group AU, with NC and PC intermediate. After minimum weight daily gains were highest for group U and followed in a descending order for PC, AU and NC.

Breeding groups.--Average daily gains according to breeding groups are given in Table A5, but none of the differences are significant. However, the interaction between breeding groups and treatments approached significance (P<.10; Table A7), and the breeding group by time interaction was significant (P<.001). Adjusted weekly body weights are shown in Figure B3.

6. Feed utilization and nutrient balance

Feed utilization.--Mild yields per kg of DM intake of the different treatments are shown in Figure 13. The overall feed

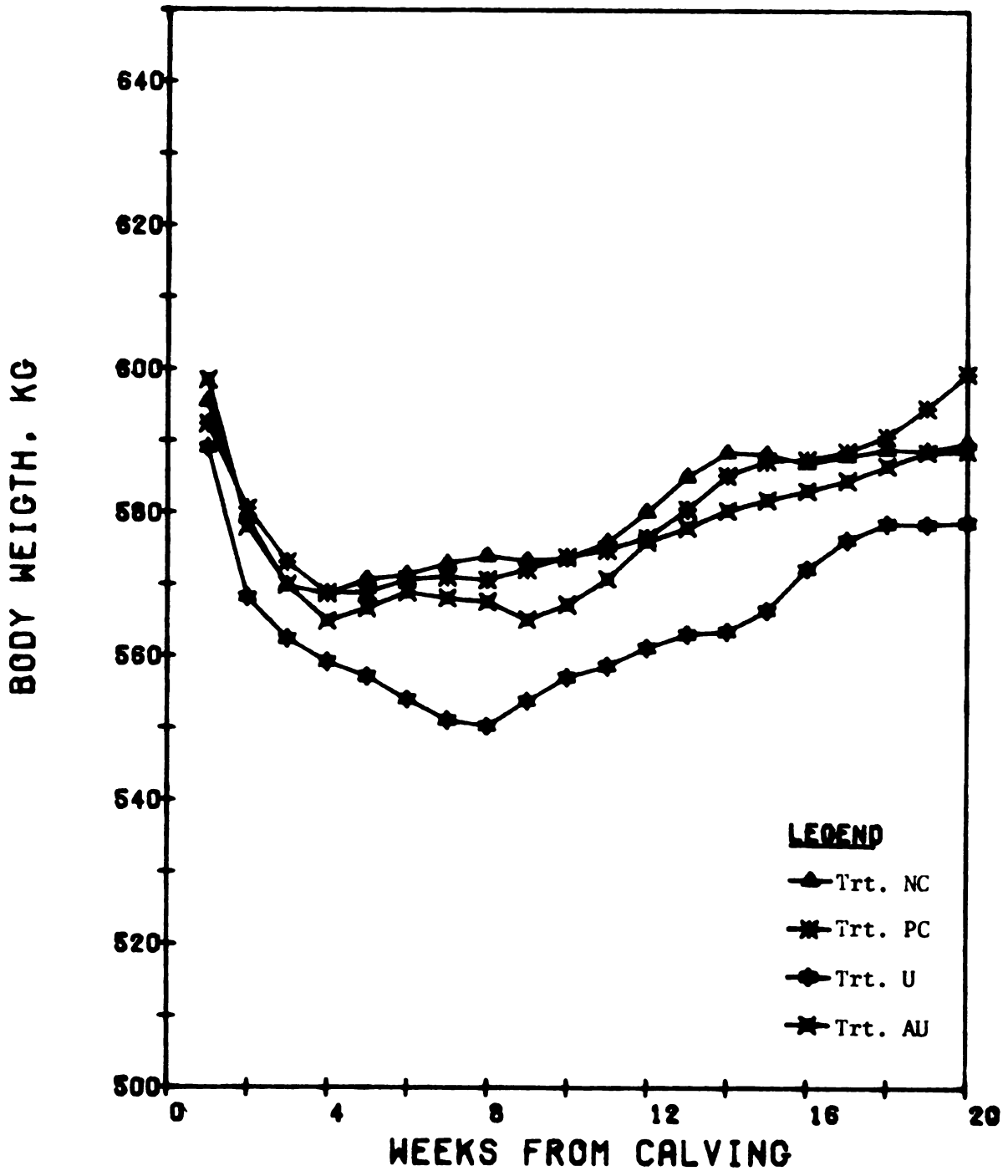

Figure 12. Average body weight for treatments per week from calving (kg; standard error ±3.6).

Table 21.--Average daily gains for treatments before and after minimum weight.[b]

	Treatment			
	NC	PC	U	AU
Minimum weight, weeks from calving:	4	4	8	4
Daily gain, kg/d:				
Before minimum BW[a]	-.954	-.843	-.692	-1.200
After minimum BW[a]	+.189	+.274	+.339	+.213

[a]Body weight.

[b]17 cows per mean.

utilization was 1.74 kg milk per kg dry matter during the second week after calving and decreased linearly at a rate of .02 per week throughout the remainder of the experiment ($P<.001$). Treatment differences and the interactions between treatments and time were not significant. The yield of FCM per kg of DM intake was also analyzed and gave results similar to those observed for milk yields.

Nutrient balance.--Intakes for NE_1 and crude protein were calculated as a percent of published requirements (NRC, 1971), and the results are shown in Figures 14 and 15. Figure 14 shows that all treatments reached 100% of NRC requirements for energy at approximately three weeks after calving. Intakes increased to about 20% in excess of requirements between weeks 3 and 7, and remained constant thereafter. Only the changes with time were significant ($P<.001$). Treatment differences in high yielding cows also were not significant.

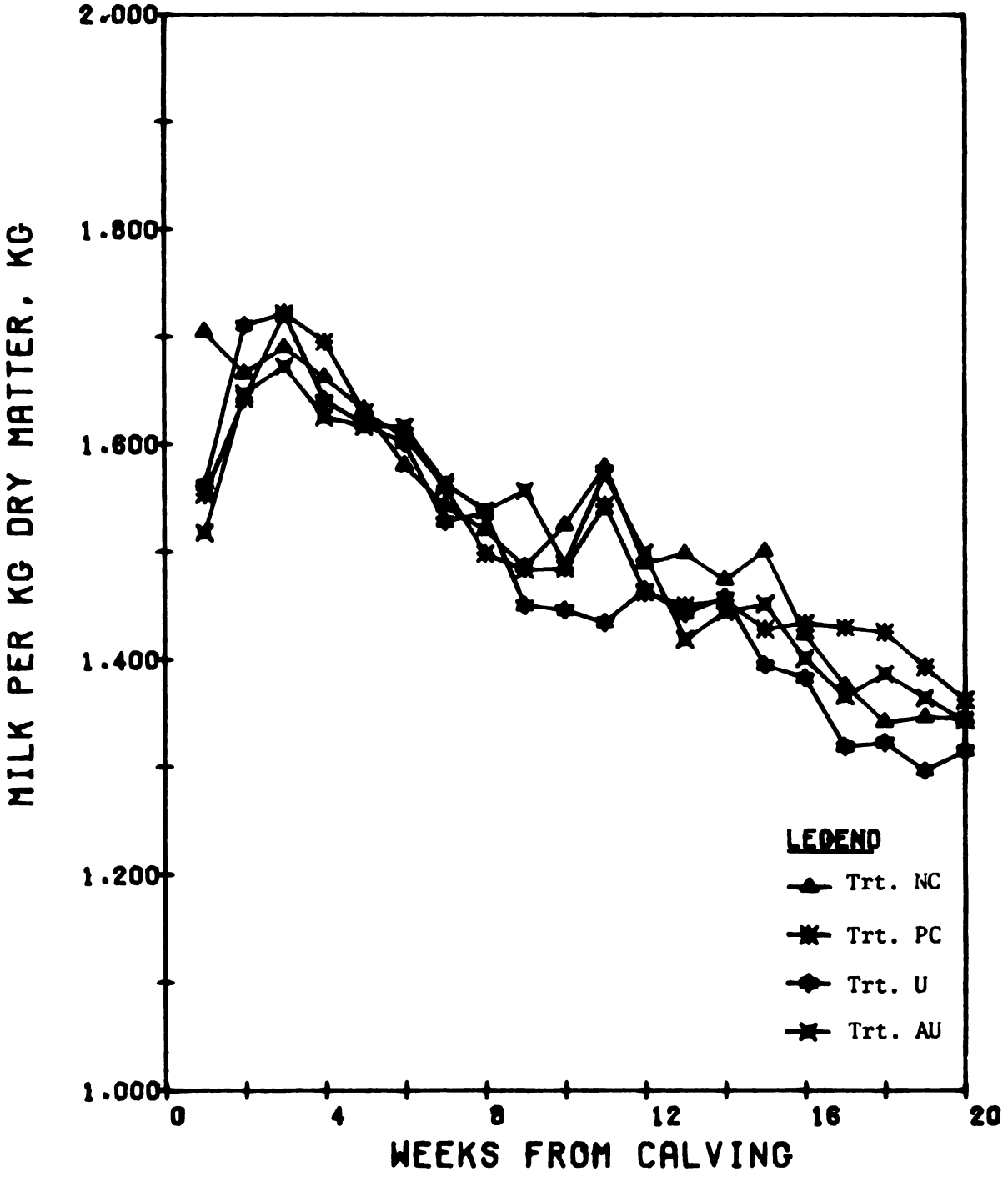

Figure 13. Milk yield per kg dry matter for treatments by week from calving (kg; standard error ±.040).

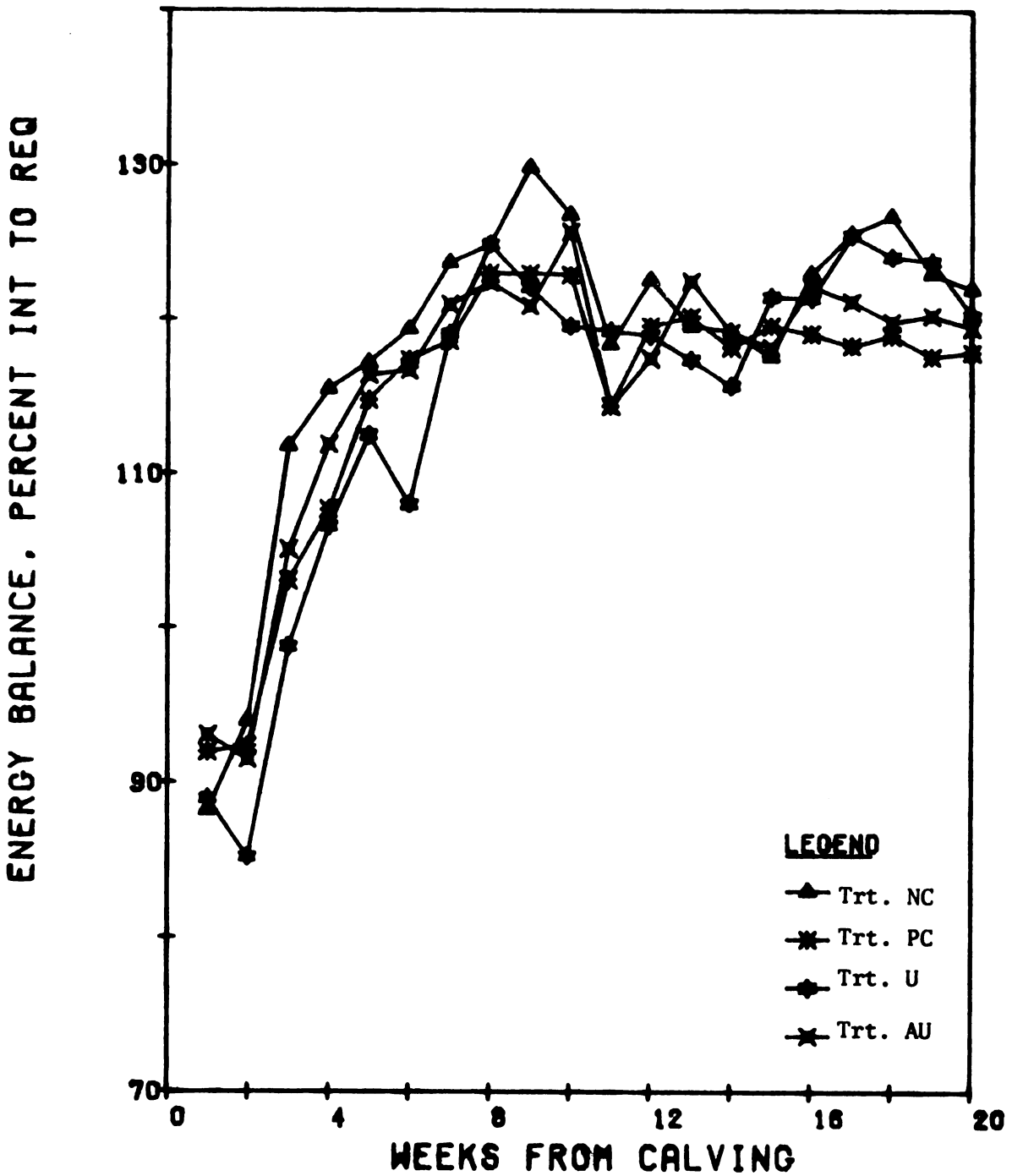

Figure 14. Balance of energy intake relative to NRC (1971) standards for treatments per week from calving (%; standard error ± 1.7).

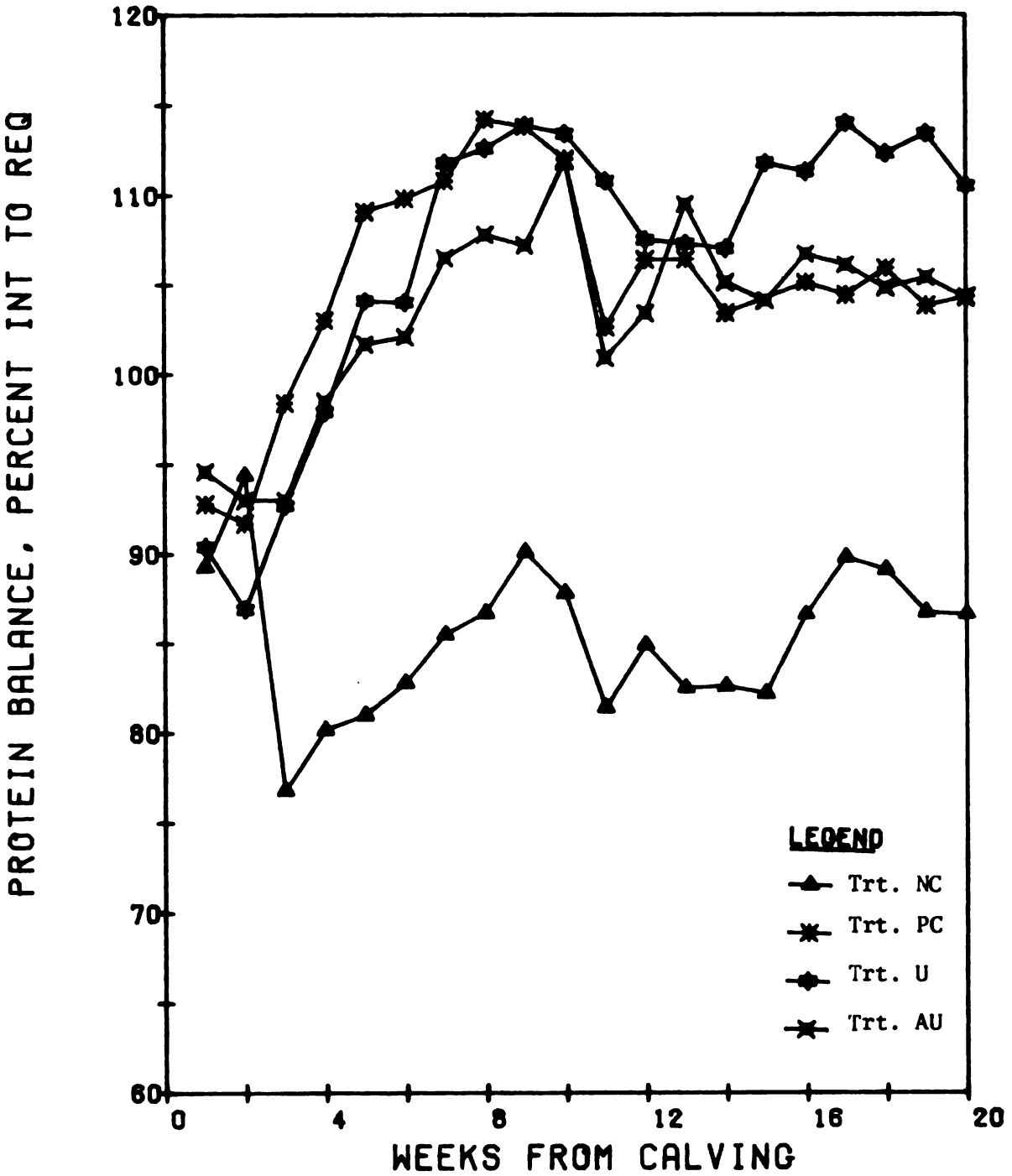

Figure 15. Balance of crude protein intake relative to NRC (1971) standards for treatments per week from calving (%; standard error ±2.3).

Data in Figure 15 shows that for treatments PC, U and AU the cows were consuming 100% of the NRC protein standard 3 to 4 weeks after calving. From that time and throughout the remainder of the trial these cows received crude protein from zero to 15% in excess of NRC requirements. For treatment NC the crude protein intake was 10 to 20% below the recommended requirements and was lower than for the other treatments ($P<.001$). The described changes with time, the differences between treatments, as well as the time by treatment interaction were significant ($P<.001$; $P<.001$ and $P<.05$, respectively). Average percents of requirements for treatments NC, PC, U and AU in weeks 3 through 10 and 11 through 20 were 83.9, 85.2; 108.9, 104.8; 106.3, 110.6; and 103.6, 105.0, respectively, with a standard error for treatments of $\pm$7.9%. Except for treatments U and AU in period one, protein intakes of high yielding cows exceeded estimated requirements for all supplemented treatments (Table 22). None of the differences between treatments PC, U and AU were significant. Treatment NC was significantly lower ($P<.001$) than supplemented treatments.

Simple correlations between production responses, body weights, and the balances for net energy and protein for high yielding cows are shown in Appendix Table A8. The correlation between milk yield and yield of milk constituents was high, but will not be further discussed. Milk yields and the yield of milk constituents were positively related to BW in period one. In period two, only the yield of milk constituents was related to the BW, and all correlations with BW were not significant after week 10. Generally milk yields and yield and content of milk constitutents were negatively correlated with the energy balance in periods one and two. In period three milk yield, and the yield of

Table 22.--Protein balance as percent of NRC (1971) requirement for high yielding cows.

	NC		PC		U		AU	
	Av.[b]	s.e.[c]	Av.	s.e.	Av.	s.e.	Av.	s.e.
Number of cows[a]	13		10		9		9	
Weeks 3 through 6	80.3[d]	2.6	102.1[e]	2.9	92.5[e]	3.1	98.0[e]	3.1
" 7 " 10	88.6[d]	3.7	114.6[e]	4.3	107.8[e]	4.5	107.5[e]	4.5
" 11 " 15	83.1[d]	2.5	108.6[e]	2.9	104.6[e]	3.0	105.6[e]	3.0
" 16 " 20	88.5[d]	2.0	106.9[e]	2.3	108.1[e]	2.4	106.5[e]	2.4

[a]Number of cows within a column.

[b]Average.

[c]Standard error.

[d,e]Means within a row without a common superscript are different ($P<.05$).

fat, total solids, SNF, FCM, and SCM were negatively related to estimated energy balances but none of these correlations were significant in period four. The yields of fat, SNF, FCM and SCM were negatively correlated with the protein balance in periods one and two, but these relationships were not significant in periods 3 and 4. The correlation between milk yield and the protein balance was negative in period 3, but the protein percentage in period 3 and the SNF percentage in period 4 were positively related to the protein balance.

Breeding groups.--Averages for milk per kg of DM, energy and CP balances are given in Table A5, but only the interaction between breeding groups and time for milk per kg of DM was significant ($P<.01$).

7. Rumen pH, volatile fatty acids, and ammonia nitrogen

Rumen pH, volatile fatty acids (VFA), and ammonia nitrogen (RAN) were measured in samples taken by stomach tube two weeks before calving and 21, 70, and 126 days after calving. The results were analyzed statistically according to the model in (9).

Rumen pH.--Pre-partum pH was higher ($P<.001$) than the post-partum values (Table 23). After calving the pH tended to decrease with time probably because of increased concentrate intake but the differences are not significant. Treatments did not significantly affect rumen pH.

Volatile fatty acid concentration (millimoles/liter).--Rumen VFA's measured included acetate, propionate, iso-butyrate, butyrate, 2-methyl-butrate, iso-valerate, valerate, and total acids. The effect

of time was significant or approached significance for all acids (Table 23). Rumen acetate was lower ($P<.005$) two weeks before and three weeks after calving than at 10 and 18 weeks post-partum. This change may be related to an increasing amount of corn silage in the diet. Pre-partum propionate concentrations were lower than the post-partum values ($P<.05$), and there was a trend toward highest levels at 70 days after calving. Propionate was lower ($P<.05$) for treatments NC and U than for PC and AU (Table 24).

Iso-butyrate was lowest 21 days post-partum ($P<.05$), and tended to be highest at 70 days and then returned to the pre-partum level at 126 days. Concentrations were higher for treatment PC than for others ($P<.05$). For butyrate, only the time was significant with lowest concentrations pre-partum. There was only a slight trend towards increasing values with time from calving. Concentration of 2-methyl-butyrate was lower prior to than after calving ($P<.05$). Iso-valerate levels were highest 70 days after calving ($P<.05$); and greater for PC than for NC, U or AU ($P<.05$). Valerate concentrations increases form pre-partum to 70 days post-partum and then decreased at 126 to a level comparable to that shown at 21 days. Group U was lower in valerate than others ($P<.05$). Rumen total acids total increased ($P<.05$) until 70 days post-partum and then remained constant.

Molar distribution of rumen volatile fatty acids.--The molar distributions of VFA were also calculated because salivary secretions may dilute the rumen sample when collected by stomach tube, thereby increasing the variation and making detection of differences more difficult.

Table 23.--Effects of time from calving on rumen pH and rumen concentrations of volatile fatty acids, m moles/liter.[a]

	Days from calving				s.e.[b]	Significance of time[c]
	-14	21	70	126		
pH	7.38[e]	7.01[f]	6.98[f]	6.96[f]	.04	P<.001
Acetate	39.40[e]	40.28[e]	45.91[f]	45.15[f]	1.90	P<.10
Propionate	10.54[e]	15.39[f]	17.86[f]	15.30[f]	.85	P<.001
Iso-butyrate	.49[e]	.45[f]	.54[e]	.50[e]	.02	P<.10
Butyrate	6.73[e]	8.09[f]	8.54[e]	8.59[e]	.42	P<.01
2-methyl-butyrate	.45[e]	.56[f]	.60[f]	.55[f]	.03	P<.025
Iso-valerate	.37[e]	.35[e]	.45[f]	.40[e]	.02	P<.05
Valerate	.67[e]	.93[f]	1.09[d]	.90[f]	.06	P<.001
Total acids	58.65[e]	66.04[de]	75.00[f]	71.39[df]	3.05	P<.005

[a]68 cows per time mean.

[b]Standard error.

[c]Determined by analysis of variance.

[d,e,f]Means within a row without a common superscript are different (P<.05).

Table 24.--Effect of protein sources on rumen pH and rumen concentrations of volatile fatty acids, m moles/liter; 68 observations per mean.

	Treatment					
	NC	PC	U	AU	s.e.	Sig of trt.[a]
pH	7.05	7.03	7.15	7.09	.06	NS
Acetate	40.32	43.71	42.84	43.87	2.32	NS
Propionate	13.77[c]	15.77[d]	12.91[c]	16.64[d]	.99	P<.01
Iso-butyrate	.46[c]	.56[d]	.51[d]	.45[c]	.03	P<.10
Butyrate	7.39	8.33	8.01	8.22	.48	NS
2-methyl-butyrate	.52	.59	.50	.54	.04	NS
Iso-valerate	.38[c]	.48[d]	.38[c]	.33[c]	.03	P<.01
Valerate	.87[c]	.99[d]	.79[c]	.95[d]	.07	P<.25
Total volatile fatty acids	63.70	70.42	65.95	71.00	3.97	NS

[a]Significance of treatment determined by analysis of variance.

[c,d]Means within a row without a common superscript are different (P<.05).

Acetate was a bigger fraction of the total acids in group U cows than in cows for the other three groups ($P<.05$), and the reverse was observed for propionate and valerate ($P<.05$; Table 25). For propionate it was found that the molar fraction was larger for AU than for NC ($P<.05$). Iso-butyrate values were higher for treatments NC, PC and U than for AU ($P<.05$). The level of 2-methyl-butyrate was higher ($P<.05$) in cows on treatments NC and PC than on treatments U and AU. Iso-valerate was higher ($P<.05$) for NC, PC and U than for AU; and higher for PC than NC and U ($P<.05$).

The effect of time on the molar fractions of individual acids was significant for all acids except 2-methyl-butyrate and iso-valerate (Table 26). Pre-partum values were highest for acetate, iso-butyrate, and iso-valerate; whereas, the reverse was observed for the other acids.

Rumen ammonia nitrogen.--Rumen ammonia nitrogen (RAN) was lower ($P<.05$) for group NC than for diets higher in CP (PC, U, AU), and was lower ($P<.05$) for PC than for the NPN treatments with little difference between U and AU (Table 27). The pre-partum value was lower for PC than the other three treatments, whereas the post-partum responses followed the overall averages. Changes with time were significant only for NC which showed a marked decrease ($P<.05$) between pre- and post-partum samplings; however, there was a trend towards increased responses for treatment AU.

Breeding groups.--Rumen pH and concentrations of VFA and RAN, as well as the molar distribution of VFA for breeding groups are given in Table A9. Differences in molar concentrations of iso-butyrate and

Table 25.--Effect of treatment on molar distribution of rumen volatile fatty acids, molar percent.[a]

Acid	Treatment				s.e.[b]	Significance of treatment[c]
	NC	PC	U	AU		
Acetate	62.87[e]	62.43[e]	65.17[f]	62.49[e]	.50	P<.001
Propionate	20.99[e]	21.85[eg]	19.55[f]	22.86[g]	.48	P<.001
Iso-butyrate	.75[e]	.81[e]	.79[e]	.66[f]	.02	P<.01
Butyrate	11.60[e]	11.90[e]	11.97[e]	11.39[e]	.23	NS
2-methyl-butyrate	.81[e]	.84[e]	.76[f]	.77[f]	.03	P<.25
Iso-valerate	.62[eg]	.71[f]	.58[g]	.47[d]	.03	P<.001
Valerate	1.37[e]	1.45[e]	1.19[f]	1.36[e]	.07	P<.10

[a]68 observations per treatment mean.

[b]Standard error.

[c]Determined by analysis of variance.

[d,e,f,g]Means within a row without a common superscript are different (P<.05).

Table 26.--Effect of time on the molar distribution of rumen volatile fatty acids, molar percent.

Acid	Days from calving				s.e.[b]	Significance of time[c]
	-14	21	70	126		
Acetate	67.39[d]	61.59[e]	61.49[e]	63.49[f]	.51	P<.001
Propionate	17.94[d]	22.79[e]	23.44[e]	21.08[f]	.54	P<.001
Iso-butyrate	.84[d]	.72[e]	.73[e]	.73[e]	.02	P<.001
Butyrate	11.27[d]	12.11[e]	11.43[d]	12.05[e]	.22	P<.025
2-methyl-butyrate	.77[d]	.85[e]	.80[d]	.77[d]	.03	P<.25
Iso-valerate	.64[d]	.55[e]	.62[d]	.57[e]	.03	P<.10
Valerate	1.17[d]	1.40[e]	1.50[e]	1.31[e]	.08	P<.025

[a]68 observations per mean.

[b]Standard error.

[c]Determined by analysis of variance.

[d,e,f]Means within a row without a common superscript are different (P<.05).

Table 27.--Effect of protein treatment and time from calving on rumen concentrations of ammonia, mg/100ml.[a]

Days from calving	Treatment			
	NC	PC	U	AU
-14	13.43[c]	10.85[d]	13.97[c]	16.36[c]
21	4.12[c]	7.14[d]	16.50[e]	13.25[e]
70	5.31[c]	12.98[d]	16.81[d]	14.69[d]
126	6.38[c]	9.35[d]	17.05[e]	13.97[e]

[a]17 cows per mean.

[b]Standard error.

[c,d,e]Means within a row without a common superscript are different (P<.05).

the interaction between breeding group and time for valerate approached significance (P<.10) (Table A10). Effects of breeding group, the breeding group by treatment interaction and the interaction between breeding group and time approached significance for molar percentages of acetate, propionate and valerate (Table A11).

8. Blood metabolites

Blood metabolite analysis reported here include glucose, urea and ammonia nitrogen, and amino acid concentrations in plasma. Plasma glucose, urea-nitrogen (PUN), and ammonia nitrogen (PAN) were determined for all cows two weeks before expected calving, and again 21, 42, 70 and 126 days after calving. In 8 cows selected for high milk yield blood was collected at 1430, 1530, 1900, 2300, 0330, 0430, 0800 and 1200 hours at approximately 59 and 112 days post-partum and samples

were assayed for PUN and PAN. Plasma amino acids are reported for a preliminary study conducted in 1971 as well as for all cows in this study at 42 days post-partum. For days 14 pre-partum and 126 post-partum plasma amino acids were assayed in samples from cows in the first seven blocks on experiment.

Plasma glucose.--Plasma glucose was highest before calving ($P<.05$), lowest 21 days after calving ($P<.05$), and remained constant for the last three samplings (Table 28). Treatment differences were not significant. The treatment by time interaction approached significance ($P<.25$) and may be attributed to crossovers between treatments.

Plasma urea- and ammonia nitrogen.--Values for PUN were lower ($P<.05$) for NC than all other treatments and PC was lower ($P<.05$) than U and AU (Table 28). Concentrations were lowest 21 days after calving ($P<.05$). The treatment by time interaction ($P<.001$) may in part be attributed to the decrease in treatment NC and slight increases of supplemented diets between pre- and post-partum samplings.

Plasma ammonia nitrogen was significantly affected by treatments and by time. The PAN concentrations were lower for groups NC, PC, and AU than for group U ($P<.05$). The PAN concentration was highest at the pre-partum sampling and decreased gradually as the cows progressed into lactation.

The hours of sampling for diurnal variation in PUN and PAN were one-half hour before and after milking at 1500 and 0400 hours and every 4 hours thereafter. On the two days of sampling the averages of milk, fat, protein, total solids and solids-not-fat were 33.6 ± 1.4, 29.2 ± 1, kg; $3.62\pm.16$, $3.55\pm.29$%; $3.32\pm.08$, $3.03\pm.08$%, $12.45\pm.14$,

Table 28.--Effects of protein treatment and time from calving on plasma concentrations of glucose, urea and ammonia nitrogen, mg per 100 ml.[a,b]

Days from calving	Treatment			
	NC	PC	U	AU
Glucose				
-14	57.45	58.30	61.69	63.47
21	45.71	49.54	45.14	50.83
42	53.42	56.68	50.00	54.68
70	53.44	56.72	57.11	50.21
126	52.93	51.64	53.61	49.86
Urea nitrogen:				
-14	13.58[c]	13.51[c]	14.05[c]	14.48[c]
21	6.06[c]	11.42[d]	14.73[e]	13.61[de]
42	5.67[c]	14.98[d]	17.10[e]	13.54[d]
70	8.04[c]	14.27[d]	16.16[d]	16.09[d]
126	7.17[c]	13.42[e]	17.54[e]	14.59[d]
Ammonia nitrogen:				
-14	.37	.40	.43	.34
21	.27	.35	.38	.33
42	.33	.30	.41	.33
70	.27	.30	.35	.29
126	.27	.27	.31	.26

[a]Standard errors: glucose = ±3.18, urea-N = ±.98, ammonia - N ±.03.

[b]17 cows per mean.

[c,d,e]Means within a row without a common superscript are different ($P<.05$).

12.43±.27% and 8.83, 8.88%, respectively. The analysis of variance was according to the model in (13). Plasma urea nitrogen was affected by all factors included in the model, except the interaction between treatment and days from calving. The three way interaction for treatments, days from calving and hour of sampling only approached significance and will not be discussed. Plasma ammonia nitrogen was significantly affected by days from calving and the interaction between days from calving and hours of sampling. The effect of hours of sampling and the three-way interaction for treatment, days from calving and hours of sampling approached significance.

The overall means for treatments and days from calving are given in Table 29. The PUN was lower ($P<.05$) for NC than for the other three treatments, and was lower ($P<.05$) at 112 than 59 days after calving. The PAN showed no particular trend for treatments but the averages were lower ($P<.05$) at 59 than 112 days after calving.

The overall change in PUN with hours of sampling was non-significant except for higher concentrations at 1200 than 0800 hours with a slight increase until 1900 hours and relatively constant concentrations, thereafter. The only significant differences were between the samplings at 0800 hours and those at 1900 and 0330 hours. The changes in PUN concentrations with time within treatments are given in Figure 16. Figure 16 shows that the PUN concentrations remained approximately constant and parallel at levels characteristic for each treatment except for a temporary increase for U at 1900 and 2300 hours. At those hours PUN was higher for U than for PC and AU. PUN concentrations tended to increase after milking.

Table 29.--Average content of urea and ammonia nitrogen in plasma from selected cows for diurnal sampling, mg/100 ml.

Item in plasma	Treatment					Days from calving		
	NC	PC	U	AU	s.e.[a]	59.	112.	s.e.[a]
Urea nitrogen	8.47[b]	15.65[c]	18.17	14.83[c]	1.65	15.64[d]	12.92[e]	.59
Ammonia nitrogen	.272[b]	.264[b]	.289[b]	.259[b]	.012	.254[d]	.288[e]	.006

[a]Standard error.

[b,c]Means within a row for treatment without a common superscript are different (P<.05).

[d,e]Means within a row for days from calving without a common superscript are different (P<.05).

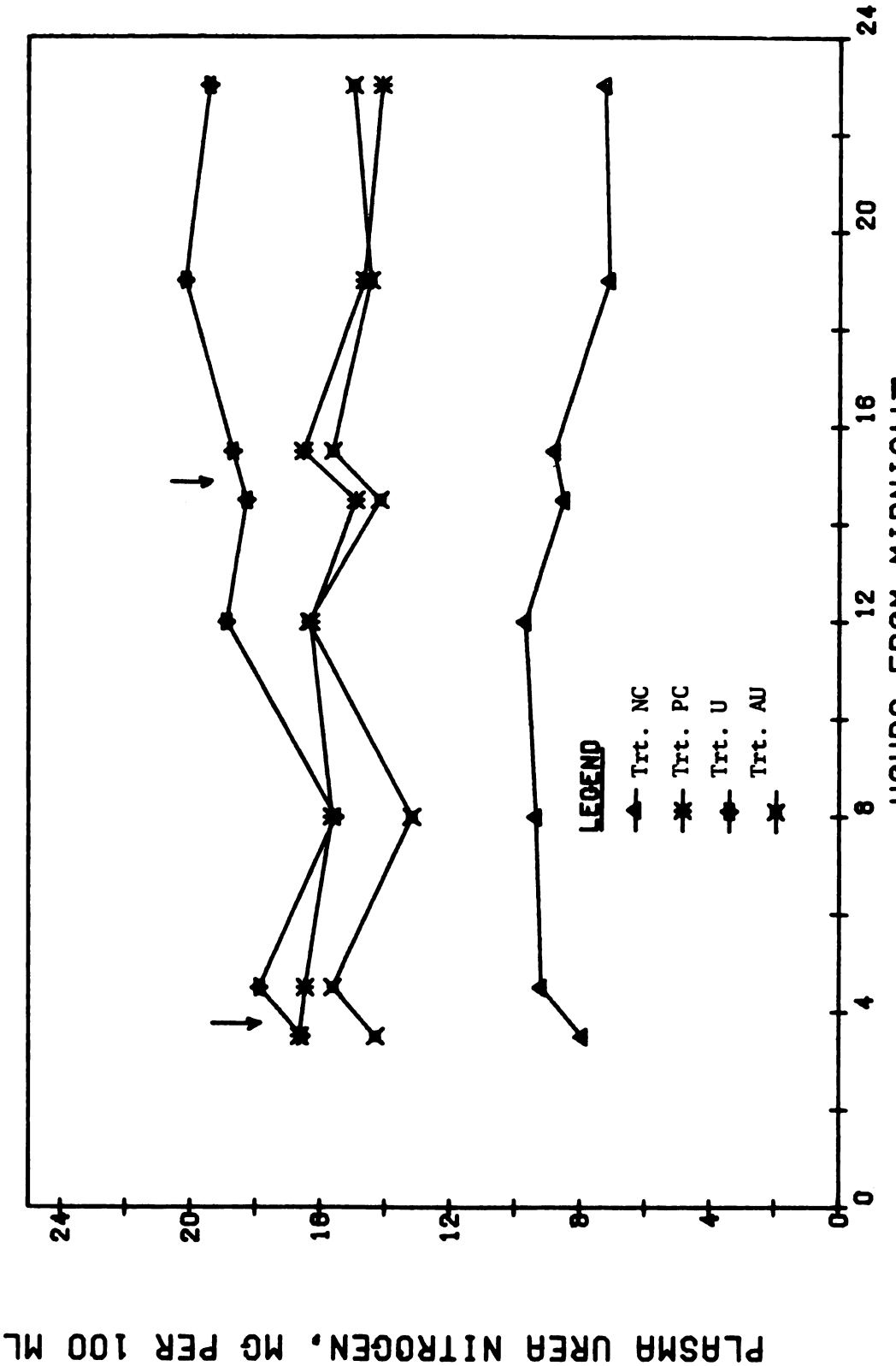

Figure 16. Effect of protein treatments on diurnal changes in plasma urea nitrogen (average for 2 cows per treatment 59 and 112 days post-partum; mg/100 ml; ↓ milking).

The interaction between days from calving and hours of sampling for PUN and PAN are illustrated in Figures 17 and 18, respectively. None of the differences between PUN concentration within a given day of sampling were significant. However, the PUN concentrations were lower ($P<.05$) at 112 than at 59 days after calving for samples taken between 1200 and 0330 hours. The PAN concentrations remained relatively constant throughout the 24-hour sampling period at 59 days after calving, and none of the differences were significant. By 112 days after calving PAN concentrations at 0330, 0430, and 1200 hours were higher than at 59 days ($P<.05$) and the concentrations at consecutive hours of sampling within 112 days after calving were different between 1900 and 1200 hours ($P<.05$). The described changes in PAN concentrations indicate a diurnal rhythm at 112 days after calving, but it is doubtful whether any rhythm was present for PUN concentrations.

Breeding groups.--Average concentrations of glucose, urea and ammonia nitrogen in plasma for breeding groups are given in Table A11, but none of the differences were significant.

8.1 Plasma amino acids

Plasma amino acid analysis to be reported include an initial experiment and the main experiment.

8.1.1 Initial experiment on factors affecting plasma amino acid concentrations

Plasma amino acid profiles were determined in 30 cows divided into two equal groups according to milk yield. The average milk yield in the two groups was 31.2 (high) and 21.0 (low) kg per day.

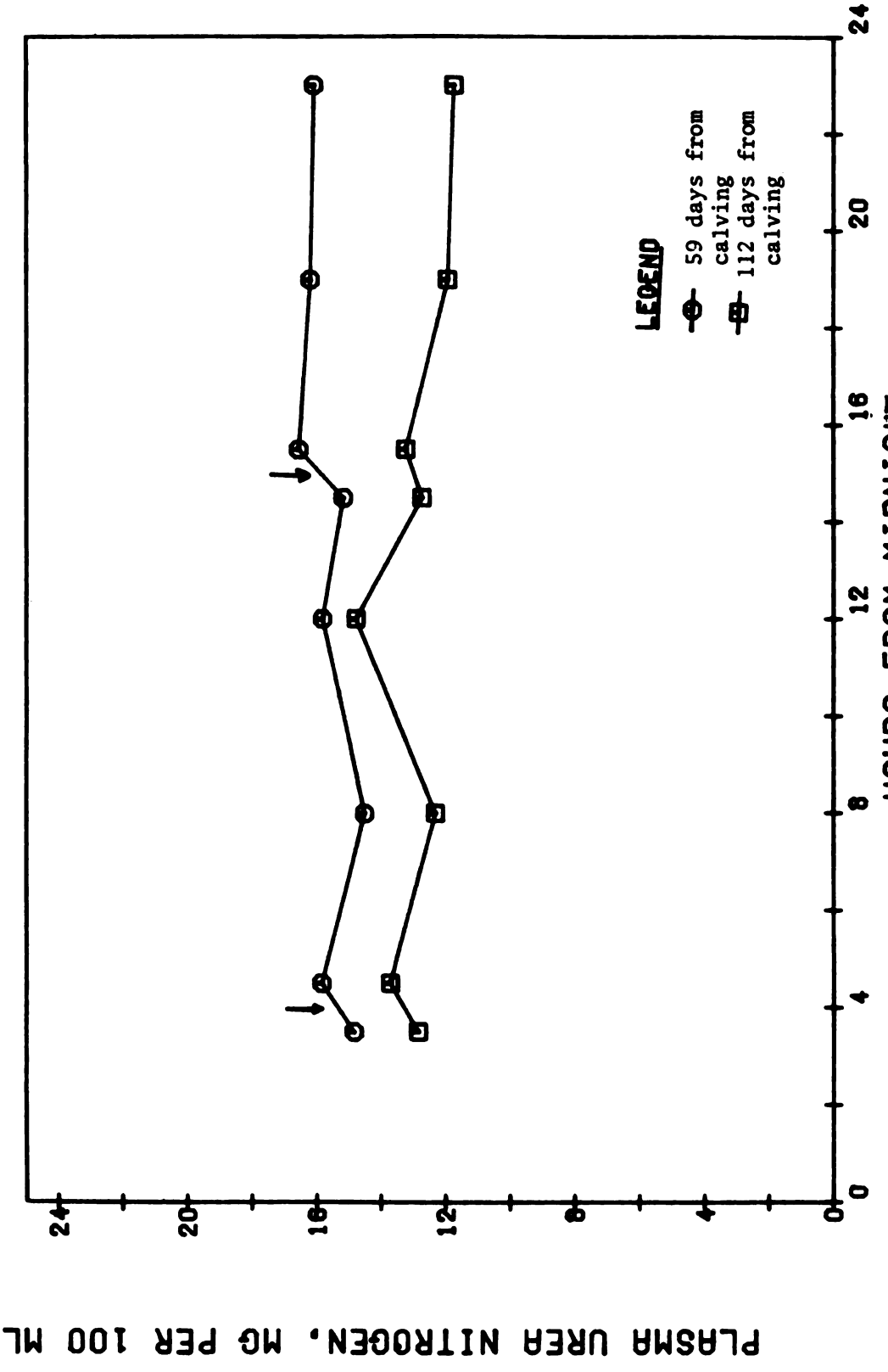

Figure 17. Effect of days from calving on diurnal plasma urea nitrogen (8 cows/mean; mg/100ml; ↓milking).

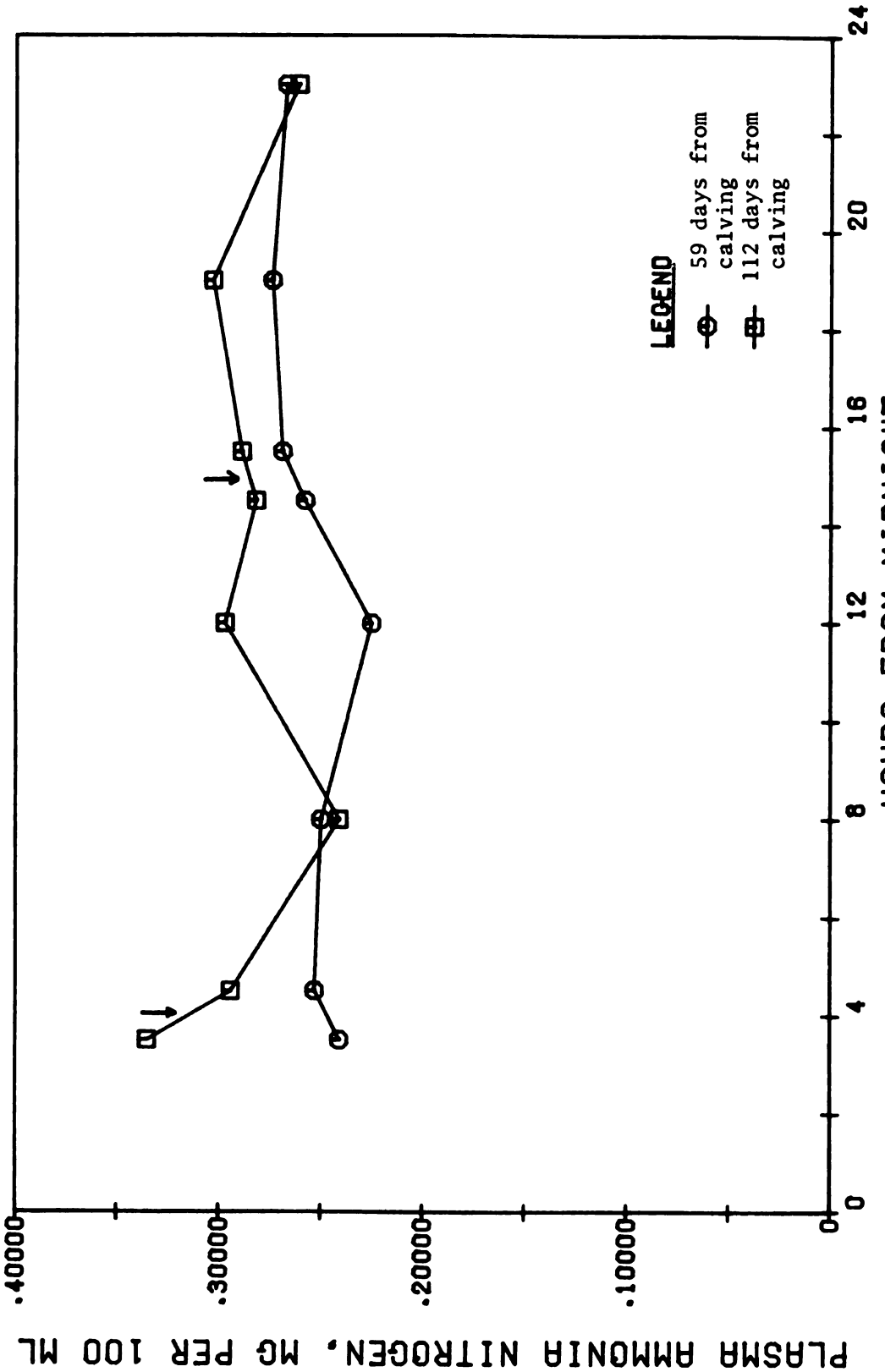

Figure 13. Effect of days from calving on diurnal plasma ammonia nitrogen (8 cows/mean; mg/100 ml; ↓ milking).

Within these two groups an equal number of cows were assigned to each of five treatments (Table 30). Two rations with all natural protein containing untreated corn silage were fed. One used a concentrate with 8% CP (group NC) and the other with 18% CP (group PC). Three diets contained NPN and consisted of corn silage treated with .5% urea (group U), 2% (group P), and 4% (group 2P) Prosil[9] at ensiling. The concentrate fed groups U and P contained 13% CP and that fed group 2P had 8% CP. All cows were bled at zero, 2 and 5 hours post-feeding after 4 and 9 weeks on trial. For the respective bleeding times plasma was pooled for the three cows within each production group within each treatment and then prepared as previously described. The experimental procedure is summarized in Table 31. Production data were reported by Huber et al. (1973). Responses for individual amino acids presented in Table 32 were statistically analyzed according to the model in (12). Proline was not affected by any of the factors studied.

Effects of treatments.--Plasma concentrations of VAL, LEU, GLU ($P<.05$), LYS, ILE, HIS, CYS, SER, and ASP ($P<.25$) were affected by treatments. However, interactions between treatments and other variables were observed for several amino acids. The interaction between treatment and week of sampling affected ARG ($P<.01$), GLY, ALA ($P<.05$) and SER ($P<.25$). The treatment by hour of sampling interaction approached significance for VAL, ARG, ASN and ASP ($P<.25$). Finally the three-way interaction between treatments, week of sampling and and hour of sampling approached significance for ARG, HIS ($P<.25$) and

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[9]A mixture containing 13.6% N (as NH_3), 55.1% molasses, 5.68% NaCl, plus Ca, P, S, Mg, Zn, Cu, Co, 1. Trade name, Prosil.

Table 30.--Protein in diets fed cows in initial experiment on factors affecting plasma amino acids.

Crude protein, % in DM	Treatment				
	NC	2P	U	P	PC
Concentrate	8.4	8.6	13.8	14.0	19.1
Corn silage	9.1	16.5	13.6	12.9	9.1
Total ration	9.5	13.2	13.9	13.7	14.1
NPN added to silage %	-	4.00[b]	.50[a]	2.00[b]	-

[a]Urea.

[b]A mixture containing 13.6% N (as NH_3) 55.1% molasses, 5.68% NaCl, plus Ca, P, S, Mg, Zn, Cu, Co, 1. Trade name, Prosil.

Table 31.--Experimental procedure for the initial study of factors affecting plasma amino acids.

Cows used:	30 Lactating dairy cows
Outcome groups:	High producers averaged 31.2 kg/day Low producers averaged 21.0 kg/day
Silage treatments:	1) Negative Control (8% C.P. Conc) 2) Positive Control (18% C.P. Conc) 3) Urea (13% C.P. Conc) 4) Pro-Sil (13% C.P. Conc) 5) Pro-sil @ 2X (13% C.P. Conc)
Bleeding schedule:	0, 2, and 5 hrs post-feeding after 4 and 9 weeks on trial

Table 32. Initial experiment on effects of treatments, level of milk production, week and hour of sampling on plasma amino acid concentrations in dairy cows, μM/100 ml.

	Treatment					Level of Production		Week of Experiment		Hour of Sampling		
	NC	2P	U	P	PC	Av	s.e.	4	9	0	2	5
Valine												
H[f]	14.65	14.50	16.56	18.17	19.11	16.60	±.48	14.46[b]	18.73[a]	16.51	15.75	17.53
L[f]	14.66	18.01	18.65	18.05	20.94	18.06	±.48	17.95[a]	18.17[a]	18.48	16.90	18.81
Av.	14.65	16.26	17.60	18.11	20.03	-	-	16.21[a]	18.45[b]	17.50[a]	16.33[b]	18.17[a]
s.e.	----------		±.77	----------		-	-	±.54	±.54	------	±.54	-----
Leucine												
H	10.56[a]	11.07[a]	10.67[a]	12.69[a]	12.42[a]	11.48[a]	±.18	10.41[b]	12.55[a]	11.47	10.50	12.48
L	10.81[a]	12.42[a]	12.32[a]	13.33[a]	13.14[a]	12.48[e]	±.18	12.59[a]	12.20[a]	12.80	11.33	13.09
Av.	10.68[a]	11.74[b]	11.50[b]	13.01[e]	12.78[bc]	-	-	11.50	12.39	12.13[a]	10.91[b]	12.79[a]
s.e.	----------		±.29	----------		-	-	±.30	-----	-----	±.38	-----
Threonine												
H	8.17	8.00	8.17	9.31	8.35	8.40	±.25	8.19[a]	8.61[a]	8.75	7.94	8.51
L	7.49	8.04	9.01	8.09	7.65	8.06	±.25	7.92[a]	8.19[a]	8.28	7.51	8.38
Av.	7.83	8.02	8.59	8.70	8.01	-	-	8.05	8.40	8.52[a]	7.72[a]	8.44[a]
s.e.	----------		±.40	----------		-	-	±.21	----	----	±.33	----

Table 32.--Continued.

	Treatment					Level of Production		Week of Experiment		Hour of Sampling		
	NC	2P	U	P	PC	Av	s.e.	4	9	0	2	8
Lysine												
H	6.02[a]	6.56[a]	6.10[a]	6.07[a]	7.09[a]	6.37[d]	±.15	5.84[a]	6.90[a]	6.79	6.17	6.14
L	6.95[a]	7.67[a]	6.89[a]	6.58[a]	6.95[a]	7.01[e]	±.15	6.80[a]	7.21[a]	6.55	7.14	7.33
Av.	6.49[a]	7.12[b]	6.49[a]	6.32[a]	7.02[b]	-	-	6.32	7.05	6.67	6.66	6.73
s.e.	----------		±.24	----------		-	-	±.27	----	----	±.33	----
Isoleucine												
H	7.57[a]	7.22[a]	8.77[a]	9.01[a]	8.40[a]	8.20	±.27	7.11[b]	9.28[a]	6.78	7.58	10.23
L	7.74[a]	8.90[a]	9.03[a]	9.70[a]	10.49[a]	9.17	±.27	8.82[a]	9.52[a]	8.60	8.37	10.55
Av.	7.66[a]	8.07[b]	8.90[c]	9.36[c]	9.45[c]	-	-	7.96	9.40	7.69[a]	7.97[a]	10.39[b]
s.e.	±.43	----------				-	-	±.28	----	±.35	----------	
Arginine												
H	4.75	6.13	5.81	5.93	6.37	5.80	±.31	4.57[a]	7.03[c]	5.77	6.00	5.62
L	5.42	5.92	5.21	5.50	4.36	5.28	±.31	4.42[a]	6.15[b]	4.89	5.53	5.43
Av.	5.09	6.03	5.50	5.71	5.36	-	-	4.47	6.59	5.33	5.76	5.52
s.e.	±.49	----------				-	-	±.07	----	±.34	----------	

Table 32.--Continued.

	Treatment					Level of Production		Week of Experiment		Hour of Sampling		
	NC	2P	U	P	PC	Av	s.e.	4	9	0	2	8
Histidine												
H	5.95	6.83	5.15	5.84	5.70	5.89	±.15	5.09[a]	6.69[a]	6.12	5.81	5.74
L		5.71	5.14	5.00	5.27	5.40	±.15	4.50[a]	6.29[a]	5.52	5.24	5.43
Av.	5.91	6.27	5.14	5.42	5.48	-	-	4.80	6.49	5.82	5.53	5.58
s.e.	±.24	----------				-	-	±.24	----	±.17	----------	
Phenylalanine												
H	3.85	3.66	2.99	3.88	4.01	3.67	±.15	3.41[a]	3.93[a]	3.65	3.46	3.92
L	3.57	3.54	3.81	3.72	3.65	3.66	±.15	3.59[a]	3.72[a]	3.62	3.37	3.99
Av.	3.71	3.60	3.40	3.80	3.82	-	-	3.50	3.83	3.63[ab]	3.41[a]	3.95[b]
s.e.	±.24	----------				-	-	±.06	----	±.11	----------	
Methionine												
H	3.22	4.04	1.93	3.08	2.70	2.99	±.29	3.31[a]	2.67[a]	3.17[a]	3.34[a]	2.48[b]
L	2.02	2.90	2.96	2.75	2.81	2.69	±.29	3.01[a]	2.37[a]	3.40[a]	2.33[b]	2.33[b]
Av.	2.62	3.47	2.45	2.91	2.75	-	-	3.16	2.52	3.28[a]	2.83[ab]	2.41[b]
s.e.	±.46	----------				-	-	±.25	----	±.22	----------	

Table 32.--Continued.

	Treatment					Level of Production		Week of Experiment		Hour of Sampling		
	NC	2P	U	P	PC	Av	s.e.	4	9	0	2	5
Cysteine												
H	1.96[a]	2.05[a]	2.20[a]	1.88[a]	2.17[a]	2.05	±.05	2.02[a]	2.09[a]	2.01[a]	1.92[a]	2.23[b]
L	2.16[a]	2.00[a]	2.02[a]	1.94[a]	2.33[a]	2.09	±.05	2.00[a]	2.18[a]	1.79[a]	2.07[b]	2.41[c]
Av.	2.06[a]	2.03[a]	2.11[a]	1.91[a]	2.25[b]	-	-	2.01	2.13	1.90[a]	1.99a	2.32[b]
s.e.	±.07	----------				-	-	±.04	----	±.07		
Glycine												
H	33.73	44.65	28.18	31.06	35.58	34.64	±2.22	36.98[a]	32.30[b]	36.58	34.11	33.24
L	32.06	34.22	32.01	28.16	21.92	29.67	±2.22	29.37[a]	29.97[a]	30.24	28.61	30.17
Av.	32.89	39.44	30.09	29.61	28.75	-	-	33.17	31.14	33.41	31.36	31.71
s.e.	±3.52	----------				-	-	±.74	-----	±1.04	----------	
Alanine												
H	21.39	25.89	21.54	26.13	22.89	23.57	±.73	22.43[a]	24.70[a]	23.58	23.59	23.54
L	24.01	23.95	24.22	24.42	24.52	24.23	±.73	23.08[a]	25.37[a]	24.94	23.70	24.04
Ave.	22.70	24.92	22.88	25.27	23.71	-	-	22.76[a]	25.04[b]	24.26	23.64	23.79
s.e.	±1.15	----------				-	-	±.31	-----	±1.05	----------	

Table 32.--Continued.

	Treatment					Level of Production		Week of Experiment		Hour of Sampling		
	NC	2P	U	P	PC	Av.	s.e.	4	9	0	2	5
Proline												
H	8.14	9.50	7.68	8.50	7.39	8.24	±.30	7.94[a]	8.54[a]	7.92	8.08	8.73
L	8.09	8.30	8.82	7.45	7.53	8.04	±.30	8.14[a]	7.93[a]	8.60	7.96	7.55
Av.	8.12	8.90	8.25	7.97	7.46	-	-	8.04	8.23	8.26	8.02	8.14
s.e.	±.48	----------				-	-	±.32	----	±.43	----------	
Glutamic acid												
H	6.58[a]	6.76[a]	5.78[a]	7.23[a]	5.64[a]	6.40[d]	±.15	5.81[a]	6.99[a]	6.89	5.80	6.51
L	6.90[a]	7.13[a]	6.60[a]	8.22[a]	7.07[a]	7.18[ee]	±.15	6.30[a]	8.06[a]	8.08	6.69	6.78
Av.	6.74[a]	6.94[a]	6.19[a]	7.73[b]	6.35[a]	-	-	6.06[a]	7.52[b]	7.48[a]	6.24[b]	6.64[b]
s.e.	±.23	----------				-	-	+.23	----	+.87	----------	
Serine												
H	7.02	8.09	5.71	5.87	6.08	6.55	±.26	7.03[a]	6.08[a]	6.69	6.52	6.45
L	5.47	6.30	5.98	5.42	5.35	5.70	±.26	5.80[a]	5.60[a]	6.01	5.41	5.69
Av.	6.25	7.19	5.85	5.64	5.71	-	-	6.42	5.84	6.35	5.97	6.07
s.e.	±.42	----------				-	-	±.25	----	±.30	----------	

Table 32.--Continued.

	Treatment					Level of Production		Week of Experiment		Hour of Sampling		
	NC	2P	U	P	PC	Av	s.e.	4	9	0	2	5
Asparagine												
H	3.55	4.23	3.80	4.21	3.96	3.95	±.13	3.67[a]	4.22[a]	4.47	3.56	3.81
L	3.72	4.20	3.82	3.38	4.03	3.83	±.13	3.60[a]	4.06[a]	3.93	3.69	3.86
Av.	3.63	4.21	3.81	3.80	3.99	-	-	3.64	4.14	4.20	3.63	3.84
s.e.	±.20	----------				-	-	±.14	----	±.15	----------	
Tyrosine												
H	3.40	4.03	3.11	4.17	3.79	3.70	±.19	3.87[a]	3.52[a]	3.87	3.19	4.03
L	3.55	4.23	4.01	3.40	3.87	3.81	±.19	4.18[a]	3.44[a]	3.73	3.37	4.34
Av.	3.47	4.13	3.56	3.78	3.83	-	-	4.03[a]	3.48[b]	3.80a	3.28[b]	4.18[c]
s.e.	±.30	----------				-	-	±.11	----	±.14	----------	
Aspartic acid												
H	.81	.96	.89	1.03	1.09	.96	±.04	.68[a]	1.23[a]	.85	.83	1.19
L	.77	.95	1.01	.83	1.02	.91	±.04	.68[a]	1.14[a]	.89	.83	1.03
Av.	.79	.95	.95	.93	1.05	-	-	.68[a]	1.18[b]	.87[a]	.83[a]	1.11[b]
s.e.	±.06	----------				-	-	±.05	----	±.05	----------	

[a,b,c]Means within a row and within treatment, week of experiment, or hour of sampling without a common superscript are different (P<.05).

[d,e]Means for level of production within an amino acid without a common superscript are different (P<.05).

[f]Level of milk production; H = high, L = low.

ASP ($P<.10$). The changes in amino acids will be described in the order of increasing complexity with respect to interactions.

Treatment was the only factor affecting LEU, LYS, CYS and GLU. Leucine was lowest ($P<.05$) for treatment NC and increased approximately linearly for treatments 2P, U, PC and 2P. Lysine remained constant for treatments NC, U and P and then increased ($P<.05$) for PC and 2P. Isoleucine increased ($P<.05$) from group NC to 2P to U and then remained constant for groups P and PC. Cystine was higher ($P<.05$) for group PC than others, whereas GLU was highest ($P<.05$) for Group P.

The effects of treatments and week of sampling on GLY, ALA and SER are shown in Table 33. In week four none of the treatment differences were significant for SER, but GLY was higher ($P<.05$) for group 2P than others, and ALA was higher ($P<.05$) for groups 2P and PC than others. In week 9 treatment differences for GLY and ALA were non-significant, but SER was higher ($P<.05$) in groups NC and 2P than in others.

The interaction between treatments and hour of sampling affected VAL and ASN ($P<.25$) and the results are given in Table 34. Plasma VAL was lower ($P<.05$) in group NC than others, but at 2 hours none of the treatment differences were significant. At 5 hours VAL was lower ($P<.05$) for groups NC, 2P and U than P and PC. The ASP response at zero hours was higher ($P<.05$) for group 2P than others, but none of the differences at 2 hours were significant, and at 5 hours it was lower ($P<.05$) in groups NC, 2P and U than P and PC.

The three-way interaction between treatments, week and hours of sampling for ARG, HIS and ASP are shown in Table 35. None of the treatment differences within hours of sampling for these three amino

Table 33.--Effects of treatments and week of sampling on plasma concentrations of glycine, alanine, serine; μM/100 ml.

Amino acid	Week of experiment	Treatment				
		NC	2P	U	P	PC
Glycine:	Week 4	32.19[a]	44.90[b]	27.84[a]	31.37[a]	29.58[a]
	Week 9	33.60[a]	33.98[a]	32.35[a]	27.85[b]	27.92[b]
Alanine:	Week 4	19.76[a]	25.62[b]	20.95[b]	25.93[b]	21.52[a]
	Week 9	24.64[a]	24.23[a]	24.81[a]	24.62[a]	24.90[a]
Serine:	Week 4	5.47[a]	6.30[a]	5.98[a]	5.42[a]	5.35[a]
	Week 9	7.02[a]	8.09[a]	5.71[b]	5.87[b]	6.08

[a,b]Means within a row without a common superscript are different ($P<.05$).

Table 34.--Effects of treatments and hours of sampling on plasma concentrations of valine and asparagine; μM/100 ml.

Amino acid	Hour of sampling	Treatment				
		NC	2P	U	P	PC
Valine:	0 hours	12.95[a]	17.04[b]	19.86[b]	17.09[b]	20.54[b]
	2 hours	14.75[a]	15.74[a]	16.36[a]	17.30[a]	17.51[a]
	5 hours	16.29[a]	15.99[a]	16.59[a]	19.95[b]	22.03[b]
Asparagine:	0 hours	3.75[a]	5.31[b]	4.33[a]	3.64[a]	3.96[a]
	2 hours	3.22[a]	3.73[a]	3.75[a]	3.75[a]	3.70[a]
	5 hours	3.93[a]	3.59[a]	3.34[a]	4.00[b]	4.32[b]

[a,b]Means within a row without a common superscript are different ($P<.05$).

Table 35.--Effects of treatments, weeks and hours of sampling on plasma concentrations of arginine, histidine and aspartic acid; μM/100ml.[c]

Amino acid	Week of experiment	Hours from feeding	Treatment				
			NC	2P	U	P	PC
Arginine:	4	0	3.64[a]	5.24[a]	4.17[a]	4.63[a]	4.91[a]
		2	4.17[a]	5.38[a]	4.48[a]	4.76[a]	4.07[a]
		5	3.73[a]	3.36[a]	7.06[b]	3.96[a]	3.88[a]
	9	0	4.68[a]	7.60[b]	5.60[a]	5.00[a]	7.86[b]
		2	7.49[a]	7.93[a]	4.96[b]	7.33[a]	7.11[a]
		5	6.82[a]	6.67[a]	6.79[a]	8.61[a]	4.38b
Histidine:	4	0	4.92[a]	5.34[a]	4.71[a]	4.83[a]	5.53[a]
		2	4.31[a]	5.44[a]	4.99[a]	4.16[a]	3.78[a]
		5	5.02[a]	4.58[a]	5.06[a]	4.24[a]	5.06[a]
	9	0	6.96[a]	8.18[a]	5.49[b]	5.55[b]	6.71[a]
		2	7.57[a]	6.98[a]	4.82[b]	7.06[a]	6.18[a]
		5	6.67[a]	7.10[a]	5.78[a]	6.70[a]	5.66[a]
Aspartic acid:	4	0	.49[a]	.95[a]	.76[a]	1.04[a]	.87[a]
		2	.46[a]	.43[a]	.48[a]	.58[a]	.55[a]
		5	.71[a]	.87[a]	.65[a]	.76[a]	.69[a]
	9	0	.85[a]	1.16[a]	.84[a]	.88[a]	.85[a]
		2	1.26[a]	1.28[a]	1.10[a]	.94[a]	1.23[a]
		5	.98	1.04[a]	1.87[b]	1.37[ab]	2.13[b]

[a,b]Means within a row without a common superscript are different ($P<.05$).

[c]Standard error: Arginine ± .73; Histidine ± .49; Aspartic acid ± .17.

acids was significant in week 4. In week 9 ARG was higher ($P<.05$) at zero hours for groups 2P and PC than others, lower ($P<.05$) at 2 hours for group U than others, and lower at 5 hours for group PC than others. Histidine was lower ($P<.05$) at zero hours for groups U and P than others, and group U remained lower ($P<.05$) than others at 2 hours, but none of the differences at 5 hours were significant. For ASP the only significant differences were at 5 hours where groups U and PC were higher ($P<.05$) than others.

Effects of level of milk production, week and hour of sampling.--The level of milk production affected GLY ($P<.01$); LEU, LYS ($P<.05$); VAL, ILE, HIS, SER ($P<.10$); and GLY ($P<.25$). Week of sampling affected all amino acids studied except THR, CYS and PRO, and the interaction between level of production and week of sampling affected LEU, ARG ($P<.05$), VAL, PHE, GLY ($P<.10$) and ILE ($P<.25$). Results are given in Table 32. Generally, EAA concentrations were lower in high than low yielding cows, and lower in week 4 than week 9 when the milk yield had decreased. For amino acids with interactions between level of production and week of sampling, the general trend was similar concentrations in weeks 4 and 9 in low yielding cows. High yielding cows had lower concentrations in week 4 than low yielders, but by 9 weeks the concentrations had increased to or above the measurement for low producers in week 4. For NEAA the trends tend to be the reverse of those observed for EAA, but the responses are more variable.

The responses with hours from feeding affected all amino acids except LYS, ARG, HIS, GLY, ALA, PRO, and SER, and the results are shown in Table 32. Generally, all amino acids remained constant or

decreased between zero and two hours and then increased at 5 hours. However, MET was lower ($P<.05$) at 5 than at zero and two hours in high producers and remained low ($P<.05$) in low producers. The interaction between week and hour of sampling was significant for GLU, SER and ASP ($P<.05$) and approached significance for ILE, CYS, GLY, and TYR ($P<.25$). The results are not reported but the trends are in agreement with those reported for high and low producers.

8.1.2 Main study of factors affecting plasam amino acid concentrations

All cows in the project were bled at 14 days before expected calving (day -14) and at days 21, 42, 70 and 126 post-partum, and individual samples were prepared for amino acid analysis. However, the data is incomplete due to limitations on the capacity for amino acid analysis, and only selected samples were assayed. Day 42 after calving was selected to coincide with peak milk yield. All samples for that period were assayed, and the results were analyzed according to the model in (10) after removal of the corvariate. Additional samples at days -14 and 126 were assayed for cows in the first 7 blocks on experiment, and their distribution within breeding groups is given in Table 36. Results for these cows were analyzed statistically according to the model in (9).

8.1.2.1 Effect of treatments for all cows 42 days post-partum

Plasma concentrations for the complete data at 42 days post-partum are given in Table 37. The data includes 17 cows per group for treatments NC and PC and 14 and 15 cows in groups U and AU, respectively. One cow in group U was deleted because of very high concentrations coinciding with diagnosed ketosis. None of the observed treatment

Table 36.--Distribution of samples assayed at days -14, 42, and 126 on breeding groups, blocks, and treatments.

Treatment	Block	Days from calving: -14 NC	-14 PC	-14 U	-14 AU	42 NC	42 PC	42 U	42 AU	126 NC	126 PC	126 U	126 AU
Best	1	+		+	+	+	+	+	+	+	+	+	+
"	2	+	+	+	+	+	+	+	+	+	+	+	+
"	3	+	+	+	+	+	+	+	+	+	+	+	+
"	4		+	+		+	+	+	+	+	+	+	
Control	1	+		+	+	+	+	+	+	+	+	+	+
Worst	1	+	+	+	+	+	+	+	+	+	+	+	+
"	2	+	+		+	+	+		+	+	+	+	+
Number of cows		6	5	6	6	7	7	6	7	7	7	7	6

Table 37.--Plasma concentrations of amino acids for all cows at 42 days post-partum μM/100 ml.

Amino acid	Treatment: NC	PC	U	AU	s.e.[a]
Number of cows	17	17	14	15	
Valine	20.23	23.58	18.93	19.78	1.76
Leucine	13.56	16.59	13.39	14.05	1.33
Threonine	8.82	8.60	7.81	9.23	.75
Lysine	7.67	7.54	6.97	6.68	.47
Isoleucine	11.78	11.54	10.03	11.18	1.03
Arginine	9.94	7.81	8.08	7.10	1.29
Histidine	8.52	7.21	7.79	7.23	.46
Phenylalanine	6.08	5.86	4.66	5.11	.74
Methionine	2.35	2.91	2.22	2.73	.27
Cysteine	1.90	1.81	1.84	1.67	.17
Glycine	38.22	34.01	32.30	35.53	4.48
Alanine	23.94	22.37	19.92	23.04	2.42
Proline	8.91	8.78	8.73	9.96	1.17
Glutamate	17.28[b]	19.49[b]	21.96[c]	22.65[c]	1.75
Serine	9.50	9.27	9.91	10.46	.83
Tyrosine	4.89	5.10	5.05	5.47	.44
Aspartate	2.56	3.26	2.70	3.19	.39

[a]Standard error for 17 cows per treatment mean.

[b,c]Treatment means within a row without a common superscript are different ($P<.05$).

means were significantly different at (P<.25), except for glutamate and tyrosine. Glutamate concentrations were lower for groups NC and PC than for groups U and AU (P<.05).

The relationship between milk production and plasma amino acid concentrations was studied by the use of stepwise multiple regression analysis. Milk yields and milk composition values used were the average for weeks 6 and 7 post-partum. The list of independent variables to be selected included all amino acids assayed, plasma urea and ammonia nitrogen and plasma glucose. The determined model for milk yield is in (14) and explained 61% of the variation in milk yield. When all essential amino acids were forced into the prediction equation the amount of explainable variation was only increased to 65% and details of the results are not reported.

(14) Milk = 47.27 + .6119 LEU + .8435 THR - 1.2290 LYS + 1.0770 HIS - 1.9439 MET - 2.7331 CYS - .5584 GLU - .2049 GLC; R = .78 (P<.005).

				Partial	Min.	Max.
Where Milk	is	milk yield,	kg/d		13.49	48.41
LEU	"	leucine,	µM/100 ml	.39	6.90	43.22
THR	"	threonine,	µM/100 ml	.30	3.42	21.56
LYS	"	lysine,	µM/100 ml	-.35	4.06	78.39
HIS	"	histidine,	µM/100 ml	.31	1.93	79.37
MET	"	methionine,	µM/100 ml	-.33	1.14	5.57
CYA	"	cysteine,	µM/100 ml	-.27	.62	5.83
GLU	"	glutamate,	µM/100 ml	-.66	8.22	44.97
GLC	"	glucose,	mg/100 ml	-.44	?	70.38
47.27	"	a constant		.86		

The milk fat percentage was not related to any of the variables studied. Of the variation in the milk protein percentage 18% was explained by the constant (r=.91; P<.001) plus PHE (r=.28; P<.05) and glucose (r=.32; P<.01). Of the variation of milk protein yield 43% was explained by a constant (r=.84; P<.001) plus GLY (r=.31; P<.05),

GLU (r=.62; P<.001) and GLC (r=-.25). The prediction equation for SNF explained 59% of the variation and included the same independent variables listed for milk yield. A prediction equation for the yield of calculated milk lactose plus ash was similar to the ones determined for the yields of milk and SNF.

8.1.2.2 Effects of treatments and time from calving on plasma amino acids in selected cows

Treatment means, averages for days -14, 42, and 126, and their interactions are given in Table 38. Significant effects of breeding groups and its interaction with treatment and time are shown in Table A13.

Arginine, MET, PRO and TYR were not affected by any of the factors studied. Treatments affected CYS, ASP (P<.001) and LYS (P<.25). Lysine was higher (P<.05) for groups NC and PC than the NPN groups, and CYS was higher (P<.05) for group NC than others. Aspartate was higher (P<.05) for group AU than others.

Changes with time from calving were significant for PHE, GLY (P<.001); ILE (P<.01); LEU, THR, SER, ASP (P<.05); CYS (P<.10); LYS, ALA and GLU (P<.25). Leucine, GLY, ALA, SER, and ASP were highest (P<.05) at 42 days post-partum. Threonine was lowest (P<.05) pre-partum. Lysine, ILE, PHE and GLU were highest (P<.05) pre-partum, and then remained low or increased slightly between days 42 and 126. Cysteine was highest (P<.05) at 126 days.

The treatment by time interaction affected PHE (P<.001), THR and ILE (P<.25) and the responses are given in Table 38. Phenylalanine was higher (P<.05) for group NC than AU at the pre-partum sampling. However, at 42 days PHE was higher (P<.05) for group PC than others

Table 38.--Plasma amino acid concentrations (mM/liter) for treatments and days from calving and/or by days within treatment; uM/ml.

Amino Acid		DFC[a]	Treatment				DFC[a]		
			NC	PC	U	AU	-14	42	126
Number of cows			20	19	19	12	23	27	27
Valine	AV		19.41[b]	21.40[b]	17.75[b]	18.30[b]	19.62[e]	18.83[e]	19.28[e]
	s.e.		1.05	1.07	1.07	1.10	.83	.76	.76
Leucine	AV		13.02[b]	13.76[b]	12.08[b]	12.69[b]	12.87[e]	13.74[f]	12.04[e]
	s.e.		.73	.75	.75	.77	.68	.63	.63
Threonine		-14	7.91	6.16	7.73	6.09	7.01	-	-
		42	8.07[b]	7.49[b]	6.53[b]	9.48[c]	-	7.96	-
		126	7.66	7.63	8.69	8.58	-	-	-
Lysine	AV		8.74[b]	8.30[b]	7.65[c]	6.87[c]	8.89[e]	7.19[f]	7.77[f]
	s.e.		.53	.55	.55	.56	.48	.44	.44
Isoleucine		-14	15.37	15.18	12.63	11.83	13.69[e]	-	-
		42	9.61	11.14	8.49	10.33	-	9.96[d]	-
		126	9.87	11.96	10.50	11.66	-	-	10.97[d]
Arginine	AV		9.11[b]	7.12[b]	7.73[b]	9.58[b]	6.42[e]	10.44[e]	8.02[e]
	s.e.		1.32	1.35	1.35	1.39	1.69	1.56	1.56
Histidine	AV		7.45[b]	6.62[b]	6.96[b]	7.01[b]	7.41[e]	7.08[e]	6.52[e]
	s.e.		.25	..26	.26	.27	.33	.31	.31
Phenylalanine		-14	6.15	5.67	5.61	4.96	5.59[e]	-	-
		42	4.32	6.43	3.90	4.93	-	4.93[f]	-
		126	4.05	3.98	3.76	3.85	-	-	3.91[g]

Table 38.--Continued.

Amino Acid	DFC[a]	Treatment				DFC		
		NC	PC	U	AU	-14	42	126
Methionine	AV	2.31[b]	2.41[b]	2.24[b]	3.40[b]	2.39[e]	2.25[e]	3.22[e]
	s.e.	.55	.56	.56	.58	.54	.50	.50
Cysteine	AV	1.93[b]	1.62[b]	1.78[c]	1.51[c]	1.65[e]	1.57[e]	1.92[f]
	s.e.	.07	.07	.07	.08	.11	.10	.10
Glycine	AV	32.11[b]	28.92[b]	28.72[b]	32.54[b]	32.35[e]	33.44[e]	22.77[f]
	s.e.	2.33	2.39	2.39	2.45	1.84	1.70	1.70
Alanine	AV	22.42[b]	20.56[b]	18.11[b]	19.57[b]	19.05[e]	21.09[e]	19.82[e]
	s.e.	1.55	1.59	1.59	1.63	1.24	1.14	1.14
Proline	AV	8.12[b]	7.74[b]	7.82[b]	8.21[b]	7.11[e]	7.87[e]	8.48[e]
	s.e.	.64	.65	.65	.67	.64	.57	.57
Glutamate	AV	23.16[b]	23.28[b]	26.43[b]	26.55[b]	27.11[e]	24.78[e]	22.79[e]
	s.e.	1.38	1.41	1.41	1.45	1.08	1.00	1.00
Serine	AV	8.67[b]	9.03[b]	8.36[b]	8.87[b]	8.90[e]	9.23[f]	7.71[g]
	s.e.	.43	.44	.44	.46	.44	.41	.41
Tyrosine	AV	5.03[b]	4.87[b]	5.01[b]	7.33[b]	5.11[e]	4.63[e]	7.18[g]
	s.e.	1.37	1.41	1.41	1.44	1.18	1.09	1.09
Aspartate	AV	2.78[b]	2.59[b]	2.73[b]	3.05[c]	2.46[e]	3.07[f]	2.70[e]
	s.e.	.11	.12	.12	.12	.21	.19	.19

[a]Days from calving.

[b,c,d]Treatment means within a row without a common superscript are different ($P<.05$).

[e,f,g]Means within a row for days from calving without a common superscript are different.

and differences at 126 were non-significant. Pre-partum differences in THR were not significant, but at 42 days it was higher ($P<.05$) for group AU than others and at 126 days groups U and AU tend to have higher concentrations than groups NC and PC. Isoleucine was lower ($P<.05$) at day -14 for groups U and AU than for NC and PC, but at 42 and 126 days groups PC and AU tend to have higher concentrations than groups NC and U.

DISCUSSION

1. Nutrients in feeds

The assayed nutrient content in concentrate was in good agreement with that planned by the use of NRC (1971) feed tables. Hay used was mainly of low quality. These feeds will not be discussed any further.

Urea and ammonia treatment of corn at ensiling increased total nitrogen 48 and 38%, respectively (Table 11). The application of crude protein equivalents was planned to be equal for the two NPN sources. Lower values for ammonia than urea treated silage may be due to loss as vapor and method of application. A flow meter for field application of liquid ammonia would be improved if the dial had finer graduations at the low end of the scale. An increase in the rate of unloading after initial matching with ammonia flow could also lower the amount applied.

The quality of nitrogen in corn silage resulting from NPN treatment at ensiling was estimated by changes in the water insoluble and water soluble fractions (Table 11). Water insoluble nitrogen decreased 32 to 33% in untreated and urea treated silages versus a 11% drop in that treated with ammonia. Bergen et al. (1974) suggested that the higher water-insoluble N in ammonia treated silage was due to decreased proteolysis of the original plant protein. This

conclusion has recently been confirmed in studies with ^{15}N-ammonia treatment of corn silage (Huber, 1977). In urea treated silage 48% of the added urea was present in that form at feeding; the remainder of the urea nitrogen was apparently changed to ammonia and other nitrogenous compounds. These data agree with previous reports (Huber et al., 1968).

Silages at feeding were all of high quality and were not adversely affected by NPN treatment. Huber et al. (1973) found increased lactic acid in NPN treated silages due to a buffering by ammonia. Lactic acid in silage DM in the present study average 3.94, 4.70 and 4.35% for untreated, urea and ammonia treated silages, but the differences were not significant. The low values and a greater than usual variation may have contributed to the lack of significant differences between treatment in lactate content.

2. Feed and nutrient intakes

The changes in intake of individual feeds with time after calving were different between treatments, but for total dry matter this interaction only approached significance. As suggested, the change from a significant interaction for individual feeds to a less pronounced effect for total dry matter was due to fixed feeding of concentrate and a compensatory intake of corn silage. However, it should be noted in Figures 6 to 8 that group NC, which received most concentrate (because of highest milk yields) also ate slightly more corn silage than other groups; whereas AU, which received the second largest quantity of concentrate, ate the least corn silage. Intake of total DM in group NC (12.5% CP) was slightly higher than in other

groups (15 to 16% CP) throughout the entire 20 weeks. These results are in agreement with those of Polan et al. (1976) who found highest intakes at 12.8% CP, but in contrast with others (Sparrow et al., 1973; Grieve et al., 1974; Cressman et al., 1977) who reported increased intake up to 17% CP. In the present study, cows fed the urea supplemented ration (U) received equivalent to 247 g urea per day (Table 39). The amount of urea consumed by the U group, intake of NPN, and the fraction of total N fed as urea or ammonia all approached the upper limits recommended by NRC (1971).

Table 39.--Intake of urea equivalents in the different periods from calving by cows fed non-protein nitrogen supplemented diets.

Period	Urea equivalents, g/d		Urea equivalents from silage, %	
	Group U	Group AU	Group U	Group AU
Weeks 3 through 6	225	208	47.4	37.0
" 7 " 10	247	216	48.5	37.1
" 11 " 15	234	192	55.5	44.8
" 16 " 20	220	188	57.2	49.3

None of the sources of supplementary N in groups PC, U and AU significantly affected DM intakes (Table 14) even though urea fed cows consumed less dry matter than other groups during weeks two to seven.

Dry matter intake per unit body weight also were not significantly affected by treatments and peaked at 3.5% during weeks 7 through 11. At no time form weeks 3 to 20 did average intakes fall

below 3% of BW. By this method of calculation group NC also tended to have highest intakes.

The intake of crude protein was approximately 500 g per day lower for group NC than others, but "true protein" (TN-SOLN-ADN) intake was equal for NC and U, with AU 100 g higher due to the suggested reduction by ammonia of plant protein proteolysis. Group PC received 400 to 500 g more true protein than other groups.

3. Production of milk and milk components

Treatments did not significantly affect the production of milk or milk components, except for a depressed production in the urea group for weeks 3 through 6 in the high producing cows. The decrease may have been due to slightly lower intake of cows fed urea. The longer period of body weight losses of the urea-fed cows also suggested more energy stress.

The similar production of high yielding cows fed approximately 12.5 and 16% CP (NC vs. PC and AU) is in sharp contrast to NRC recommendations (NRC, 1971) and with results or suggestions of Gardner and Parker (1973), Sparrow et al. (1973), Grieve et al. (1974), Cressman et al. (1977) and Satter and Roffler (1975). However, these data agree with Thomas (1971) who reported normal persistencies in cows producing about 30 kg milk per day fed rations containing 12.5 to 13.6% CP. Also, Chandler et al. (1976) obtained similar milk yields in cows fed 12.5 and 15.5% CP in ration DM. In the present experiment, dry matter intakes and energy densities were not significantly affected by treatments. In trials by Sparrow et al. (1973), Grieve et al. (1974), and Cressman et al. (1977) the energy intakes increased with increasing

CP in the ration DM. Lamb et al. (1974) showed increased milk yields when the rate of grain feeding was increased from .25 to .63 kg per kg milk, thus raising the intake of total dry matter. Schwab et al. (1971) and Moe and Tyrrell (1977) reported increased digestibilities with increased protein in the diet.

The urea fermentation potential (UFP), as defined by Burroughs et al. (1973, 1974a,b, 1975a,b), are estimated for rations used in the present experiment in Table 40. These calculations suggest that 244 to 267 g urea could be utilized if bypass of true protein is 40%, but UFP is decreased to 237 to 259 g urea if rumen bypass of true protein is decreased to 10%. Tables 39 to 40 shows that only group U in weeks 7 through 10 received urea equal to the UFP if 10% bypass is considered.

The system of metabolizable protein suggested by Satter and Roffler (1975) was applied to results of the present study. Crude protein in DM for group NC was less than 13% and RAN values were in agreement with the suggested critical value for rumen ammonia utilization. Thus metabolizable protein for group NC may be calculated according to (4). Satter and Roffler (1975) suggested zero utilization of supplementary NPN if RAN concentrations exceeded 5 mg per 100 ml fluid, whereas 30% of true protein may be utilized. Therefore, metabolizable protein for groups PC, U, and AU was calculated as in (15):

$$(15) \quad MP = MP_{NC} + (TP - TP_{NC}) * .30.$$

where MP is metabolizable protein for groups PC, U or AU;

MP_{NC} is metabolizable protein for group NC;

TP is "true protein" intake for groups PC, U or AU (Table 15);

TP_{NC} is "true protein" intake for group NC; and .30 is a constant

(see Satter and Roffler, 1975).

Metabolizable protein used for milk protein synthesis is calculated in Table 41. By this method, coefficients may be expected equal for all diets. However, Table 41 shows that the nutritive value of the plant protein (groups PC, U and AU) was depressed when protein in DM exceeded 13%. Moreover, protein was less efficiently utilized in cows receiving the most natural protein (PC) compared to those on NPN (U and AU).

Production results of this experiment suggest that the CP requirement of high yielding dairy cows fed corn, corn silage, limited hay rations is not more than 13% CP in DM. Protein reserves, released early in lactation (Coppock et al., 1968; and Paquay et al., 1972), may have compensated for a dietary deficit, but weight changes before and after minimum BW and the number of weeks until minimum weight was attained were not different for groups NC, PC and AU. Had the NC cows incurred a protein deficit of 3% of the dry matter intake, compared to others, then body weight changes should have been sensitive enough to detect the differences. For example, to increase dietary CP from 12.5 to 15.5% for group NC would require approximately 600 g CP per day in weeks 3 through 20. If the efficiency of utilization of dietary protein for milk protein synthesis is 33%, as in this experiment (Table 42), then protein already assimilated should be approximately three times more efficient for milk protein production. Thus, the net tissue protein loss that would equal a 600 g/d dietary deficiency might be estimated at 200 g per day. If body weight were 20% protein

Table 40.--Urea fermentation potential (UFP;[a] g urea/d) of high concentrate, corn silage, limited hay rations used for treatments in the different periods from calving.

Period	Treatment			
	NC	PC	U	AU
40% bypass of true protein:				
Weeks 3 through 6	264	262	263	261
" 7 " 10	267	263	259	258
" 11 " 15	263	258	252	251
" 16 " 20	259	256	244	248
10% bypass of true protein:				
Weeks 3 through 6	259	247	252	248
" 7 " 10	256	245	248	246
" 11 " 15	253	245	242	239
" 16 " 20	253	244	234	237

[a]Burroughs et al. (1975a,b).

Table 41.--Utilization[a] of metabolizable protein[b] for milk protein production in dairy cows in the different periods from calving; %.

	Treatment			
	NC	PC	U	AU
All cows:				
Weeks 3 through 6	54	46	49	51
" 7 " 10	51	42	47	48
" 11 " 15	54	49	51	50
" 16 " 20	51	47	49	48
High producers:				
Weeks 3 through 6	53	48	49	53
" 7 " 10	52	46	47	51
" 11 " 15	51	48	50	50
" 16 " 20	55	53	54	54

[a](Milk protein*100)/(metabolizable protein).

[b]Satter and Roffler (1975).

Table 42.--Protein availability at the abomasal level, its relation to present standards, and utilization for milk protein production in dairy cows in the different periods from calving.

Period	Treatment			
	NC	PC	U	AU
Protein at abomasum,[a] kg/d:				
Weeks 3 through 6	3.03	2.99	2.87	2.96
" 7 " 10	3.17	3.24	2.81	2.83
" 11 " 15	2.78	2.95	2.70	2.63
" 16 " 20	2.75	2.77	2.50	2.50
Protein balance,[b] %:				
Weeks 3 through 6	105	101	93	103
" 7 " 10	105	111	97	105
" 11 " 15	100	108	100	100
" 16 " 20	105	108	110	102
Utilization of crude protein,[c] %:				
Weeks 3 through 6	39	30	31	33
" 7 " 10	38	28	29	31
" 11 " 15	39	32	30	32
" 16 " 20	37	32	30	31
Utilization of protein at abomasum,[c] %:				
Weeks 3 through 6	33	31	31	32
" 7 " 10	31	29	32	34
" 11 " 15	33	31	32	33
" 16 " 20	31	31	33	33

[a]16.5 g microbial protein per 100 g digestible organic matter (Bucholtz and Bergen, 1973).

[b]Balance = (protein per abomasum)/(NRC(19/1) requirement).

[c]Utilization = (milk protein)/(available protein).

(Huber, 1975) then a weight loss of 1000 g might be incurred for every 200 g of protein loss. The weight loss in the first four weeks approached the above value for group NC but similar losses were also observed for the other groups. However, weight gains were encountered for groups NC, PC and AU after the second week on trial. Therefore one might conclude that the protein deficits for group NC was less than estimated, or that changes in tissue composition actually occurred which were not detected. The important question of how dependent are early lactation cows on body reserves to sustain the milk protein yields still remains unanswered.

The in vivo production of microbial protein have been estimated at 32 g per kg fermentable organic matter (e.g., Allen and Miller, 1976). Bucholtz and Bergen (1973) suggested 16.5 g microbial protein per 100 g organic matter digested when the rumen turnover of protein was at 25%. Protein at abomasum in the present study were calculated as in (16) and (17):

$$(16)\quad DOM = \sum_{i=1}^{3} (IT^* DM * OM * TDN)$$

$$(17)\quad ABPR = [DOM - (TP * BP)] * .165 + (TP * BP)$$

where DOM is intake of digestible organic matter, kg;

i is 1 for concentrate; 2 for corn silage; and 3 for hay;

IT is intake of feed on as is basis, kg;

DM is dry matter in feed, %;

OM is dry matter minus ash %;

TDN is total digestible nutrients, %;

ABPR is total protein at abomasal level, kg;

TP is true protein, kg;

BP is plant protein bypassing rumen fermentation, %; and

.165 is kg microbial protein per kg DOM.

Estimates of total protein available at the abomasal level as well as the balance of abomasal protein in relation to recommended standards (NRC 1971), and the utilization of CP and abomasal protein for milk protein synthesis are given in Table 42. By this method of calculation available protein and its utilization for milk protein synthesis were equal for all groups, and were equal to or slightly higher than present standards. However, for group NC available protein exceeds the CP intake and cannot occur if the pool of body protein is constant. Utilization of CP and protein at the abomasal level was the same in groups PC, AU and U; but in group NC the CP was utilized more efficiently than protein at abomasum. The discrepancy may be explained by the amount of plant protein bypassing rumen fermentation (Table 43). If only 10% true protein bypass the rumen, then protein at the abomasum would be equal to the CP intake for group NC. In groups PC, U and AU protein at the abomasum is equal to NC when 10 to 20% of true protein bypass the rumen, and the utilization for milk production is 40% for all groups.

The production results obtained and metabolic factors considered all support the previous statement that high yielding cows in early lactation fed corn, corn silage, limited hay rations require no more than 13% CP in the diet. This conclusion is supported by Thomas (1971), Chandler et al. (1976), and Huber (1976). The value of NPN in early lactation rations still remains unsolved; but Roffler and Satter (1975b), Burroughs (1975a,b) and Huber (1975, 1976) all

Table 43.--Total protein and true protein available at the abomasal level in dairy cows in weeks 7 through 10 post-partum.

True protein bypassing the rumen, %	Treatment			
	NC	PC	U	AU
Total protein at abomasum, kg/d:				
0	2.48	2.35	2.23	2.23
10	2.65	2.57	2.40	2.42
20	2.82	2.79	2.56	2.60
30	3.00	3.02	2.73	2.78
40	3.17	3.24	2.90	2.96
True plant protein at abomasum, kg/d:				
0	0	0	0	0
10	.21	.27	.20	.22
20	.41	.54	.40	.43
30	.62	.80	.60	.65
40	.82	1.07	.80	.87

reported that the utilization of some NPN in rations containing 12 to 13% CP was as high as for plant protein, particularly when high energy rations are fed. Huber (1975) provided evidence that high yielding cows fed less than 12% CP rations had lesser persistencies than those fed 13 to 14% CP. These trials also showed that milk production in cows fed rations with ammonia treated corn silage was as persistent as in cows given plant protein as the supplementary N source. Polan et al. (1976) reported that urea supplementation was advantageous at low levels of CP (9.4%) in the basal ration, but had a negative effect at increased protein.

4. Rumen metabolities

The molar concentration of total rumen VFA's increased from the pre-partum sampling until 70 days post-partum and then decreased slightly. The increase was due to increased concentrations of all

acids. The molar percentage of acetate decreased between pre- and post-partum sampling, and other acids increased. The observed changes are characteristic for changes from all forage to high grain rations. Generally, molar concentrations of rumen VFA's and total acids were highest for group PC and AU. However, iso-butyrate and iso-valerate for group AU were equal to those in groups NC and U. The increased iso-acid concentrations for group PC may be due to greater deamination of amino acids from soybean meal.

Rumen ammonia nitrogen (RAN) in pre-partum samples was similar for groups NC, U and AU and higher than in group PC. This may be explained by the distribution of calvings. Table 44 shows that RAN was lower in dry cows at pasture than in those fed indoors, and that the lower pre-calving value for group PC can be attributed to unequal numbers in the groups. The values for dry cows indoors fed corn silage with added urea are in agreement with treatment values reported by Polan et al. (1976), and higher than all post-partum values found in the present trial (Table 27).

Post-partum concentrations of RAN for group NC was in close agreement with the critical value of 5 mg per 100 ml suggested by Roffler and Satter (1975). For supplemented groups, RAN was lowest for group PC and highest for group U with AU intermediate. The slightly higher RAN concentrations for group PC than NC at days 21 and 126 suggests that considerable amounts of the supplementary soybean meal given group PC escaped rumen degradation. A greater rumen bypass of plant protein on PC than NC is in contrast to the calculations for protein available at the abomasal level (Table 42). This

Table 44.--Rumen ammonia nitrogen in dry cows at pasture and indoors fed corn silage plus urea added at feeding (mg/100 ml fluid).

	Treatment				All cows
	NC	PC	U	AU	
Pasture:[a]					
Number of cows	9	12	10	10	41
Average	5.59	4.87	7.66	7.96	6.46
Standard error	1.76	1.34	3.12	2.51	1.10
Indoors:[b]					
Number of cows	8	5	6	7	26
Average	19.64	24.71	27.57	27.65	24.60
Standard error	2.50	3.30	4.92	2.32	1.68

[a]Samples taken before October 23, 1976.

[b]Samples taken after October 23, 1976.

discrepancy may be related to time of sampling in relation to time of feeding.

Despite the fact that total NPN intake was higher for group U and AU after than before calving, RAN concentrations were higher pre-partum. This was probably because more grain was fed post-partum which increased efficiency of microbial utilization of ammonia. The insoluble nitrogen levels in silage treated with NPN at ensiling as opposed to those treated at feeding might also have contributed to the lower RAN in post-partum cows.

The RAN values for NPN diets in the present study did not exceed those reported by Roffler and Satter (1975b) for all plant protein diets with comparable CP contents. The relatively low RAN concentrations should not be attributed to a decreased rate of feed

consumption, but to a synchronous release of ammonia and energy for microbial utilization.

5. Blood metabolites

Plasma glucose was lowest at 21 days post-partum but was not affected by treatments nor the interaction between treatment and time after calving. Plasma glucose concentrations in the present experiment are in agreement with arterial concentrations reported by Bickerstaffe et al. (1974) and Annison et al. (1974), but lower than those reported by Derring et al. (1974) and Vik-Mo et al. (1974a) who infused glucose and/or casein per abomasum. The concentrations at 21 days post-partum, when the energy intake was still slightly less than the NRC recommendations, approached values for fasting cows (Kronfeld et al., 1968).

Post-partum PUN values were lowest for group NC, highest for group U with groups PC and AU intermediate. The low PUN concentrations for group NC are in agreement with lowest RAN values and highest coefficients for utilization of metabolizable protein. Furthermore, PUN values for group NC are in agreement with those reported by Huber and Thomas (1971) when cows were fed corn silage treated with urea at ensiling. Comparable PUN concentrations for groups PC and AU, despite differences in RAN concentrations indicate similar losses in urine whether the supplementary protein source is soybean meal or NPN. Whether the discrepancy in RAN and PUN concentrations for group PC is due to deamination of amino acids from soybean meal bypassing rumen degradation, or is an effect of sampling hour, is unknown. Highest PUN concentrations in group U indicate less efficient utilization of urea than ammonia when added at ensiling, and may be related to the

presence of non-hydrolyzed urea in the silage and less protection of plant protein. Plasma urea nitrogen (PUN) concentrations in the selected cows were in general agreement with overall treatment values and showed little diurnal variation. The PUN concentrations were less sensitive than RAN to time of sampling or to differences in feeding.

Plasma ammonia nitrogen (PAN) in selected cows tended to show diurnal rhythm at 112 days post-partum with lowest concentrations at 0800 hours (Figure 21). Group U maintained slightly higher PAN concentrations than the other groups for the samplings at 1530, 1900 and 2300 hours. Pre-partum PAN were higher than post-partum concentrations and decreased throughout lactation. Differences between treatments were small but group U tended to have the highest concentrations. This is in agreement with treatment effects on PUN. Similar PAN concentrations for groups PC and AU suggests similar utilizations of N in soybean meal and corn silage treated with ammonia at ensiling. Highest PAN values for group U support the suggested decreased utilization of urea in corn silage when compared to ammonia treated silage.

The higher PUN concentrations for groups PC and AU than NC are in agreement with more efficient use of dietary protein for production of milk protein. Elevated PUN levels were also reported for cows receiving high levels of abomasally infused casein (e.g., Vik-Mo et al., 1974b). The high PUN and PAN levels in plasma and the lower coefficients of utilization of metabolizable protein for the groups fed supplementary protein reemphasize our suggestion that about 13% CP is sufficient for high yielding cows in early lactation consuming the type of diet fed in these studies. The observed discrepancies between

RAN and PUN concentrations for cows fed supplemental N suggest that PUN might be a better measurement of nitrogen utilization in dairy cows than RAN as suggested by Roffler and Satter (1975a,b). Derring et al. (1972) observed decreased PUN when cows fed according to NRC feed standards received abomasally infused casein. However, Vik-Mo et al. (1974b) fed cows above NRC feed standards and observed increased PUN when casein was infused into the abomasum.

Factors affecting plasma amino acid concentrations were studied in an initial experiment and the main experiment. Factors studied included treatments, level of milk production, stage of lactation and sampling hours in relation to feeding. In the initial experiment VAL, LEU, THR, LYS, ILE, ARG, HIS, PHE, ALA, GLU, ASN and ASP were lowest for high yielding cows or for the samples taken during week 4 versus week 9 of treatment. Methionine, GLY, SER and TYR decreased, whereas, CYS and PRO remained constant. In the main experiment amino acids were generally highest at the pre-partum sampling, decreased at 42 days and then increased at 126 days. However, LEU, GLY, SER and ASP were highest at 42 days, and PHE continued to decrease throughout lactation.

Plasma amino acid concentrations arise from the balance between rates of digestion, absorption, and synthesis, as well as tissue demand and breakdown (Albanese, 1959; Gitler, 1964; Harper, 1968; Munro, 1970; McLaughlan, 1974). Lowest PAA concentrations in high yielding cows and at 42 days post-partum are in agreement with highest demands for milk synthesis. However, dramatic increases with decreasing milk yield are prevented by normal decreases in feed intake with advancing lactation. Decreasing plasma concentrations of some

amino acids with decreasing milk yield may be related to an increased availability of the limiting amino acid relative to milk yield; hence, an increased demand for these amino acids would arise. The constant CYS concentrations cannot be explained by either a decreased demand or an increased availability.

Neither Halfpenny et al. (1969a,b) nor Vik-Mo et al. (1974) observed diurnal rhythms for PAA in dairy cows milked twice daily. In the initial experiment PAA concentrations generally remained constant or decreased slightly at two hours when compared with samples taken before feeding, and then increased at 5 hours. The described trends with time may be attributed to changes in demand for milk synthesis or in the availability of amino acids.

In the initial experiment treatments significantly affected plasma concentrations of VAL, LEU, LYS, ILE, HIS and CYS and non-significant trends were observed for other EAA. None of the treatment differences in the main experiment at 42 days post-partum were statistically significant. Increased PAA concentrations have been related to increased availability of amino acids in cows given abomasal infusions of casein (Spires et al., 1973; Hale et al., 1972; Broderick et al., 1970; Derring et al., 1974; Vik-Mo et al., 1974b) and IV infusion of individual amino acids (Fisher, 1969, 1972; Teichman et al., 1969; Fisher and Erfle, 1974).

Interpretation of amino acid profiles without quantitative estimates of the availability of amino acids may be improved by adaptation of the methods used in amino acid requirement studies in nonruminants. In such experiments the amino acid under investigation remains constant in plasma until the requirement is met and then

increases linearly (two-phase response; e.g., Zimmerman and Scott, 1965). The feasibility of this method in ruminants was demonstrated by Fenderson and Bergen (1975) in steers given graded levels of an amino acid per abomasum. However, it should be emphasized that the method may give erroneous results if the availability and the demand are changed simultaneously.

The plasma concentrations of EAA in high and low yielding cows in the initial experiment and at 42 days post-partum in the main study are plotted in Figures 19 to 22. In these figures arrangement of treatments on the abcissa was in order of ascending plasma concentrations, and an assumed increased availability with increasing plasma concentrations. Figure 19 to 22 shows that plasma concentrations of these amino acids in the main experiment were higher than in both high and low yielding cows in the initial experiment, except for THR, MET and CYS. In the initial experiment THR, MET and HIS were lower in low than high yielding cows, and CYS tended to remain constant. Tentative two-phase responses are seen for VAL, LEU and LYS in high yielding cows in the initial experiment; and in the main study VAL, LEU and HIS may be implemented. None of the amino acids showed two-phase responses in low yielding cows.

Those three or four amino acids which might be limiting in the various groups are in Table 45. In the initial study VAL or LEU appeared co-limiting in the negative control and NPN groups, except for group P. Lysine was limiting or co-limiting for groups NC, U and P in the initial experiment. Histidine was identified as limiting in group PC in both experiments and co-limiting in group AU. The limiting amino acids identified by the above method are also those

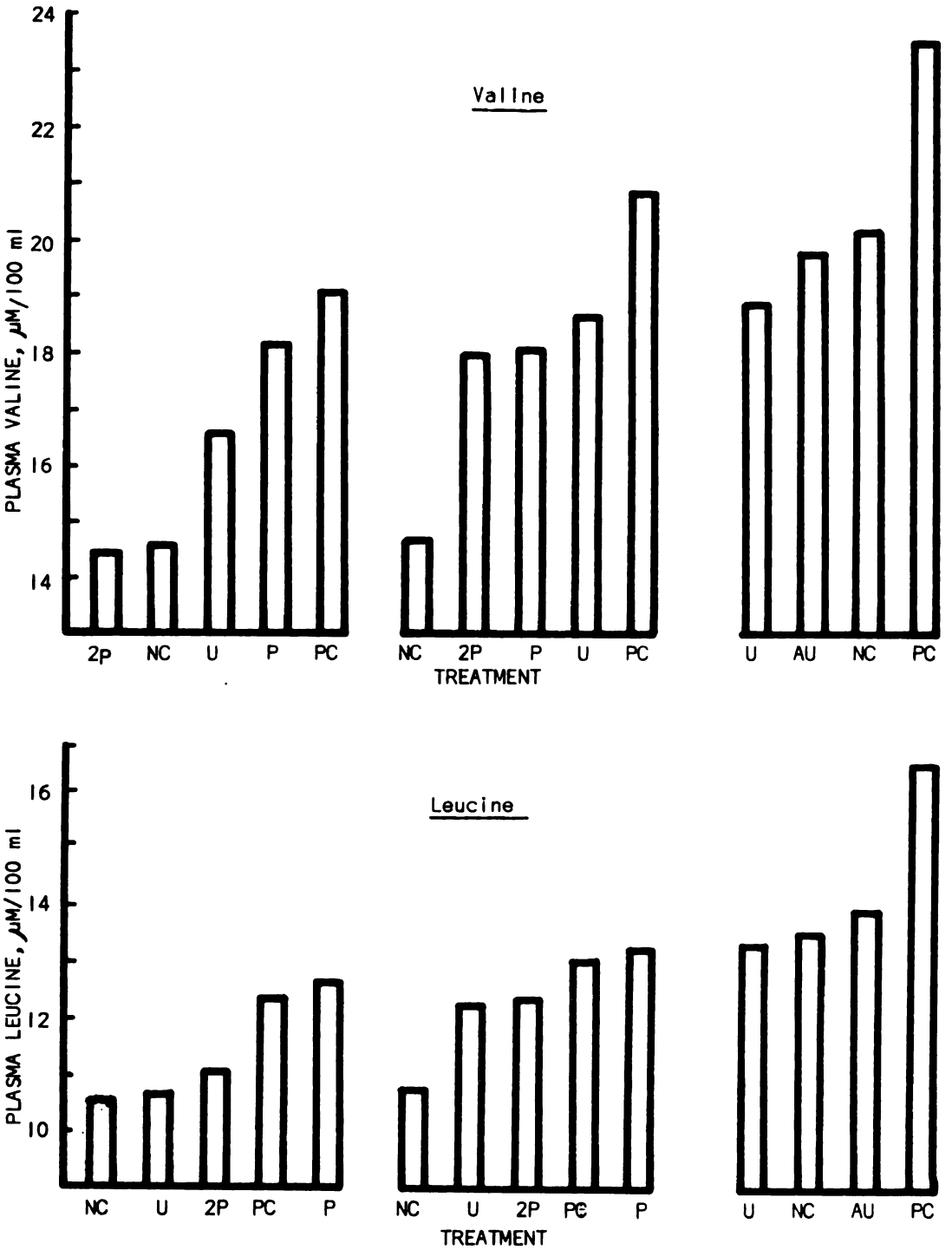

Figure 19. Plasma valine and leucine responses to treatments in the initial and the main experiment.

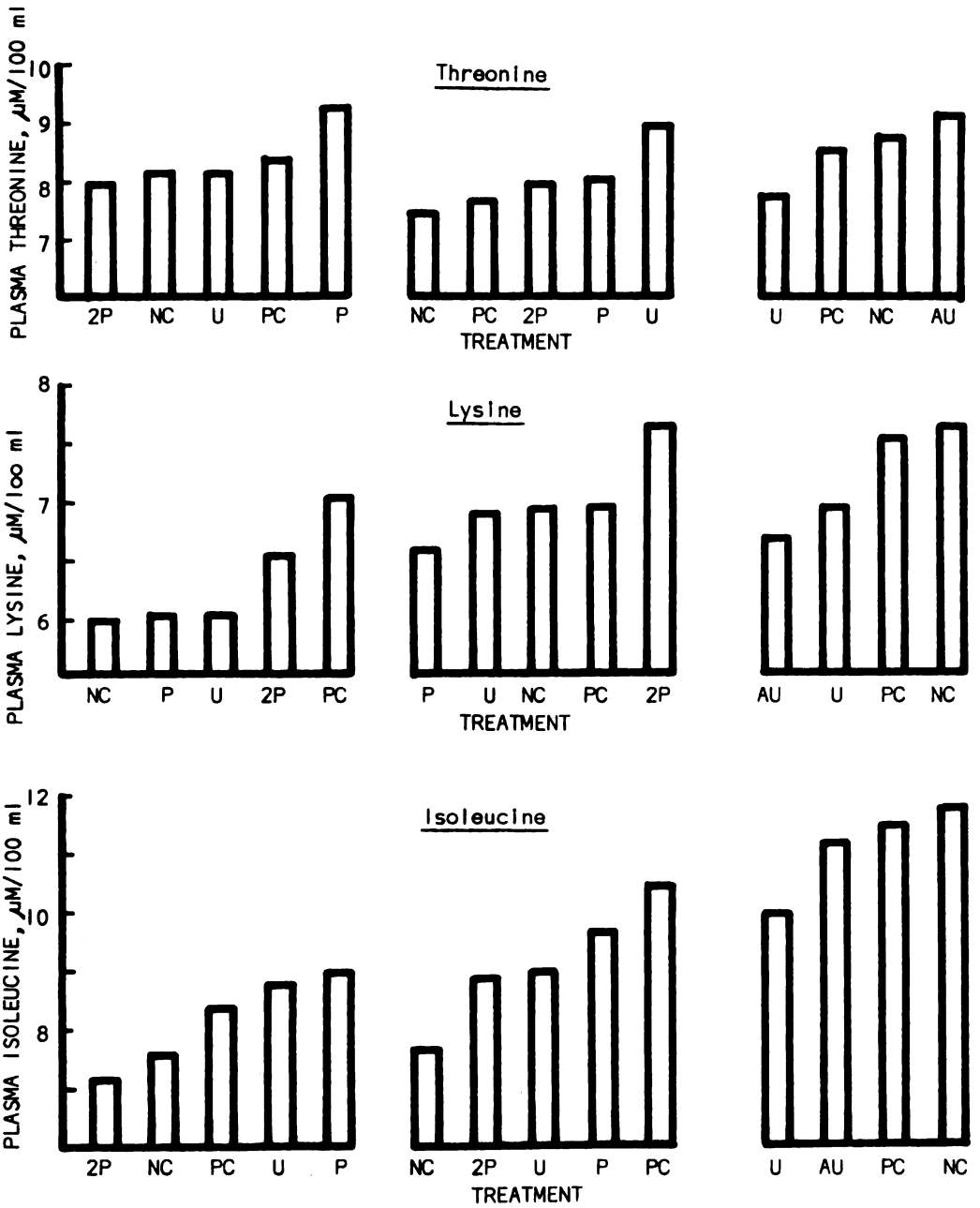

Figure 20. Plasma threonine, lysine and isoleucine responses to treatments in the initial and the main experiment.

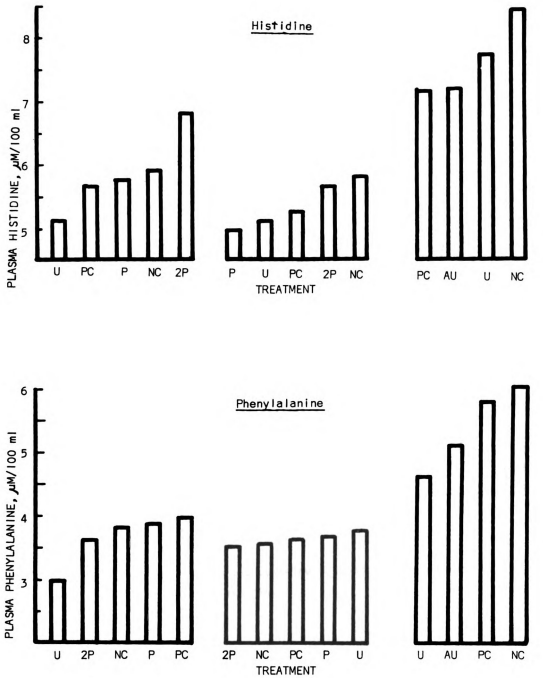

Figure 21. Plasma histidine and phenylalanine responses to treatments in the initial and the main experiment.

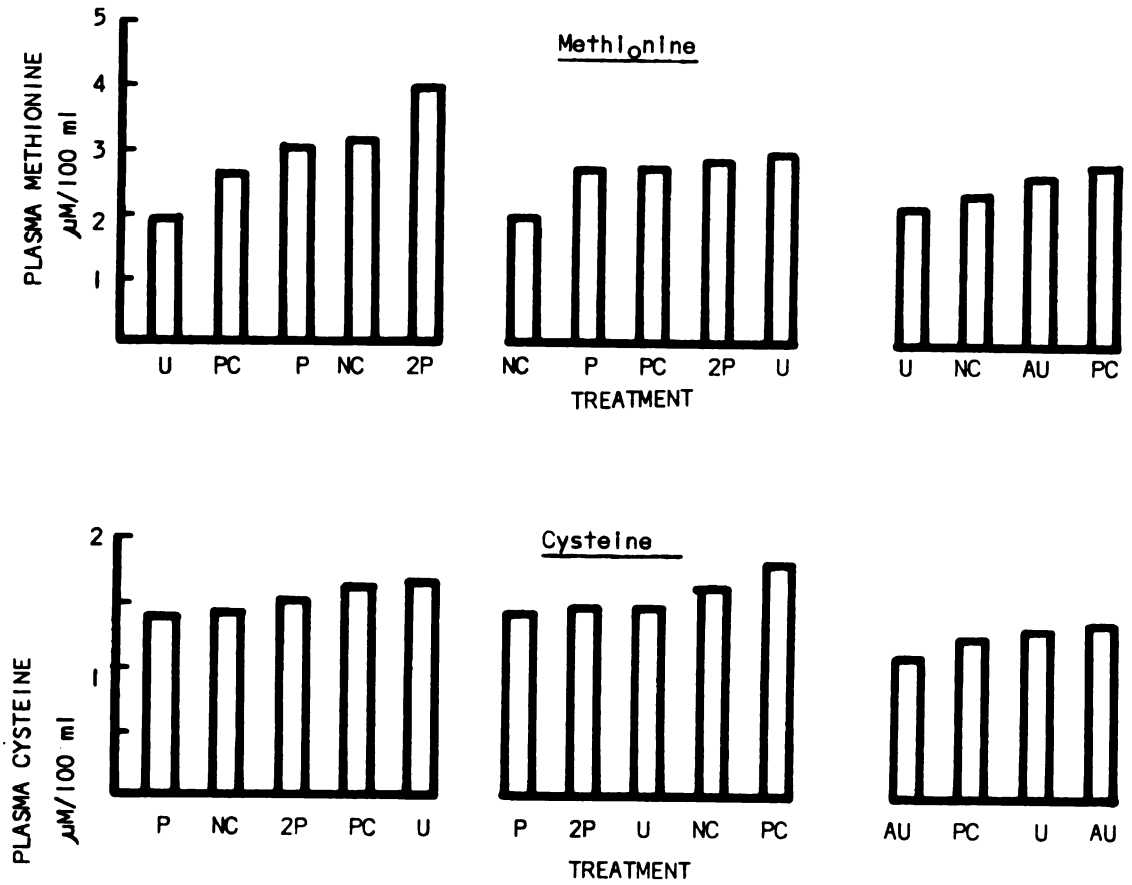

Figure 22. Plasma methionine and cysteine responses to treatments in the initial and main experiment.

Table 45.--Amino acids potentially limiting milk protein production of dairy cows in the initial and the main experiment, as well as in abomasal protein.

Treatment	Amino acid									
	VAL	LEU	THR	LYS	ILE	ARG	HIS	PHE	MET	CYS?
Initial experiment[a]:										
NC	*	*	*	*			*			
2P	*	*	*				*			
U		*	*	*			*			
P				*						
PC			*				*			
Main experiment[a]:										
NC	*	*								
PC							*			
U	*	*								
AU	*	*					*			
Main experiment[b]:										
NC		(2)[g]	(3)						(1)	(1?)
PC			(2)					(3)	(1)	(1?)
U		(3)						(2)	(1)	(1?)
AU				(2)				(1)	(3)	(1?)
Calculated[c]:										
Bacteria	(3)	(2)					(1)			
Corn	(3)			(1)	(2)					
SBM[d]	(3)			(2)					(1)	
75%+25%[e]	(2)				(3)		(1)			
75%+25%[f]	(2)						(1)		(3)	

[a]Determined by two-phase response in plasma amino acid concentrations.

[b]Determined by minimum plasma flow at the mammary gland for output in milk.

[c]Determined by the ratio between amino acid in "feed protein" and milk protein.

[d]Soybean meal.

[e]Abomasal protein is 75% microbial protein and 25% corn protein.

[f]Abomasal protein is 75% microbial protein and 25% soybean meal protein.

[g]Ranking of limiting amino acids (one first limiting).

which are generally in least supply when bacterial protein constitute the major fraction of protein available at the abomasal level, or when abomasal protein consists of 75% bacterial protein plus 25% corn or soy protein (Table 45). However, the amino acids identified by this method are in contrast to previous investigations (Table 7); except for LYS and HIS reported by Spires et al. (1973).

These discrepancies may be related to arterio-venous differences. Verbeke and Peters (1965), Clark et al. (1974), Derring et al. (1974), and Bickerstaffe et al. (1974) reported similar A-V for individual amino acids, but their research also showed highly variable A-V among amino acids. Chandler and Polan (1972) suggested minimum transfer coefficients as a method of comparison for the availability of amino acids for milk protein synthesis. In the present main study the drain on the amino acid pool was considered largest at 42 days post-partum. The minimum plasma supply for amino acid output in milk was calculated form average A-V for individual amino acids (Verbeke and Peters, 1965; Clark et al., 1974; Derring et al., 1974; and Bickerstaffe et al., 1974), amino acid composition of milk (NRC, 1969; NDC, 1965; Smith, 1971), and determined plasma amino acid concentrations and milk protein yields. The calculations are given in Table A14 and Table 46. Table 46 shows that MET was the most limiting in groups NC, U and PC, whereas PHE was indicated for group AU. These amino acids are also those most often indicated by other researches (see Table 7). However, it should be noted from Table 46 that the required plasma flow per day for several amino acids are very close to those for the amino acid identified as first limiting. Thus only slight changes in A-V may rearrange the three or four most limiting amino acids. Davis

Table 46.--Estimated minimum flow of plasma to the mammary gland for the output of amino acids in milk protein produced at 42 days post-partum in the main experiment, and the ranking of amino acids.

Amino acid	Treatment							
	NC		PC		U		AU	
	MPF[a]	Rank[b]	MPF	Rank	MPF	Rank	MPF	Rank
Essential amino acids:								
Valine	91	6	75	7	88	6	90	6
Leucine	111	2	70	8	108	3	104	4
Threonine	98	3	97	2	100	4	91	5
Lysine	96	4	93	4	96	5	107	2
Isoleucine	83	7	81	6	88	7	84	7
Arginine?	36	9	44	9	40	9	49	9
Histidine	74	8	83	5	73	8	81	8
Pheynlalanine	95	5	94	3	111	2	110	1
Methionine	122	1	99	1	121	1	105	3
Non-essential amino acids:								
Cysteine	140		140		132		156	
Glycine	102		109		109		106	
Alanine	123		126		134		124	
Proline	346		338		319		300	
Glutamic acid	136		115		97		101	
Serine	150		148		130		132	
Tyrosine	126		117		110		110	
Aspartic acid	624		472		532		490	

[a]Mammary plasma flow; hl/d.

[b]Ranking of amino acids according to required MPF. One for highest flow.

and Bauman (1974) failed to identify a pathway for the generation of CYS from MET. The required plasma flow for the output of CYS in milk was larger than for any of the EAA, but the flow also exceeded that expected for the output of milk if one were to assume a constant of 500 liters of blood per liter milk and a packed cell volume of 30%.

The limiting amino acids identified by plasma amino acid concentrations were also those included in a stepwise multiple regression (14). Amino acids with positive partial correlations (HIS, LEU, THR) indicate they are limiting, whereas those with negative partial correlations are those removed from plasma when the availability of the limiting nutrient is increased. Negative effects of increased methionine have previously been reported by Fisher (1972), Vik-Mo et al. (1974), and Schwab et al. (1976).

SUMMARY AND CONCLUSIONS

The review of literature showed that mammary blood flow, arterial metabolite concentrations, and the *in situ* metabolism are major determinants of milk yield. The mammary blood flow to milk ratio remain constant at approximately 500:1. The uptake of glucose and amino acids remain constant as a percentage of arterial blood concentrations. The biosynthesis of milk constituents appear to be interrelated, and the yield was increased during increased substrate availability (abomasal and/or intravenous infusion); but there has not been a concensus on the nutrient which is most limiting. Recent studies of the protein requirement in high yielding cows have not shown conclusive evidence of increased yields by protein supplementation beyond the amounts recommended by the National Research Council (1971). Crude protein has been used extensively in dairy cattle ration formulation but metabolizable protein and urea fermentation potential have been introduced as alternatives which are more in agreement with digestible protein in nonruminants. These methods allow extensive use of NPN in cows producing less than 20 kg milk per day, but certain assumptions and a weak data base render them useless in high producing dairy cows.

The present experiment was conducted: (1) to test protein requirements and the feasibility of substituting two sources of

nonprotein nitrogen (NPN) for natural protein in rations for high yielding cows early in lactation, and (2) to measure the effect of protein source on critical rumen and blood metabolites. This was tested in 68 lactating Holstein cows in weeks 3 through 20 post-partum. All cows were fed the same ration, containing NPN, from four weeks pre-partum through the second post-partum. An equal number of cows were assigned to four treatments. The first two groups received plant protein as the only nitrogen source, one 12 to 13% CP (group NC) and the other 15 to 16% (group PC). Two groups were also fed rations with 15 to 16% CP with a approximately 25% of total nitrogen as NPN. Corn silage treated with .65% urea (group U) or .40% ammonia (group AU) at ensiling was fed with concentrate containing 1.25% urea. Corn silage was fed ad libitum, hay was restricted to 2.2 kg per day, and grain was fed at one kg per 2.5 kg milk in the first 10 weeks post-partum and then reduced to one per 3 kg milk during weeks 11 to 20.

Dry matter and nutrient intakes were not statistically different between treatments except for crude protein, water soluble nitrogen and crude protein content in dry matter. However, group NC tended to have highest intakes of total dry matter and of dry matter per unit body weight. Actual milk yields for all cows average 29.9, 27.9, 27.0 and 27.7 kg per day for groups NC, PC, U and AU, respectively. Adjusted average milk for cows producing more than 25 kg milk per day in the second week post-partum were 32.6, 34.8, 32.1 and 32.8 kg per day in groups NC, PC, U and AU. Neither milk yield nor milk components were affected by treatments except for slightly lower yields for high producers in group U during weeks 3 through 6, post-partum.

Because average production for groups did not differ it was concluded that the crude protein requirement of high yielding cows fed corn, corn silage, and limited hay does not exceed 13% CP in DM. This conclusion was supported by rumen and blood metabolite concentrations. This study did not solve the question of the feasibility of using NPN as a substitute for plant protein in rations for high yielding cows. However, numerous experiments and theoretical considerations have demonstrated that NPN and plant protein are equally beneficial in high energy rations containing less than 13% crude protein in dry matter.

Rumen volatile fatty acids, plasma glucose, plasma ammonia nitrogen and amino acids were not significantly affected by protein treatments. Rumen ammonia and plasma urea nitrogen were lowest in group NC highest in U with PC and AU intermediate. The higher values for group U than AU were attributed to higher losses of ammonia during ensiling and to the presence of more plant protein in ammonia than urea treated silages. Plasma urea nitrogen in groups PC and AU were equal, despite lower rumen ammonia nitrogen in PC than AU. The calculated supply of protein at the abomasal level was equal in all groups when 10 to 20% of true protein consumed bypasses rumen fermentation. The equal plasma urea nitrogen concentrations in groups PC and AU, irrespective of the site of deamination, suggest similar losses of urea, whether supplementary nitrogen was from soybean oil meal or ammonia added to corn at ensiling plus urea in the grain. Based upon these observations and the effects of site and hours of sampling upon rumen ammonia nitrogen, it is concluded that plasma urea nitrogen is

superior to rumen ammonia nitrogen as a measurement of protein availability.

Factors affecting plasma amino acid concentrations were studied in two experiments. Essential amino acid concentrations were generally lowest when the demand was highest (high versus low producers; and early versus late lactation) and/or the supply was lowest (one vs. five hours after feeding). The method used in amino acid requirement studies with non-ruminants was adapted to these experiments. Based upon this method histidine and threonine were limiting or co-limiting on all treatments during the initial experiment and for PC and AU in the main experiment. Valine and leucine were co-limiting in all groups fed negative control or NPN rations except one in the initial experiment. In the initial experiment, lysine was also co-limiting for cows fed the negative control diet and those supplemented with urea- or ammonia-treated silages.

From the ratio of an amino acid in abomasal and milk protein, histidine is first limiting when rumen bypass of plant protein is less than 25%. Milk production was regressed on plasma amino acid concentrations and positive partial correlations were found only for histidine, valine and threonine. Lysine, methionine, cysteine and glutamic acid concentrations were negatively correlated with milk yield. The minimum plasma supply at the mammary gland for the output of amino acids in milk protein produced was calculated for results at 42 days post-partum in the main experiment. By this method the first limiting amino acid was methionine in groups NC, PC and U, but phenylalaline in group AU. The failure to clearly identify the limiting amino acid for a given treatment may be related to a nearly

identical minimum blood flow to the mammary gland for several essential amino acids and slight changes in the arterlovenous differences would change the rankings and suggested degree of limitation.

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APPENDIX A

Table A1.--Amounts of corn silage harvested by year and silo and its nutrient content.

Treatment	None		Urea				Ammonia			
Year of harvest	1975	1976	1975		1976		1975		1976	
Date of harvest	9/18	9/1-9/2	9/17	9/18	9/2	9/13	9/19	9/18	9/2	9/13
Silo no.	6	2	4	8	6	8	5	7	3	7
Fresh weight, t.	65.6	122.9	64.2	57.5	42.7	42.3	54.2	59.4	43.4	37.9
Dry matter, t.	17.1	36.9	17.4	15.3	13.3	16.2	14.6	15.7	13.5	15.2
Dry matter, %	26.1	30.0	27.1	26.6	31.2	38.3	26.9	26.4	31.1	40.1
Nitrogen: Total, % in DM	1.374	1.474	1.424	1.462	1.418	1.358	1.129	1.519	1.374	1.196
Soluble "	.454	.433	.355	.546	.418	.321	.292	.399	.449	.293
Insoluble,[a] "	.920	1.041	1.069	.916	1.000	1.037	.837	1.120	.925	.903
Crude protein; "	8.59	9.21	8.90	9.14	8.86	8.49	7.06	9.49	8.59	7.48
CPE;[b]	--	--	6.74	6.86	5.82	4.78	5.60	6.84	6.22	4.42
Expected CP;[c]	8.59	9.21	15.64	16.00	14.68	13.27	12.66	16.33	14.81	11.90
ADF;[d]	ND[e]	25.16	24.51	25.56	25.70	23.12	26.55	23.86	30.58	25.44

[a]By difference: Total N - Soluble N.

[b]Crude protein equivalent added as urea or ammonia $\{^{\text{urea}}_{NH_3} = 2.81 \atop = 5.14$

[c]Crude protein.

[d]Acid detergent fiber.

[e]Not determined.

Table A2.--Date of birth, record in the previous lactation, actual date of calving, genetic group and assignment to blocks within genetic group of cows used.

Cow No.	Date of Birth	Date of Calv. Prev.	Date of Calv. Actual	Age at Actual Calv.[c]	Calv. interval[c]	Previous Lactation Days in Milk	Previous Lactation Milk kg	Previous Lactation Fat kg	Previous Lactation Milk/d kg	Genetic Group[b]	Block Within br. group
Treatment NC											
1351	11-5-73	3-26-75	5-2-76	909	403	343	6459	215	18.8	B	1
1350	11-3-73	5-13-75	6-2-76	942	386	339	6114	250	18.0	B	2
1224	3-17-71	7-18-75	6-2-76	1940	320	273	5928	207	21.7	B	3
1457	6-25-74	-	7-7-76	743	-	-	-	-	-	B	4
1408	10-11-73	12-16-75	12-5-76	1151	355	300	6690	246	22.3	B	5
1334	11-30-72	1-9-76	1-13-77	1505	370	313	6563	229	21.0	B	6
1340	11-30-72	7-26-75	6-27-76	1305	337	305	4310	159	14.1	C	1
1460	7-9-74	-	10-19-76	833	-	-	-	-	-	C	2
1393	7-17-73	12-8-75	12-3-76	1235	361	307	6440	287	21.0	C	3
1415	10-28-73	12-26-76	1-30-77	1190	401	349	7142	238	20.5	C	4
1440	3-22-74	-	7-9-76	840	-	-	-	-	-	W	1
1452	6-12-74	-	5-28-76	716	-	-	-	-	-	W	2
1385	7-3-73	8-25-75	11-7-76	1223	440	387	7170	251	18.5	W	3
1302	5-26-72	11-5-75	10-31-76	1619	361	315	7175	255	22.8	W	4
1417	11-4-73	12-3-75	11-17-76	1109	350	287	5853	225	20.4	W	5
1356	1-28-73	3-2-76	2-20-77	1484	355	330	5914	202	17.9	W	6
1432	1-14-74	11-25-75	3-3-77	1144	464	398	7155	263	18.0	W	7

Table A2.--Continued.

Cow No.	Date of Birth	Date of Calv. Prev.	Date of Calv. Actual	Age at Actual Calv.[c]	Calv. interval[c]	Previous Lactation Days in Milk	Previous Lactation Milk kg	Previous Lactation Fat kg	Previous Lactation Milk/d kg	Genetic Group[b]	Block Within br. group
Treatment PC											
1264	10-21-71	10-2-75	9-26-76	1802	360	294	4440	186	15.1	B	1
1438	3-9-74	-	5-20-76	803	-	-	-	-	-	B	2
1444	4-30-74	-	5-28-76	759	-	-	-	-	-	B	3
1459	7-3-74	-	7-6-76	734	-	-	-	-	-	B	4
1369	3-20-73	11-8-75	10-26-76	1316	353	296	5754	212	19.4	B	5
1154	3-9-70	3-4-76	2-22-77	2542	355	305	6670	251	21.9	B	6
1220	3-8-71	6-17-75	6-2-76	1913	351	281	4122	160	14.7	C	1
1469	9-16-74	-	10-16-76	761	-	-	-	-	-	C	2
1203	12-12-70	10-29-75	10-1-76	2120	338	272	3780	197	17.6	C	3
1421	11-24-73	12-24-75	1-11-77	1144	384	315	7524	261	23.9	C[a]	4
1450	6-7-74	-	6-7-76	731	-	-	-	-	-	W	1
1466	8-10-74	-	7-1-76	691	-	-	-	-	-	W	2
1390	7-11-73	8-23-75	10-27-76	1204	426	368	6390	231	17.4	W	3
1263	10-12-71	10-17-75	11-12-76	1858	392	284	5246	188	18.5	W	4
1462	7-24-74	-	10-10-76	809	-	-	-	-	-	W	5
1322	10-27-72	1-2-76	1-2-77	1528	366	306	5128	192	16.8	W	6
1195	10-26-70	2-24-76	3-9-77	2326	379	302	8473	346	28.1	W	7

Table A2.--Continued.

Cow No.	Date of Birth	Date of Calv. Prev.	Date of Calv. Actual	Age at Actual Calv.[c]	Calv. interval[c]	Previous Lactation Days in Milk	Previous Lactation Milk kg	Previous Lactation Fat kg	Previous Lactation Milk/d kg	Genetic Group[b]	Block Within br. group
Treatment U											
1218	12-1-71	7-22-75	9-1-76	1745	416	316	6989	283	22.1	B	1
1359	2-6-73	4-3-75	5-17-76	1196	410	335	7039	248	21.0	B	2
1374	4-22-73	7-15-75	6-15-76	1150	336	-	-	-	-	B	3
1456	6-25-74	-	7-3-76	739	-	-	-	-	-	B	4
1387	7-3-73	9-7-75	10-28-76	1213	417	348	6877	273	19.2	B	5
1347	1-1-73	2-2-76	3-7-77	1526	399	324	5906	200	18.2	B	6
1424	12-5-73	-	6-13-76	921	-	-	-	-	-	C	1
1458	6-25-73	9-21-75	9-15-76	1178	360	303	6735	237	22.2	C	2
1282	1-19-72	10-24-75	1-11-77	1819	445	353	7418	255	21.0	C	3
1419	11-14-73	12-3-75	11-14-76	1096	347	287	4756	176	16.6	C	4
1451	6-10-74	-	7-11-76	762	-	-	-	-	-	W	1
1416	10-28-73	-	5-19-76	934	-	-	-	-	-	W	2
1379	5-6-73	8-25-75	10-12-76	1255	414	371	7985	318	21.5	W	3
1321	9-16-72	11-19-75	11-11-76	1517	358	301	4473	176	14.9	W	4
1434	1-26-74	-	9-18-76	966	-	-	-	-	-	W	5
1358	1-29-73	1-24-76	12-22-76	1423	333	261	4035	157	15.5	W	6
1475	10-27-74	-	1-1-77	797	-	-	-	-	-	W[a]	7

Table A2.--Continued.

Previous Lactation

Calv

Block

Table A2.--Continued.

Cow No.	Date of Birth	Date of Calv. Prev.	Date of Calv. Actual	Age at Actual Calv.[c]	Calv. interval[c]	Previous Lactation Days in Milk	Previous Lactation Milk kg	Previous Lactation Fat kg	Previous Lactation Milk/d kg	Genetic Group[b]	Block Within br. group
Treatment AU											
1345	12-26-72	6-2-75	5-15-76	1236	348	311	7282	253	23.4	B	1
1411	10-12-73	-	6-9-76	971	-	-	-	-	-	B	2
1419	5-27-74	-	7-6-76	771	-	-	-	-	-	B	3
1465	8-1-74	-	12-14-76	866	-	-	-	-	-	B	4
1377	5-3-73	8-12-75	10-25-76	1271	440	400	7347	245	18.4	B	5
1331	10-25-72	2-29-76	2-20-77	1579	357	309	9213	283	29.8	B	6
1367	3-11-73	6-7-75	3-3-76	1088	270	270	4322	158	16.0	C[b]	1
1463	7-24-74	-	10-12-76	811	-	-	-	-	-	C[b]	2
1254	8-25-71	11-28-75	12-5-76	1929	373	308	5479	208	17.8	C[b]	3
1439	3-18-74	1-31-76	3-17-77	1095	411	371	6196	221	16.7	C[a]	4
1364	3-3-73	6-19-75	5-19-76	1173	335	258	3655	132	14.2	W	1
1386	7-3-73	8-20-75	7-10-76	1103	325	240	4638	184	19.3	W	2
1404	9-14-73	-	7-3-76	1023	-	-	-	-	-	W	3
1260	9-29-71	12-2-75	11-24-76	1883	358	237	4248	176	17.9	W	4
1418	11-9-73	12-3-75	11-3-76	1090	336	271	5851	200	21.6	W	5
1400	8-23-73	1-25-76	1-5-77	1231	346	283	7061	232	25.0	W	6
1474	10-22-74	-	12-12-76	782	-	-	-	-	-	W	7

[a]Best cow.

[b]Worst cow.

[c]Days.

Table A3.--Simple correlations among nutrient and quality measurements on corn silage.[a,b]

	DM	% in DM									
	%	TN	SOLN	ADN	ADF	Et	Ac	Pr	I-bu	Bu	Lact
% in DM:	--										
TN	-.43	--									
SOLN	-.35	.80	--								
ADN	NS	NS	NS	--							
ADF	.37	.21	NS	NS	--						
Et	-.36	.32	.45	NS	NS	--					
Ac	NS	NS	NS	NS	.23	NS	--				
Pr	NS	NS	NS	NS	NS	.30	.22	--			
I-bu	NS	NS	NS	NS	NS	NS	NS	NS	--		
Bu	NS	NS	.27	.30	-.41	.31	NS	NS	.35	--	
Lact	NS	NS	NS	NS	NS	NS	.27	NS	-.34	NS	--

[a]Correlation coefficients exceeding +.207 are significant (P<.05). Non significant (NS) correlations were deleted.

[b]Abbreviations: DM, dry matter; TN, total nitrogen; SOLN, water soluble nitrogen; ADN, acid detergent nitrogen, ADF, acid detergent fiber; Et, ethanol; Ac, acetate; Pr, propionate; I-bu, iso-butyrate; Bu, butyrate; Lact, lactate.

Table A4.--Partial correlations between the dependent variable and independent variables selected by multiple regression analysis.[a]

Dependent variable (y)	Independent variables (x_i)					Constant	R	R^2	Significance of R
	DM %	TN	SOLN	ADN	ADF				
Ethanol, % in DM	-.37	NS[b]	.36	NS	-.36	.40	.59	.35	P<.0005
Acetate, % in DM	NS	NS	NS	NS	.23	-.07	.23	.05	P=.031
Propionate, "	NS	NS	NS	NS	NS	NS	NS	NS	NS
Iso-butyrate, "	NS	NS	NS	NS	NS	NS	NS	NS	NS
Butyrate, "	-.30	NS	NS	.35	-.51	.45	.57	.33	P<.005
Lactate, "	NS	NS	NS	NS	NS	NS	NS	NS	NS

[a]Independent variables were included in the prediction equation if P<.050 and they were deleted if P<.051.

[b]Not significant at P<.050.

Table A5.--Effects of breeding groups on feed and nutrient intake, milk yield and composition and body weight.

Item	Breeding group			s.e.[a]	Significance of[b]		
	Best	Control	Worst		B	BxT	BxTime
Number of cows	24	16	28				
Feed and nutrient intake:							
Concentrate, kg/d	9.50	10.43	9.93	3.11	NS	P<.001	NS
Corn silage, kg/d	22.93	21.87	23.52	5.92	NS	P<.001	NS
Hay, kg/d	2.26	2.17	2.29	.38	NS	P<.001	NS
Dry matter, kg/d	17.86	18.35	18.62	4.13	NS	P<.10	NS
Net energy, Mcal/d.	32.68	33.95	34.17	8.16	NS	P<.10	NS
Crude protein, kg/d	2.62	2.79	2.79	.71	NS	P<.01	NS
Soluble nitrogen, kg/d	.082	.078	.082	.018	NS	NS	NS
ADF, kg/d	3.23	3.24	3.39	.71	NS	NS	P<.10
Crude protein in DM,%	14.67	15.20	14.98	.83	P<.25	P<.25	P<.05
Milk production							
Milk, kg/d	26.61	29.23	27.42	4.40	NS	NS	NS
Fat, kg/d	.96	1.03	1.03	.25	NS	NS	NS
Protein, kg/d	.85	.95	.92	.17	NS	NS	NS
Total solids, kg/d	13.28	3.59	3.42	.45	NS	NS	P<.25
Solids non fat, kg/d	2.33	2.55	2.39	.52	NS	NS	NS
Fat corrected milk, kg/d	24.99	27.18	26.43	4.46	NS	NS	P<.25
Solids corrected milk, kg/d	25.02	27.27	26.27	3.56	NS	NS	NS
Fat , %	3.64	3.58	3.77	.46	NS	NS	NS
Protein , %	3.23	3.28	3.37	.12	P<.01	NS	P<.05
Total solids , %	12.41	12.35	12.47	.48	NS	NS	P<.25
Solids non fat, %	8.78	8.77	8.70	.21	NS	P<.05	P<.05
Body weight, kg	564.	582.	581.	33.	NS	P<.10	P<.001
Energy balance % of NRC	117.	113.	117.	6.60	NS	NS	NS
Protein balance % of NRC	99.	97.	100.	5.18	NS	NS	NS

[a]Standard error for 24 cows per group.

[b]B is breeding group; T is treatment; and Time in days from calving.

Table A6.--Interaction between breeding groups and treatments for the solids non fat percentage in milk.[a]

Breeding group	No.[b]	Treatment				Overall	
		NC	PC	U	AU	Av.[c]	s.e.[d]
Best	24	8.64	8.85	8.70	8.92	8.78	.21
Control	16	8.82	8.53	8.79	8.95	8.77	.26
Worst	28	8.60	8.67	9.07	8.47	8.70	.20
Overall Av.	68	8.67	8.70	8.87	8.74	8.75	--
s.e.		.32	.32	.32	.32	--	.20

[a]17 Cows per treatment mean.

[b]Number of cows per breeding group.

[c]Average.

[d]Standard error.

Table A7.--Interaction between breeding groups and treatments on the average bodyweight.[a,c]

Breeding group	No.[b]	Treatment				Overall	
		NC	PC	U	AU	Av.	s.e.
Best	24	591	561	564	542	564	33
Control	16	587	574	575	593	582	40
Worst	28	568	601	560	596	581	30
Overall Av.	68	581	581	565	576		--
s.e.		23	23	23	23	--	23

[a]17 cows per treatment group.

[b]Number of cows per breeding group.

[c]The number of cows per breeding group within treatment are 6 best, 4 control, and 7 worst and the standard errors are 39, 47 and 36 kg, respectively.

Table A8.--Simple correlation among milk parameters, body weight and the balances for net energy and protein in high yielding cows within periods f,g.

	milk kg/d	F %	P %	TS %	SNF %	F kg/d	P kg/d	TS kg/d	SNF kg/d	FCM kg/d	SCM kg/d	BW kg	Balance NE	% Prot.	Correlation with covariate[e] wk.3-6[a]	wk 7-10[b]
Milk, kg/d.	-	-	-	-	-	.69	.78	.93	.94	.84	.88	.34	-.31	-	.86	.68
F%	-	-	-	.78	.25	.81	-	.43	-	.66	.58	.34	-.62	-	.32	-.07
P%	-.28	.31	.55	.57	.71	-	.52	-	-	-	-	-	-	-	.48	.26
TS%	-	.84	.51	-	.40	.56	.34	.35	-	.42	.45	.40	-.29	-	.27	.16
SNF%	-	-	-	.49	-	-.32	.26	-	-	-.32	-	-	-.46	-	.38	-.17
F kg/d	.61	.78	.34	.62	-	-	.58	.85	.59	.97	.93	.47	-.61	-.34	.55	.31
P kg/d	.80	-	-	.28	-	.64	-	.85	.89	.69	.79	.37	-	-	.67	.60
TS kg/d	.92	.31	-	.33	-	.82	.86	-	.92	.94	.98	.47	-.38	-.26	.79	.69
SNF kg/d	.95	-	-	-	-	.58	.85	.94	-	.74	.84	.38	-	-	.70	.44
FCM kg/d	.82	.56	-	.43	-	.95	.77	.94	.78	-	.98	.46	-.56	-.32	.66	.49
SCM kg/d	.85	.48	-	.45	-	.91	.83	.98	.86	.98	-	.49	-.47	-.29	.72	.63
BW, kg	-	-	-	-	-	.32	.31	.25	-	.31	.28	-	-.26	-	.97	.91
Balance NE %	-.32	-.43	-	-.26	-	-.55	-	-.41	-.25	-.52	-.47	-	-	.35	.56	.15
Protein %	-	-	-	-	-	-.29	-	-.27	-		-.29	-	.43	-	.16	-.03
Wk 11-15[c] wk 16-20[d]															wk 11-15	wk 16-20
Milk kg/d.	-	-	-.27	-.29	-.34	.68	.84	.93	.97	.88	.87	-	-.41	-.26	.60	.55
F%	-.50	-	.41	.89	.45	.60	-	-	-	.31	.30	-	-	-	-.06	-.21
P%	-.31	.47	-	.63	.70	-	.29	-	-	-	-	-	.40	.35	.42	.41
TS%	-.25	.64	.46	-	.80	.42	-	-	-	-	-	-	-	-	.10	.26
SNF%	-	-	-	.71	-	-	-	-	-	-	-	-	-	-	.18	.25
F kg/d	.52	.47	-	.34	-	-	.74	.87	.74	.95	.94	-	-.27	-	.36	.25
P kg/d	.88	-.28	-	-	-	.58	-	.90	.90	.85	.88	-	-	-	.63	.62
TS kg/d	.94	-.28	-	-	.36	.67	.89	-	.97	.97	.99	-	-.36	-	.64	.63
TS kg/d	.95	-.49	-	-	.43	.47	.87	.97	-	.90	.93	-	-.37	-	.46	.39
SNF kg/d	.84	-	-	-	-	.90	.82	.91	.78	-	.99	-	-.36	-	.52	.48
FCM kg/d	.89	-	-	-	.32	.80	.87	.98	.91	.96	-	-	-.34	-	.59	.60
BW kg	-	-	.34	-	-	-	-	-	-	-	-	-	-	-	.88	.84
Balance NE %	-	-	-	-	-	-	-	-	-	-	-	-	-	.44	.23	-.05
Protein %	-	-	-	-	.32	-	-	-	-	-	-	-	.34	-	-.03	-.12

[a]Upper triangle of correlation matrix is for wk 3-6.

[b]Lower triangle of correlation matrix is for wk 7-10.

[c]Upper triangle of correlation matrix is for wk 11-15.

[d]Lower triangle of correlation matrix is for wk 16-20.

[e]Observation in second week after calving.

[f]68 observation $r \geq .249$ $P<.05$, $r \geq .309$ $P<.01$.

[g]Abbreviations F, P, TS, SNF, FCM, SCM, BW are fat, protein total solids, solids non fat, fat corrected, and fat solids corrected milk, and body weight, respectively. NE and CP are net energy for lactation and crude protein.

Table A9.--Effects of breeding groups on rumen pH and rumen concentrations of volatile fatty acids and ammonia nitrogen.

Fatty acid	Breeding group				Significance of		
	Best	Control	Worst	s.e.	B	B*T	B*Time
Number of cows	24	16	28		P<.10	NS	NS
Rumen pH.	7.03	7.08	7.13	.11	NS	NS	NS
Millimoles per liter							
Acetate	44.50	40.14	42.59	3.28	NS	NS	NS
Propionate	15.44	15.04	14.06	1.91	NS	NS	NS
Iso-butyrate	.53	.45	.50	.04	P<.10		
Butyrate	8.26	7.57	8.00	.69	NS	NS	NS
2-methyl-butyrate	.55	.52	.54	.06	NS	NS	NS
Iso-valerate	.40	.36	.41	.05	NS	NS	NS
Valerate	.88	.94	.89	.10	NS	NS	P<.10
Total acids	70.45	65.14	67.06	5.73	NS	NS	NS
Molar present:							
Acetate	63.63	61.98	64.25	1.17	P<.10	P<.05	P<.25
Propionate	21.42	22.83	20.38	1.30	P<.10	P<.05	P<.25
Iso-butyrate	.76	.72	.77	.04	NS	NS	NS
Butyrate	11.66	11.60	11.82	.44	NS	NS	NS
2-methyl-butyrate	.77	.81	.81	.07	NS	NS	NS
Iso-valerate	.57	.57	.63	.06	NS	NS	NS
Valerate	1.25	1.49	1.34	.02	P<.25	P<.25	P<.25
Rumen ammonia-N mg/100ml.	10.67	13.31	12.37	2.50	NS	NS	NS

Table A10.--Effect of breeding group on the rumen concentrations of iso-butyrate and valerate, mMoles/liter.

Breeding group	Iso-butyrate[e]			Valerate[f]					
				Days from calving					
	No[a]	Av.[c]	s.e.[d]	No[b]	-14	21	70	126	s.e.
Best	96	.53[g]	.02	24	.63[gi]	.83[gij]	1.16[gk]	.90[gj]	.10
Control	64	.45[h]	.02	16	.64[gi]	1.26[hj]	.96[hk]	.87[gik]	.12
Worst	112	.50[g]	.02	28	.71[gi]	.81[gi]	1.11[gj]	.92[gi]	.09

[a]Number of observations per mean for iso-butyrate.

[b]Number of observations per mean within a row for valerate.

[c]Average.

[d]Standard error.

[e]$p<.10$ by analysis of variance.

[f]$p<.10$ by analysis of variance.

[g,h]Means within the column without a common superscript are different ($P<.05$).

[i,j,k]Means within a row for valerate within a common superscript are different ($P<.05$).

Table A11.--Effect of breeding group on the molar distribution of acetate, propionate and valerate, molar percent.

Acid		Breeding group			Significance of breeding group[d]
		Best	Control	Worst	
Number of obs.[a]		96	64	112	
Acetate	Av.[b]	63.63[e]	61.98[f]	64.25[e]	P<.10
	s.e.[c]	.59	.72	.54	
Propionate	Av.	21.42[ef]	22.83[e]	20.38[f]	P<.10
	s.e.	.65	.80	.60	
Valerate	Av.	1.25[e]	1.49[f]	1.34[ef]	P<.25
	s.e.	.07	.09	.07	

[a]Number of observations per breeding group.

[b]Average.

[c]Standard error.

[d]Determined by analysis of variance.

[e,f]Means within a row without a common superscript are different (P<.05).

Table A12.--Effects of breeding group on plasma concentrations of glucose, urea and ammonia nitrogen; mg/100ml.

	Breeding group				Significance of[b]		
	Best	Control	Worst	s.e.[a]	B	B*T	B*Time
Number of cows per mean	24	16	28				
Glucose	53.51	52.59	54.26	2.37	NS	NS	NS
Urea nitrogen	12.51	13.72	12.94	1.39	NS	NS	NS
Ammonia-N	.33	.32	.33	.06	NS	NS	NS

[a]Standard error for 24 cows per mean.

[b]B is breeding group; T is treatment and Time is days from calving.

Table A13.--Plasma amino acid concentrations (μM/100 ml) in breeding groups and by treatment and/or days from calving within breeding groups.

Amino acid	DFC/ TRT	Breeding group			Amino acid		Breeding group		
		B	C	W			B	C	W
VAL		18.71	18.05	20.65	GLY	-14	31.66	30.46	34.45
	-14	20.43	18.42	18.61		42	30.54	46.83	32.93
	42	17.24	18.85	22.18		126	22.00	23.23	23.98
	126	18.86	17.17	21.12					
LEU		12.44	12.50	14.05	SER	-14	8.88	6.87	9.80
	NC	13.28	14.58	11.70		42	8.60	11.85	9.26
	PC	12.92	10.27	17.05		126	7.43	7.59	8.28
	U	11.61	12.06	13.62					
	AU	11.70	13.09	13.78	ASP	NC	3.03	2.51	2.41
						PC	2.40	2.47	3.00
ILE		11.06	10.23	12.37		U	2.57	2.67	3.30
						AU	2.65	4.85	2.87
His	NC	7.80	7.84	6.56		-14	2.43	2.08	2.68
	PC	6.31	7.45	6.80		42	2.98	3.26	3.19
	U	6.88	7.81	6.60		126	2.47	3.81	2.56
	AU	6.75	6.88	7.39					
PHE		5.63	4.76	5.13					
CYS	NC	1.79	2.80	1.76					
	PC	1.63	1.31	1.76					
	U	1.73	2.08	1.70					
	AU	1.55	1.46	1.47					

Table A14.--Minimum plasma flow at mammary gland for output of individual amino acids in milk protein produced.

	Avd[a]		Milk prot.	Molecular	Plasma amino acids[e], g/hl				Milk amino acids,[e] g/d				Plasma,[g] hl/d			
					Treatment				Treatment							
	Av	s.e.	g/16gN[b]	weight, g	NC	PC	U	AU	NC	PC	U	AU	NC	PC	U	AU
	(A)		(B)	(C)					986[f]	944[f]	891[f]	956[f]	161.65[h]	152.53[h]	143.63[h]	152.13
ARG	51.5	2.4	3.25	174.21	1.73	1.36	1.41	1.24	32.05	30.68	28.95	31.07	35.97	43.80	39.87	48.65
HIS	27.0	1.8	2.66	155.16	1.32	1.12	1.21	1.12	26.23	25.11	23.70	24.43	73.60	83.04	72.54	80.70
ILE	42.5	2.2	5.52	131.18	1.55	1.51	1.32	1.47	54.43	52.11	49.18	52.77	82.63	81.20	87.66	84.47
LEU	45.1	1.2	9.07	131.18	1.78	2.72	1.76	1.84	89.43	85.62	80.81	86.71	111.40	69.80	101.81	104.49
LYS	59.6	.9	8.14	182.65	1.40	1.38	1.27	1.22	80.26	76.84	72.53	77.82	96.19	93.42	95.82	107.02
MET	56.5	3.4	2.54	149.21	.35	.43	.33	.41	24.04	23.98	22.63	24.28	121.57	98.70	121.37	104.81
PHE	42.6	1.8	4.10	165.19	1.00	.97	.77	.84	40.42	38.70	36.53	39.20	94.88	93.65	111.37	109.55
THR	35.8	1.3	3.75	119.12	1.05	1.02	.93	1.10	36.98	35.40	33.41	35.85	98.38	96.94	100.35	91.04
VAL	28.3	1.1	6.19	117.15	2.37	2.76	2.22	2.32	61.03	58.43	55.15	59.18	90.99	74.81	87.78	90.14
TRP	-	-	1.25	204.23	-	-	-	-	12.33	11.80	11.14	11.95				
ALA	12.2	2.3	3.24	89.09	2.13	1.99	1.77	2.05	31.95	30.59	28.87	30.97	122.95	126.00	133.69	123.83
ASN	31.0	-	-	-	-	-	-	-	-	-	-	-				
ASP	31.0	5.4	6.67	132.12	.34	.43	.36	.42	65.77	62.96	59.43	63.77	624.00	472.32	532.53	489.78
LYS	20.5	1.5	.67	120.15	.23	.22	.22	.20	6.61	6.32	5.97	6.41	140.19	140.13	132.37	156.34
GLN	24.0	-	-	-	-	-	-	-	-	-	-	-				
GLU	60.9	2.3	21.35	147.13	2.54	2.87	3.23	3.33	210.51	201.54	190.23	204.11	136.09	115.31	96.71	100.65
GLY	6.2	1.4	1.83	75.07	2.86	2.55	2.42	2.67	18.04	17.28	16.31	17.49	101.74	109.30	108.70	105.65
PRO	26.0	-	9.39	115.13	1.03	1.01	1.01	1.15	92.59	88.64	83.66	89.77	345.74	337.55	318.58	300.23
SER	34.2	4.2	5.19	105.09	1.00	.97	1.04	1.10	51.17	48.99	46.24	49.62	149.62	147.68	130.00	131.90
TYR	47.2	2.9	5.38	181.19	.89	.92	.92	.99	53.06	50.79	47.94	51.43	126.31	116.96	110.40	110.06
ORN	46.4	5.7	-	-	-	-	-	-								
CIT	9.0	4.4	-	-	-	-	-	-								

[a]Arterio-venous difference as % of arterial concentration from Clark et al., 1974; Bickerstaffe et al., 1974; Verbeke and Peeters, 1965.

[b]NRC, 1969; NDC, 1965; Smidth, 1971

[c]Plasma amino acids = $\mu M/l * MW * 10^{-5}$.

[e]Milk amino acid = Milk protein*(A)/100.

[f]Milk protein, g/d.

[g]$\text{Blood} = \frac{\text{Milk amino acids}}{\text{Plasma amino acid} * \text{AVd.}}$

[h]hl blood /d for milk yield; 500 liters/kg milk.

APPENDIX B

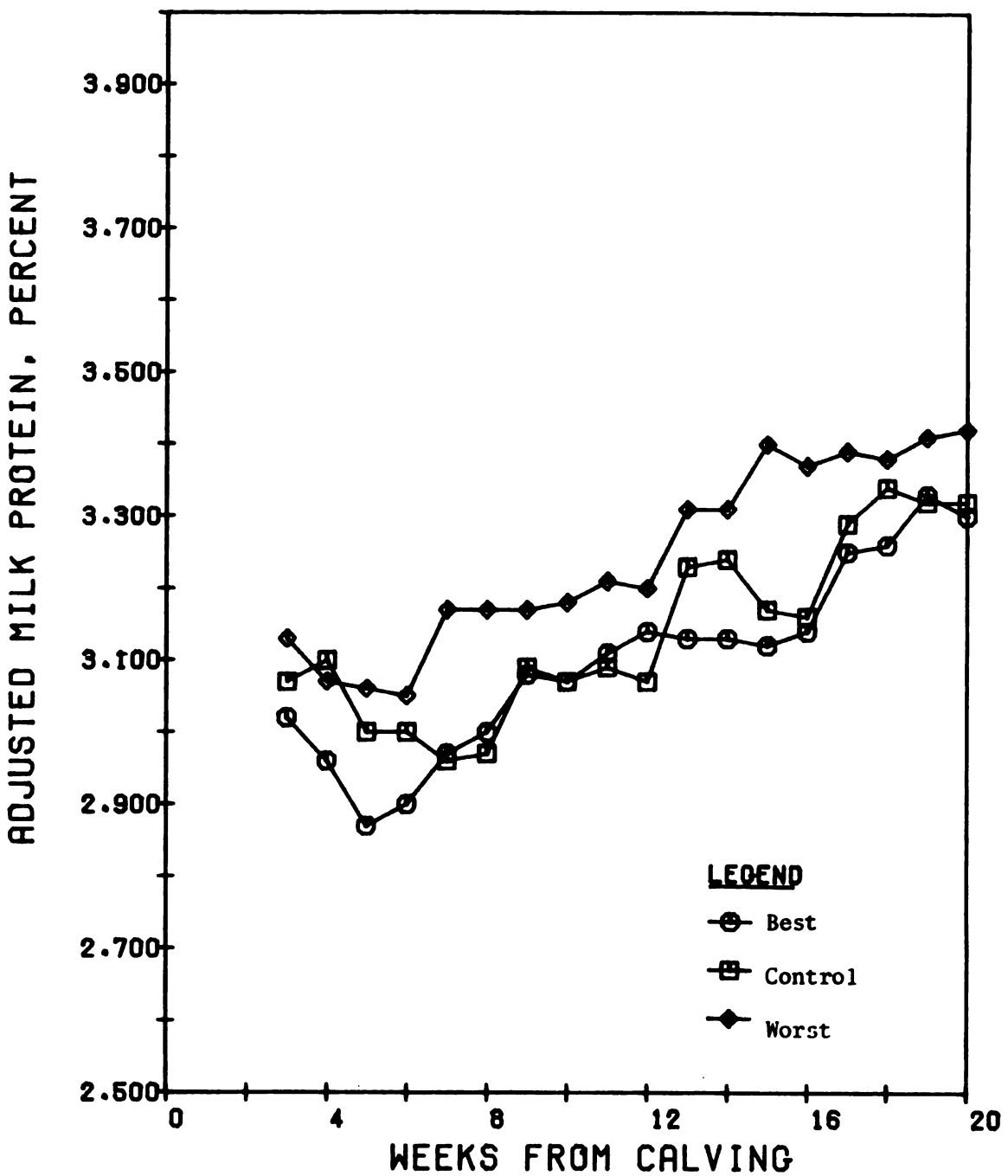

Figure B1. Adjusted content of protein in milk for breeding groups per week from calving (standard error ± .05).

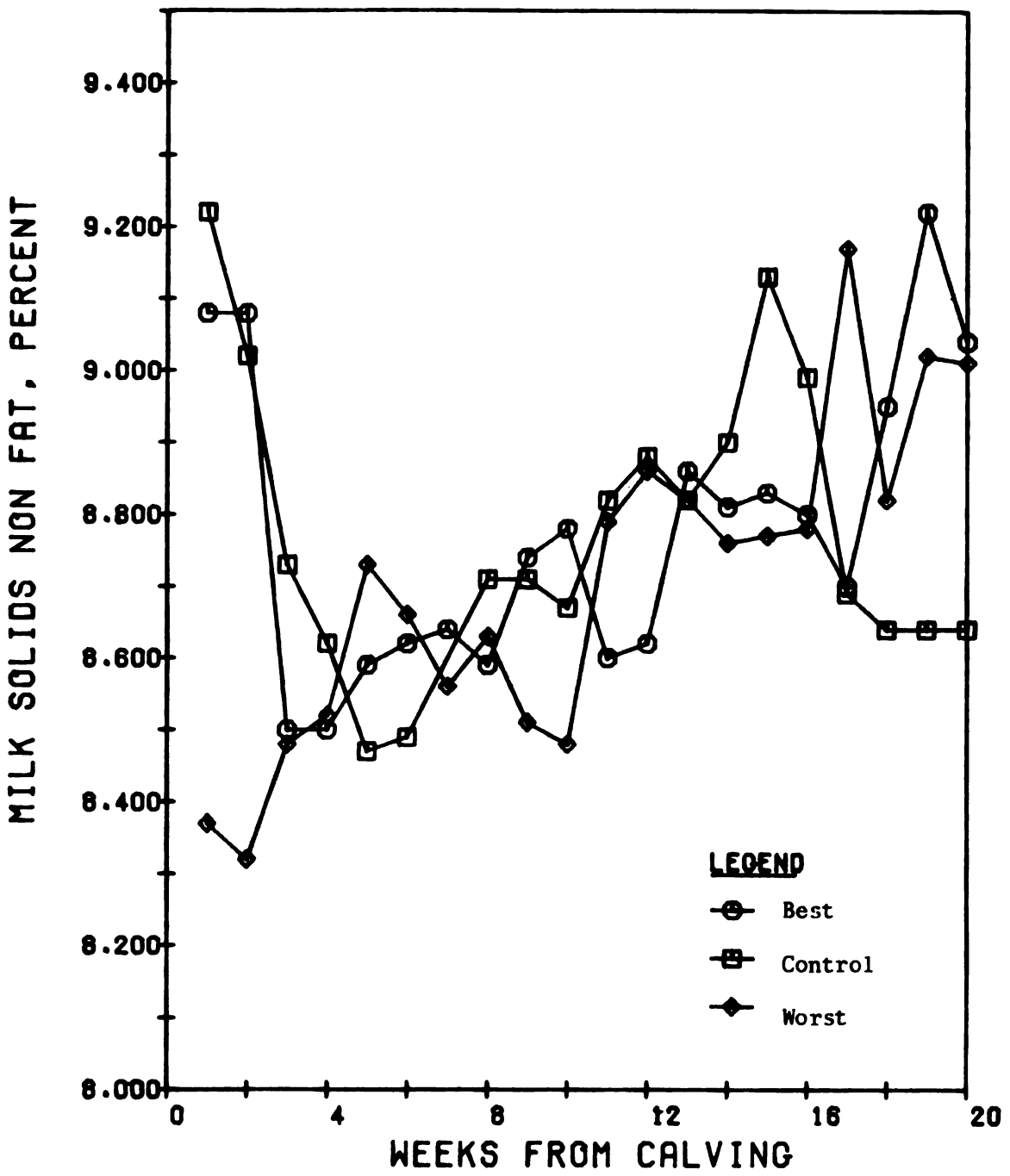

Figure B2. Concentrations of solids-non-fat in milk for breeding groups per week from calving (standard error ± .13).

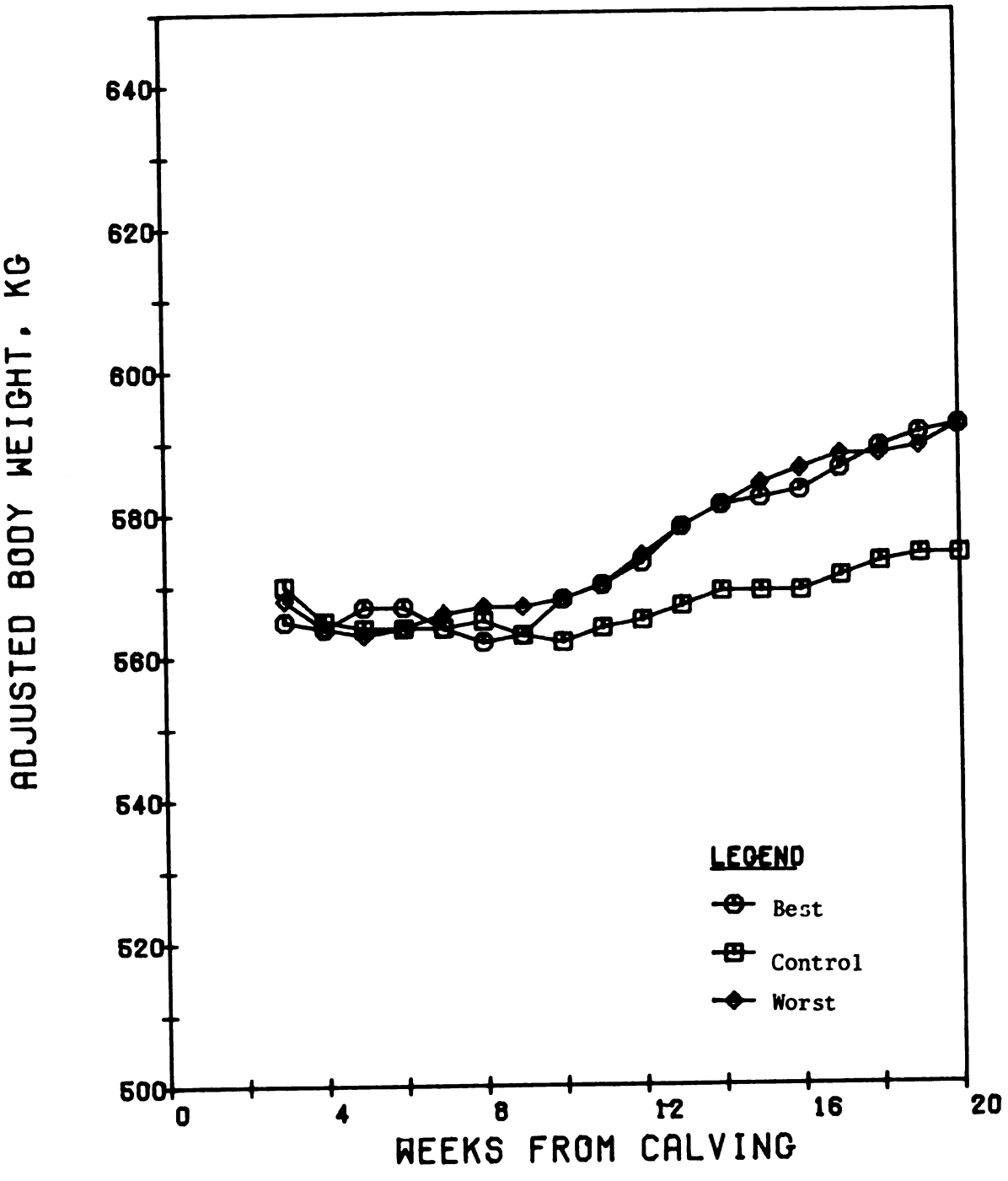

Figure B3. Adjusted body weight for breeding groups per week from calving (standard error ± 3.0).