ABOMASAL INFUSION OF PROTEIN AND GLUCOSE IN LACTATING COWS

Thesis for the Degree of Ph. D.
MICHIGAN STATE UNIVERSITY
LARS VIK-MO
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This is to certify that the

thesis entitled

Abomasal Infusion of Protein And

Glucose In Lactating Cows

presented by

Lars Vik-Mo

has been accepted towards fulfillment
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ABSTRACT

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The influence of amino acid availability on milk protein production in cows fed above NRC standards of energy and protein was studied by infusions through abomasal cannulas, using three and four Holsteins, respectively, in two trial series (years). In every trial, all of the cows received all treatments. Performance during substrate infusion was compared to controls before and after in four out of five trials.

Supplying 300g casein per day for six days increased ($P<.05$, N=5 cows) milk yield 1.1kg/day compared to controls (15.3kg/day) and milk protein (N×6.38) production increased ($P<.01$) 49g/day with slight change in milk protein content (+0.07%, $P>.05$). During glucose infusion (300g/day) milk yield increased slightly (0.9kg/day, $P<.05$), and response in protein production was smaller ($P<.05$) than with casein.

Na-caseinate + 3% dl-methionine (K) at 75% of milk protein output was compared to equicaloric amounts of glucose (G) and a mixture (M), 1:1 of K+G, in a 3×3 Latin square design with saline infusions (O) before and after. Periods were 7 days. K and M increased ($P<.05$) daily milk yield 1.8kg over O (24.0±.7kg) and 1.9kg over G, while milk protein content was increased ($P<.05$) 0.20% over O and 0.15% over G. Hence, daily milk protein yield was increased more by K (101g, $P<.01$) and M (79g, $P<.10$) than by G (40g, NS) when compared to adjacent controls (trial 2.I).

Caseinate + 3% dl-methionine infused at 50, 100, and 200% of milk protein yield depressed ($P<.01$) feed intake compared to saline controls in a 4×4 Latin square design with 4-day periods and 2 days between periods (trial 2.II). Milk yields (17.8±1.1kg/day) and protein yields did not differ between treatments but estimated true protein (ETP=(N-NPN)×6.38) content of milk increased non-linearly ($P<.05$) with level of treatment.

Feed NPN at 38% and 14% of total N (120% of NRC (1971) standards) in a cross over design (N=4 cows) did not influence responses in milk protein to caseinate + 3% dl-methionine infusion which equalled 20% of the CP in the feed (trial 2.III). This infusion increased milk ETP content 0.25% ($P<.01$) and daily ETP yield 50g ($P<.05$)

with a nonsignificant increase (0.4kg) in milk volume over saline controls (12.9±1.3kg/day).

Multiple regression analyses showed responses in milk protein yield to casein infusion (N=25) depended on control yield (P=.002) and level of casein infused (P=.072) (R=0.78, P<.01). The control protein yields explained twice as much of the variance in responses in protein yield as did level of infusion. The mean response of 64±6g milk protein per day was 11.6% above control yields (556±25g/day) and accounted for 18% of the infused protein (360±17g/day).

While NPN content in milk generally increased (P<.05) with protein infusion, this did not change the ranking in traetment responses between milk CP and ETP. Infusion treatments generally depressed milk fat content 0.2 to 0.3%. Plasma or blood urea N and plasma glucose increased during protein infusion. Urea N was correlated positively with NPN concentration in milk.

Plasma α amino N in trial series 1 and the molar % ratio of essential to nonessential amino acids in trial series 2 increased with protein infusion. In two different trials the molar % of threonine and phenylalanine decreased (P<.05) by the protein infusion. Branched chain amino acids were high and methionine increased (P<.01) several fold from the lowest to the highest level of treatment (trial 2.II). When relating the output of essential

amino acids by milk protein to plasma concentration (trial 2.III), phenylalanine appeared as the least abundant amino acid.

The consistently higher responses in milk protein production by abomasal protein than glucose infusion, and a tendency towards more response with increasing levels of infused protein, suggest that milk protein synthesis was enhanced through improved amino acid supply. Blood parameters support this interpretation.

ABOMASAL INFUSION OF PROTEIN AND GLUCOSE IN LACTATING COWS

By

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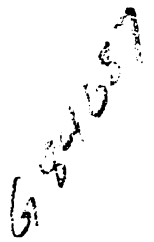

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A. INTRODUCTION

In view of a rapidly increasing world population it has been considered a matter of time whether production of meat and milk based on cereals and high quality plant proteins can continue as today. Already the proteins are the most costly part of the feed for livestock.

These circumstances place ruminant nutrition in a unique position due to the ruminant's ability to utilize nonprotein nitrogen (NPN) to a far larger extent than other farm animals. To what extent this relationship can be drawn upon is a matter of economics for the educated farmer. But it continues to be a great challenge for animal scientists to expand the knowledge of ruminant nutrition such that nonprotein nitrogen can be utilized extensively with enhanced efficiency in overall protein nutrition.

A most outstanding yet often forgotten feature of protein nutrition in ruminants is its dualistic nature: the microbial metabolism in the forestomachs on one hand, and the metabolism in the remainder of the alimentary tract and tissues of the animal on the other hand. Although distinctive in place and character, the events of these

two phases will mutually influence each other. Thus the fate of the crude protein fed will depend upon digestive processes as well as the physiological status of the animal.

It is now well accepted that protein metabolism is very dependent on energy metabolism. This also applies to the rumen, as utilization of NPN is intimately linked to the energy source. Thus, the other great asset of ruminant animals; the ability to thrive on substantial amounts of cellulose for energy, is also influenced by the nitrogen metabolism. Extensive utilization of NPN and cellulose, however, are not totally compatible because highly digestible carbohydrates, preferably starch, are necessary for maximal use of NPN; but bacterial growth on these readily available nutrients will depress digestion of cellulose. In any event, there is a limit for how fast microbes in the rumen can turn over; and this limits the amount for microbial protein which can be supplied to the lower gut of the host animal (Hungate 1966).

Some of the feed protein normally passes by the rumen and supplements the microbial protein, but variable amounts will be degraded in the rumen. The degradation compounds can potentially be used for synthetic purposes in the rumen but a rapid and extensive disintegration of feed protein to ammonia will easily result in a loss of nitrogen to the animal. All factors considered, it is hard to

assure that ruminants receive an amino acid mixture which allows full expression of their capacity for protein synthesis.

Since dairy cows produce large amounts of high quality proteins, in the form of milk, it is questionable whether the amino acid supply to the mammary gland is sufficient for milk protein synthesis; even when cows are fed a diet which, by conventional standards, is considered adequate. Apparently a more precise evaluation of diets for dairy cows with better use of available resources could be aided by greater knowledge of the cow's demands for amino acids. Understanding the cow's potential for milk protein synthesis is particularly important in view of the high nutritive value of milk proteins.

It was the aim of this thesis work to study protein nutrition of lactating cows by delivering high quality protein (casein) directly into the abomasum of cows fed an apparently adequate diet and measuring various response criteria.

B. LITERATURE REVIEW

1. Assessment of protein demands in lactating cows

Earlier debates about appropriate terms for expressing protein need and supply in ruminants reflect the qualitative as well as quantitative aspects of protein nutrition of dairy cows (Tyler, 1959). While crude protein (CP = N × 6.25) gradually has been accepted as a more appropriate term than true proteins it still remains controversial how crude protein should best be expressed; and statements about level of protein supplement invariably raise the question of quality. Variables in feed and physiological status of the animal evidently should be considered in a discussion of these relationships. This review, however, will only briefly deal with the topic of protein quality in lactating cows and mention the main factors critical in studies of their protein supply. With reference to the tempering effect of rumen metabolism on absorbable amino acids, some recent findings on rumen bypass of protein or amino acids will be presented. Finally, there will be brief discussions of nongenetic influence on milk protein content.

1.1 Protein quality: The concept of biological value and essential amino acids in lactating cows.

The biological value (BV) of feed protein can, with some qualifications, be obtained from an N balance

experiment. Properly, the term expresses the efficiency of absorbed proteins in supplying amino acids needed for the synthesis of body protein, thus taking account of metabolic losses computed on the basis of truly digested protein (Maynard & Loosli, 1962). It is now generally accepted that BV of protein is determined primarily by its content of essential amino acids (EAA) and, specifically, the content of that essential amino acid which is in greatest deficit relative to the animal's requirement (Block & Mitchell, 1946). Due to digestive processes in the rumen it is not valid to specify the BV of a given feed protein for a ruminant. It is the BV of nitrogen (N) in a given ration that is nutritionally significant (McDonald, 1968).

For a time following the work of Loosli, et al. (1949), showing that rumen microorganisms can synthesize all the essential amino acids, it became a general feeling that protein quality was relatively unimportant in ruminant nutrition (Jacobson, et al., 1970; Purser, 1970). In this period McNaught, et al. (1954) found rumen microbial protein to be of rather high nutritive value. Data for amino acid composition of microorganisms presented by Duncan, et al. (1953) and Weller (1957) indicated small variations even at different feeding regimens, an impression confirmed by later investigators (Purser, 1970).

Black, et al. (1955,1957) and Downes (1961), however, showed that ruminant tissue is dependent upon an exogenous supply of the same amino acids considered essential for other mammals. According to the definition of Rose (1938), these are the amino acids which must be supplied by the diet in order to support the demands for protein anabolism.

Rose, et al. (Rose, 1938) singled out the essential amino acids for the rat and dog by applying a "deletion technique" in which individual amino acids were successively removed from the complete diet. Because of the comprehensive microbial synthesis in the rumen, an indirect approach making use of tracers was particularly valuable for identification of amino acids essential for ruminant tissues. Furthermore, the rumen microbes make quantitative determination of EAA requirement by regular feeding trials a difficult and hardly relevant task.

By intravenously injecting ^{14}C-labelled glucose and volatile fatty acids (VFA) to lactating cows Black, et al. (1955, 1957) were able to largely circumvent the metabolism in the rumen. The amount of ^{14}C incorporated into various amino acids ofmilk casein revealed two groups: those with low levels of ^{14}C, which corresponded well with the EAA, and those with much higher levels which were generally the non-essential amino acids.

Downes (1961) applied a similar method in a lactating ewe and confirmed that tyrosine, phenylalanine, leucine, isoleucine, valine, methionine, lysine, threonine and histidine all had neglible radioactivity when isolated from casein. The amino acids mentioned include 8 of 10 considered essential for the rat. Arginine is evidently not essential for the sheep or the cow. Tyrosine is not classified as an essential amino acid from nutritional studies in rat and dog. However, since it is synthesized *in vivo* by the hydroxylation of phenylalanine, which is essential, no ^{14}C would be expected in tyrosine unless phenylalanine was also radioactive (Downes, 1961).

This interpretation was confirmed by Black, *et al.* (1972) who pulse-labelled jugular blood with U-C^{14}-tagged individual amino acids from casein and found tyrosine gained 10-12% of the original phenylalanine activity. Other EAA did not label other amino acids, even NEAA remained largely unlabelled. The NEAA, however, labelled each other in characteristic pairs, but only for aspartate and glutamate was interconversion extensive.

Tryptophan and cystine were not isolated in the first experiments with C^{14} labelled precursors, but Marston (1935) had already stimulated wool growth by subcutaneous injection of cystine which is present in large amount in keratine. Other workers have

confirmed this exceptional role of cystine by postrumen supply of protein and S-amino acids (Reis and Schinckel, 1964) and studies with ^{35}S-cystine (Downes, et al., 1970).

Land and Virtanen (1959) applied ^{15}N to the feed of two cows and observed less enrichment in EAA than other amino acids in milk proteins. When the relative label of glutamic acid was set to 100, valine was 59, lysine 54, phenylalanine 50, arginine 41 and histidine 15. When ^{15}N was used in a cow adapted to purified diet with urea as N source for 16 months there was more labelling of EAA than observed earlier. Label introduced by $(^{15}NH_4)_2SO_4$ and ^{15}N-urea gave similar results. Virtanen (1966) suggests the low label of histidine may indicate a united supply of milk protein synthesis.

Because tryptophan is destroyed in commonly used amino acid assays less data is available for this amino acid, but according to Fenderson and Bergen (1972) tryptophan is an essential amino acid in ruminants. Studies by Piana and Piva (1969[1], according to Virtanen 1971) evidently showed a very low synthetic rate of tryptophan in the rumen of sheep when $^{15}NH_4$ was used as a marker.

After years of shifting opinions, the use of radioactive tracers showed that milk proteins are largely (over

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[1]As cited, the source not available.

90%) synthesized in the mammary gland from free amino acids in the blood (Barry, 1964; Larson and Gillespie, 1957). Arterio-venous (AV) differences of EAA over the mammary gland have correlated more closely to output in milk proteins than have the other amino acids (Mepham, 1971).

No single EAA, however, is present in milk proteins in an extra high proportion. Thus, it is not simple to identify any one as first limiting for maximum milk protein synthesis (Thomas and Clapperton, 1972). The most abundant amino acid in milk proteins is glutamate (Porter, et al., 1968); and from experimental evidence, glutamate has been suggested as a possible candidate for limiting milk protein synthesis (Halfpenny, et al., 1969) although it is not essential as defined above.

On the other hand, Verbeke and Peeters (1965) concluded that a group of amino acids whose concentrations in milk proteins showed a close linear relationship to their AV differences should be considered essential for the mammary gland. Included in this group was valine, leucine, isoleucine, threonine, arginine and glutamate. They did not succeed in determining a reliable AV difference for histidine. Tyrosine, phenylalanine, methionine and alanine formed a group with some relation between milk content and AV difference, but not as closely as the former

group. An explanation for these differences were not suggested, but might be the consequence of various extent of metabolism within the gland.

Using conventional figures for blood flow and milk protein content Verbeke and Peeters (1965) calculated an uptake of amino acids 15-25% short of the output by milk protein. This discrepancy was probably due to a deficiency in the blood sampling techinque (Mepham, 1971). Mepham and Linzell (1966) measured simultaneously the AV difference adn blood flow in goats with considerable attention to possible sampling errors. They found the uptake of EAA agreed closely with output of milk proteins. The uptake of total amino N was apparently sufficient for the protein synthesis.

Discussing amino acid supply in cows (as well as other animals), McCarthy, et al., (1970), suggested that a distinction should be made between an amino acid deficiency and what might be called an amino acid insufficiency. That is, a cow may be free of any deficiency symptoms yet milk production may be limited by available substrate rather than enzymatic reactions in the secretory tissue (McCarthy, et al., 1970).

1.2 Factors influencing response to protein supply of lactating cows.

Body protein reserves may buffer the effect of various feed proteins in short term experiments (Reid,

et al., 1966, 1967) and prevent response to diet over a wide range (10-20%) of CP in the ration (Jacobson, et al., 1970). Coppock, et al., (1968) considered protein reserves quantitatively inferior to energy reserves, but Paquay, et al., (1972) found larger capacities for protein storing than formerly thought possible; more than 15kg protein could be lost and regained in cows around 600kg body weight.

Digestibility has been criticized as a misleading description of protein availability in ruminants (Chalmers, et al., 1954; Thomas, 1966) because extensive breakdown to NH_3 in the rumen may lead to large N losses in the urine. Low N digestibility, however, as for heat damaged forage (Thomas, et al., 1972) will be detrimental for utilization of the whole ration. While the reliability of N balances in ruminants have also been criticized (Agricultural Research Council (ARC) 1965) these are nevertheless used as basis for estimation of the protein requirements expressed in feeding standards (ARC, 1965).

The level of energy intake, as well as its ratio to protein, will generally influence protein requirements (Reid, et al., 1966; Balch, 1967; Thomas, 1966, 1971). Protein needs can be adequately ascertained only after energy requirements are met (Perkins, 1957), and several experiments have shown that protein requirement

was minimal at a high energy allowance (Thomas, 1971). Jacobson, et al. (1970) maintained that high levels of protein encourage greater voluntary feed intake; and while milk production was related more closely to net energy than protein intake, the values for protein requirements (based on empirical results) have been confounded with feed consumption. Balch (1967) advocated that different levels and qualities of protein should be tested at different levels of energy. Gordon and Forbes (1970) registered a greater response in milk yield to increased dietary protein at an energy level 20% above standards than at an energy level 20% below standards.

The porportion of non protein nitrogen (NPN) in the feed that will not depress milk yields evidently varies with the protein need of the cow, which depends primarily upon milk production (Huber, et al., 1967, 1972; Conrad and Hibbs, 1968). Maximum levels of urea in lactating cows therefore should be expressed on an absolute basis rather than as a proportion of the total dietary nitrogen (Huber, et al., 1967; Conrad and Hibbs, 1968). Special preparations can allow for higher than usual levels of urea (Meyer, et al., 1967; Helmer, et al., 1970a,b; Conrad and Hibbs, 1968). These are based on a favourable

timing of the release of NH_3 and energy for protein synthesis in the rumen (Chalupa, 1970), but the energy source is still crucial for extensive NPN utilization (Chalupa, 1970; Virtanen, 1966). A noticeable effect on productivity and feed efficiency of source of NPN, apparently through interaction with other feed constituents, has resulted from NH_3 additives to corn silage (Huber and Santana, 1972; Henderson, et al., 1972).

2. The role of rumen metabolism in amino acid supply to the host animal

In order to find the amount of amino acids available for absorption it is necessary to know: (1) the flow rate of digesta from the rumen; and (2) the amino acid content of the digesta. The latter would depend upon the amount of microbial protein produced and its quality as well as the amount of feed protein passing unaltered by the rumen. Knowledge about the factors influencing these entities apparently could be valuable for a systematic manipulation of ruminant nutrition (Hutton and Annison, 1972). Still, the importance of maintaining a viable microbial population in order to obatin effective digestion of forages should not be overlooked (Hutton and Annison, 1972).

2.1 The fate of protein and N in the rumen.

The cycling of N between rumen and the tissues as described by McDonald (1948) makes it difficult to assess in quantitative terms the contribution made by dietary protein versus microbial protein to the amino acid mixture of the digesta in the small intestine (Ellinger and Phillipson, 1964). When the dietary N (CP) level is low, the recycling of N by urea to the rumen will lead to conservation of N for the animal. However, when high N levels are fed, the rumen NH_3 and the blood urea concentrations are elevated, and increasing amounts of urea are excreted by the urine. Ruminants thus appear to be less efficient than mono-gastric animals in utilizing high protein diets (McDonald, 1968). Weston and Hogan (1967) found that the amount of microbial protein passing to the lower gut in wethers was 8.8 and 8.1g per day when the feed contained about 20 and 8g of CP. Clarke, et al. (1966) reported similar results.

While it is well established that hydrolysis of urea in the rumen usually proceeds at a faster rate than NH_3 assimilation into microbial protein, rumen bacterial growth can also be limited by a low availability of NH_3-N; thus, replacement of dietary protein with urea may in certain instances increase microbial growth (Allison, 1970).

The concentration of NH_3 in the rumen critical for bacterial growth has not been clearly defined, but optimal NH_3 concentration will probably vary with shifts in microbial populations and growth rates (Allison (1970). Chalmers (1971) implied that when grass or grass products are used for feeding of dairy cows, there is hardly ever a limit in bacterial growth due to low NH_3 concentrations in the rumen; thus, energy is the main limiting factor.

Waldo (1968) stated that the concentration of rumen N fractions, size of the rumen pool, or N turnover rates, have mathematical relationship to each other; and although they are frequently measured and discussed as distinct entities, it must not be ignored that a change in one of these parameters is frequently a cause or a result of a change in another. The turnover rates of N in the rumen, however, are considerably below the growth potential of most bacteria (Hungate, 1966). The rate of N removal in the intestinal tract is influenced by the ratios of particulate and soluble N, and the relative rates of particle passage and water passage (Waldo, 1968). Because bacteria and protozoa (Waldo, 1968) have different retention times, it can be surmized that changes in the

relative biomass of the two types of microbes will have impact on the amino acid supply to the animal beyond the differences in protein quality for the two fractions.

Naturally, the proportion of feed protein reaching the lower gut will be the inverse of the extent of NH_3 production in the rumen. Thus, McDonald (1952, 1954) fed purified proteins to sheep and showed that a large part (about 40%) of the highly insoluble protein zein passed into the abomasum unchanged. Soluble casein, however, was nearly all (about 96%) degraded and replaced by microbial protein (McDonald and Hall, 1957). Feeding casein was associated with far higher levels of NH_3 in the rumen than was observed for zein. Chalmers, et al. (1954) confirmed these relationships by showing less NH_3 formation and less degradation of casein after making it less soluble by treatment with sodium hydroxide and heating. Herring meal also resulted in lower NH_3 concentrations in the rumen and higher N retention than did casein in growing lambs (Chalmers and Synge, 1954) and in lactating goats and cows (Chalmers and Marshall, 1964).

Addition of starch or cereal meal reduced rumen NH_3 levels in animals fed highly-soluble groundnut meal (McDonald, 1954; Annison, et al., 1954). Therefore, differences in NH_3 formation from different proteins need not be of great practical importance when liberal

amounts of grain are fed, as with fattening of beef cattle (Annison, et al., 1954).

Chalmers (1971) stated that no bench-test reflects the rate of dissolution of a solid protein in the rumen. Others reviewing work in this area, however, seem to agree that solubility of feed protein is an important factor in the rate of its breakdown and consequent NH_3 accumulation in the rumen (Waldo, 1968; Tillmand and Sidu, 1969; Smith, 1969). While Little, et al. (1963) found a poor association in an artificial rumen system between microbial attack on proteins and protein solubility in water or diluted NaOH; a fairly close relationship prevailed between the in vitro NH_3 produciton and protein solubility in rumen fluid at pH 7.

Proteins rapidly converted to NH_3 also appeared readily available as N sources for in vitro cellulose digestion. Even though heated soybean meal was less soluble than untreated meal, there was no difference in growth rate of lambs on these protein supplements. On the other hand, insufficient N-release for potential protein synthesis in the rumen obviously hampered the growth of lambs fed corn gluten meal. Adding urea to a corn gluten diet markedly improved weight gains while supplementing with lysine and methionine had little effect although corn gluten meal is low in lysine (Little, et al., 1963).

A relatively low solubility of corn gluten meal in rumen fluid was reported also by Chalupa, et al. (1963) but they found isolated soy protein much less soluble than did Little, et al. (1963); (7% vs. 63%). This discrepancy might be related to processing methods, but Chalupa, et al. (1963) did not disclose the pH of the system they used for testing.

Significant effects of rumen fluid acidity on protein solubility was demonstrated in vitro by Isaacs and Owen (1972), suggesting that ruminal pH influences the bypass of proteins. They showed that within a pH range of 5 to 7, the higher pH favoured solubility of casein and soybean meal while corn protein appeared relatively more soluble at the lower pH. The authors (Isaacs and Owen, 1972) proposed solubility curves for calculating nitrogen availability to rumen microbes. Thus, for assumed rates of rumen turnover it might be possible to estimate protein degradation, and by difference, assess the passage of intact protein through the rumen.

Protein degradation rates in vitro indicated to Isaacs and Owen (1972) that rumen proteolytic enzymes are saturated under common feeding conditions, although ruminal proteolytic rate may decrease with time after feeding.

Since the concentration of free amino acids in rumen fluid is quite low (McDonald, 1952; Blackburn, 1965),

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the importance of free amino acids in ruminal microbial metabolism is hard to assess. By concentrating rumen fluid from sheep and removing its NH_3, Lewis (1955) observed fifferent concentrations of individual amino acids. These findings per se would not prove amino acids to be intermediates in protein catabolism; but the extent of NH_3 production following the introduction of amino acids into rumen indicated hydrolysis is an intermediate step that probably regulates the rate of protein breakdown (Lewis, 1955).

Far lower rates of NH_3 production occurred from amino acids added to washed cell suspensions compared to those in vivo (Lewis, 1955). Cells from casein-fed sheep deaminated protein more vigorously than from hay-fed sheep. This ranking among the diets was also shown in vivo. Various rates for the different amino acids prevailed in vitro as well as in vivo. Aspartate was attacked fastest in both solutions with an optimum in vitro pH of 6.5 (Lewis, 1955).

Lewis and Emery (1962a) found the proteolytic capacity of washed-cell suspensions infereior to rumen fluid in vitro; but largely the same sequence of dissimilation rates among individual amino acids persisted. On the basis of resistance, the amino acids fell into three groups; largely confirming the results of Lewis (1955). Generally, d- and l-enantiomorphs differed in

catabolic resistance, but not so for serine and tryptophan. The production of NH_3 resembled the rate of disappearance of amino acids determined by chromatography (Lewis and Emery, 1962a). The evolvement of NH_3 from amino acids *in vitro* fell with the pH of the medium from a maximum at 6.5 to neglible release at 4.5 (Lewis and Emery, 1962b). Deamination rates *in vivo* (Lewis and Emery, 1962c) paralleled the *in vitro* results. The highest NH_3 level in the rumen occurred 6 hours after arginine administration, whereas tryptophan and lysine yielded smaller, yet detectable, amounts of NH_3. Lysine concentrations decreased to 50% in 6 hours, but ruminal tryptophan was not reduced until 8 to 10 hours after it was supplied.

While arginine and lysine addition to the rumen resulted in elevated plasma levels of several amino acids within one hour, plasma lysine itself did not increase until four hours following the administration (Lewis and Emery, 1962b). Conversion of arginine to ornithine in the rumen yielded equal concentrations of these amino acids 6 hours after introducing arginine (Lewis and Emery, 1962c).

Arginase activity in the rumen wall has been demonstrated *in vitro* and *in vivo* (Harmeyer, *et al.*, 1968). Increased ornithine concentrations in ruminal venous blood after arterial arginine injection was, however, not

detectable unless ample amount of starch was present in the rumen (Harmeyer, et al., 1968). This observation is in line with marked decrease of urea recycled to the rumen of sheep deprived of easily fermentable carbohydrate (Houpt, 1959). Harmeyer, et al. (1968) suggested combined activities of the arginase and urease associated with rumen mucosa participate in regulating recycling of urea N to the rumen.

Actual transport of amino acids across rumen epithelium has been demonstrated in steers and goats (Cook, et al., 1965), but Blackburn (1965) contends that the low ruminal amino acid levels observed on continuous feeding make it unlikely that direct absorption should affect the nutrition of the animal. Even casein hydrolysate placed into sheep rumen failed to increase the α amino N content in portal or arterial blood (Annison, 1956). But Cook, et al., (1965) state that amino N is not sufficiently sensitive to reflect changes in ruminal absorption of amino acids.

Cook, et al.,(1965) found added amino acids to be quite stable in the rumen fluid for one to two hours. Thereafter, concentrations fell which were accompanied by an increase in the NH_3 level. Emery (1971) found the mean half life for 50g doses of dl-methionine to be 2.4 hours in the rumen of mature cows, with no difference in persistency between dl-methionine and other forms of the amino acid.

Thus, discrepancies in amino acid resistance to ruminal destruction may largely be a consequence of differing concentrations. Large experimental dosages evidently saturate catabolic pathways; for amino acids are rapdily degraded at physiological concentrations (Emery, 1971).

Attempts to protect rumen amino acids with antibiotics carries potential hazards to vital fermentations; but is, nevertheless, a challenging approach. Penicillin, examined for this purpose *in vitro*, was not effective in concentrations that prevented bloat, but levels ten-fold higher markedly decreased amino acid disintegration (Lewis and Emery, 1962a). Retarded ruminal deamination of methionine and lysine has recently been reported after addition of oxytetracycline hydrochloride (OTC), without noticeable effects on the microbial activity (Schelling, *et al.*, 1972).

2.2 Quantification of microbial synthesis in the rumen.

The extent to which food can be converted into cell material is limited by anaerobiosis, and rarely exceeds 20% under such conditions (Hungate, 1966; Walker, 1965). Thus, as degradation rate of feed protein exceeds the rate of re-synthesis in the rumen, its anaerobic state imposes a thermodynamic limit on the extent of host protein synthesis (Hungate, 1965, 1966).

In vitro cell yields have been related to ATP produced by pure cultures of bacteria (Bauchop and Elsden,

1960) and extended to *in vivo* conditions (Walker, 1965). From these findings, Hutton and Annison (1972) concluded that about 20g bacterial CP was synthesized per 100g DM digested.

Based on Hungate's (1966) anaerobic data, Purser (1960) calculated that 18.3g digestible microbial protein could be synthesized per Mcal feed digested. Chalupa (1972) applied this value to commonly accepted figures for feed intake and estimated that microbial protein can support maintenance and a daily production of 10kg milk. However, research data indicate that the rate of microbial synthesis might be higher than the theoretical one used by Chalupa (Purser, 1970). This would allow support of a larger production of milk on microbial protein alone. The work of Virtanen (1966) also indicates a higher potential for microbial protein synthesis than theoretical calculations held possible.

Incubating rumen contents *in vitro*, Al-Rabbat, *et al*. (1971b) confirmed there is good agreement between microbial growth and fermentation. However, microbial protein syntehsis estimated by ^{15}N incorporation was higher than calculated from VFA production, using 2 ATP/mole VFA; but the rate of VFA production might have influenced the result. Tracer data showed 9.2g microbial cells were synthesized from NH_3 per 100g digestible

organic matter (DOM) fed (Al-Rabbat, et al., 1971a). This was 61% of total cell production, the remaining N presumably coming from amino acids and peptides. Similar figures have been obtained by continuous ruminal infusion of $^{15}NH_4$-salts (Mathison and Milligan, 1971; Pilgrim et al., 1970) or ^{15}N-urea (Nolan and Leng, 1972); but recycling of NH_3 may obscure estimates by this approach (Mathison and Milligan, 1971).

Bucholtz (1972) measured cell synthesis from incorporation of ^{33}P into phospholipids based on specified properties of polar lipids in microbes. By frequently sampling the rumen of sheep fed at 9 hour intervals combined with in vitro incubations, microbial protein synthesis was estimated at 26g/100g organic matter (OM) digested.

Utilization of ^{35}S as a marker is based on its incorporation in sulfur-containing amino acids (Walker and Nader, 1968). In a study with lactating cows, Conrad, et al. (1967a,b) combined ^{35}S-sulfide and fish mealsas markers after finding 91% of the fish meal withstood degradation in the rumen. Fish meal is rich in methionine, and apparently should serve as an indicator of the fraction of total rumen protein of feed origin. The high resistance of fish meal to ruminal degradation, based on nylon bag incubations, might have biased upwards the estimates of total methionine synthesis; which were 1.5g per kg feed (Conrad, et al., 1967a,b). In the same

laboratory, Mugerwa (1969) found daily protein synthesis in rumen of a cow on a 70% NPN diet to be about 72g per kg DM digested, regardless of whether cellulose or starch was the main carbohydrate source.

Little NH_3 is found in duodenal contents; and more than 80% of the non-ammonia N has been accounted for as amino acids (Clarke, et al., 1966; Weston and Hogan, 1970). The remaining is presumably found in the nucleic acids (Ellis and Pfander, 1965; Weston and Hogan, 1970). Because nucleic acids entering rumen are not rapidly degraded, their concentration in digestsa can also estimate the amount of microbial protein (Smith, 1969). Nucleic acid N accounts for 14-19% of microbial N, mostly RNA (Allison, 1970); and since RNA associates directly with protein sythesis and DNA levels vary considerably, it appears that the RNA fraction is a better marker for microbial cell growth (Smith, 1969).

Because α-ε diamino pimelic acid (DAPA) is unique to bacteria it also has been used to estimate bacterial protein (Weller, et al., 1958, 1962; Hogan and Weston, 1970) Hutton, et al., 1971); although Synge (1953) showed that DAPA/total N varied considerably between strains of ruman bacteria. But Weller, et al. (1958) found the ratio of DAPA/N on a fixed dietary regimen was quite constant. The ratio of DAPA to non-ammonia N in digesta

leaving sheep's stomach, however, tended to decrease as the crude protein in the diet increased (Hogan and Weston, 1970). This marker showed bacterial protein synthesized corresponded to 3.7g N/100g OM apparently digested in the stomach (Hogan and Weston, 1970).

A decrease in the amount of DAPA was generally accompanied by decreased VFA production (Hogan and Weston, 1970); another demonstration of the quantitative relation between energy transactions and bacterial growth in the rumen. The flow of total and bacterial protein, estimated by DAPA, also has been found positively correlated with the molar % of propionic acid in the rumen (Jackson, et al., 1971; Ishague, et al., 1971) and circumstantial evidence suggests this relationship may have more general applicability (Thomas and Clapperton, 1972).

By summarizing a multitude of metabolic data in a computer simulation system, Baldwin, et al. (1970) arrived at 12 to 18g dry cells produced per 100g DOM fed, consistent with many direct experimental results.

Hutton, et al. (1971) stressed that an accurate evaluation of the animals N economy requires information on the magnitude of protozoal and endogenous N, as well as bacterial N in the digesta. The ratio between protozoal and bacterial N varies greatly with dietary conditions from 1:10 on poor hay to 1:4 on lucerne (Weller, et al.,

1962). Values of 1:2.5 have been reported in grain-fed animals (Hungate, 1966), and absence of protozoa was shown on semipurified diets devoid of true proteins (Virtanen, 1966).

2.3 Quality of protein in the digesta.

Most researchers agree that 50 to 80% of dietary N is converted to microbial N by the time the digesta reaches the small intestine (Smith, 1969). Nevertheless, quantity and quality of crude protein, as well as the overall ration markedly influence absorbable amino acid. This point was confirmed in several studies with zein which largely passes unaltered through rumen (McDonald, 1952, 1954; Ely, et al., 1967; Amos, et al., 1971; Little, et al., 1968), and is not well digested in the intestine either (Little and Mitchell, 1967). Moreover, N-retention was lower after zein administration per abomasum than per os, while the opposite was observed for casein, soybean and gelatin (Little and Mitchell, 1967). Feeding of these proteins yielded similar concentrations of amino acids in abomasal hydrolysates but EAA in animals fed zein were quite variable (Little, et al., 1968).

While the amino acid patterns for mixed abomasal protein were similar on soybean meal and urea feeding (Potter, et al., 1969), more of the total N in digesta was present as protein when steers received soybean. Total N

was also higher on soybean than urea. This strengthened the impression that the quantity of amino acids reaching the lower gut, rather than the amino acid pattern, limits performance on high NPN rations (Potter, et al., 1969). However, adaptation to urea in lambs (Webb, et al., 1972) increased total abomasal N from values lower than soybean after 10 days feeding to higher levels after 20 days, regardless of CP content (9 to 20%) in the diet. Others have shown a higher percent of the consumed N passing out of rumen on low CP diets than high CP diets (Clarke, et al., 1966; Hume, et al., 1970). Thus, the quantities of amino acids passing out of the stomach may differ substantially from those consumed, but differences between diets become smaller, as one samples farther down the digestive tract (Clarke, et al., 1966).

Confirming findings in sheep, Hale and Jacobson (1972) reported the N flow through the abomasum of cows was positively correlated with DM intakes, but recovery of dietary protein in relation to level of feed consumed was not reported. Because feed intakes in high yielding dairy cows are higher than in other ruminants, digestive observations in other classes of livestock will not describe ruminal protein bypass in high producing cows.

Amino acid balances in lactating cows combined with observations of digesta in ewes on similar rations (Bigwood,

1964) revealed that ruminal synthesis was highest for lysine, followed by leucine, which is particularly important when considering milk protein composition. Methionine and phenylalanine were not increased in digesta compared to the feed, but the adequacy of EAA in digesta differed with the rations (Bigwood, 1964).

In recent studies with sheep the amount of individual amino acids absorbed showed a high positive correlation with the amounts entering the small intestine. Both parameters were influenced by physical form and level of forage intake, (Coelho da Silva, et al., 1972a,b). Microbial N/total N in digesta decreased with level of feeding.

Studies involving portal blood flow in wethers indicated that with some exceptions amino acids were absorbed in a ratio similar to their occurrence in rumen bacterial protein (Hume, 1971b). These results apparently contradict older ones (Clarke, et al., 1966) which led Armstrong and Prescott (1971) to contend that the amino acid content of duodenal digesta is not a satisfactory indicator of absorbable amino acids.

Microbial protein quality cannot be assessed in the ruminant animal under normal feeding conditions; therefore, laboratory tests have been sought. Although informative, such investigations can only approximate the situation in well-fed ruminants (Smith, 1969).

McNaught, et al. (1954) found the true digestibility in rats was 75% for isolated bacteria and 90% for protozoa. These coefficients have been confirmed by many other workers (Purser, 1970), but values as low as 55% have been reported for digestibility of bacteria (Hatfield, 1970). Bergen, et al. (1967) found wide variations between strains of bacteria grown in pure cultures. Changes in microflora therefore may influence amino acids available to the host. Likewise, low concentrations of protozoa will adversely affect microbial protein quality (Klopfenstein, et al., 1965), but such impacts on amino acid availability is not easily separated from digestion in the rumen (Smith, 1969). Larger losses of fecal N in ruminants than simple stomached animals, particularly at low N intakes, have been related to the low digestibility of bacteria (Smith, 1969). Their protein appears protected by cell walls (Hoogenraad and Hird, 1970); which is also responsible for the low digestibility of nucleic acids (Smith, 1969).

Although microbial protein syntehsis leads to N looses in the animal (Hatfield, 1970), this should not be considered a serious problem in N economy if a cheap NPN source supplies the NH_3 for bacterial growth (Hungate, 1966; Purser, 1970). Likewise, the merits of conversion of feed protein to microbial protein must be considered on basis of feed protein quality in terms of absorbable amino acids (Armstrong and Prescott, 1971).

Reviewing the topic of amino acids in protein of ruminal microbiota, Purser (1970) concluded that the bulk composition shows such uniformity that variations in animal performance cannot be explained on this basis. Excellent agreement was shown (Purser, 1970) between workers in different countries, between strains of bacteria, and between microbial protein obtained under diverse dietary conditons. Also bacterial and protozoal protein were similar in this amino acid content, although protozoa were higher in lysine, leucine and phenylalanine (Purser, 1970).

The similarity in amino acid composition of bacterial and protozoa proteins suggests an equal BV. Reed, et al., (1949) found BV for both types of microbes in sheep to be slightly below 80 when fed to rats; and McNaught, et al., (1954) found BV 81 and 80 for bacterial and protozoal proteins, respectively. These values have been confirmed by others (Purser, 1970). Due to higher digestibility, the protozoal protein will be of higher NPU value (BV×N digestibility). Enzymatic digestion in vitro (Bergen, et al., 1967) released from 2.5 to 52.6% of EAA in protein of different strains of bacteria. Compared to amino acids released from egg protein the quality of bacterial proteins ranged from 37 to 80% (NPU_{enz}).

The data of Clarke, et al. (1966) show higher absorption of EAA than remaining amino acids in digesta. These differences might reflect selective absorption but could also be due to type of protein presented for digestion (Abidi, et al., 1967; Purser, 1970).

3. Postrumen supply of proteins and amino acids, and rumen bypass through protective treatments

3.1 Studies in sheep and growing cattle.

a. Effects of chemically prepared proteins.--Because of differences in acidity between the rumen and abomasum, Ferguson, et al. (1967) treated casein with formaldehyde in order to channel it past the rumen for digestion in the abomasum. A previous in vitro test showed treated casein was virtually insoluble at pH 6 and highly protected against breakdown to NH_3 during in vitro incubation with rumen contents. Daily addition of 60g of formaldehyde treated casein into a sheep rumen did not change ruminal NH_3 concentration, whereas similar amounts of untreated casein resulted in a substantial increase. While formaldehyde thus had rendered casein resistent to ruminal metabolism, the protein was still 80% digestible; and good availability of amino acids from this supplement was evident by more wool growth than controls fed untreated casein (Ferguson, et al., 1967).

Reis and Tunks (1969) confirmed the positive effects of rumen bypass when sheep received threated or untreated casein in the diet or casein infused into the abomasum treated casein and casein per abomasum stimulated wool growth equally well and far better than untreated casein in the feed. Formaldehyde-treated casein in this trial was 90% digestible compared to 98 and 96% for untreated casein per abomasum or per os, respectively.

Sampling of the small intestine revealed that lambs on a diet with 10% formalinized-casein digested 60% more protein and 50% more starch in the lower gut than did lambs on untreated casein (Faichney and Weston, 1971). Accordingly, less organic material was digested in the rumen when casein was treated with formaldehyde, and this diet provided 24g DCP to the intestine per 100g DOM compared to 15g on the casein diet. Similarly, a larger proportion of formaldehyde-treated than untreated groundnut meal was digested in the intestine (Miller, 1972). However, this difference was observed for all components of the meal.

While 50% of soybean meal N could be accounted for in the duodenum at a low level of feed intake, the recovery was 80% after formaldehyde treatement (McLaughlin, et al., 1972). Doubling the level of soybean meal increased recovery to 65% for untreated and 100% for formaldehyde protected protein.

An extensive digestion study involving formalinized casein by Macrae, et al., (1972) provided quantitative data on amino acid absorption from treated versus untreated casein added to a basic grass diet. Twice as much of the amino acids in treated casein passed into the small intestine and net retention of supplementary N was increased by the formaldehyde treatment.

A corollary to these results are the findings of Faichney and Weston (1971) that α amino N and insulin concentrations of blood plasma were increased and urea concentration was decreased in lambs on formaldehyde treated casein compared to untreated controls. An enhanced flow of protein into the intestine acted as a trigger for hormonal mechanisms and might explain the depressed flow rate of digesta in lambs on formalinized casein. Altered endocrine balance might also partially explain differences in blood levels of metabolites in lambs on treated versus nontreated casein.

Downes, et al., (1970), however, demonstrated with blood proteins labelled by ^{35}S that the extent of formalin treatment decisively influences the digestability of the processed proteins. While wool growth involves only protein with a very low demand for extra feed energy, body growth cannot be stimulated by an elevated protein to energy ratio beyond certain limits, which depends upon the protein quality and physiological stage of the animal. Ruminants, after an

early age, will generally not respond to an excess of 18g absorbed amino acids per 100g DOM. Any common ration, even when low in N, will usually provide this amino acid to energy ratio (CSIRO)[2] (1971). Assuming a well-balanced amino acid mixture, it is therefore not likely that addition of protected protein will produce the same relative increases in weight gains as in wool growth.

Faichney (1971), however, found that lambs on a diet with 10% formalinized casein gained significantly more than lambs on similar diet with untreated casein (165.5g versus 154g/day); but growth of wool was not different in these lambs. An experiment with calves (Faichney and Lloyd Davis, 1972) demonstrated that formaldehyde-treated peanut meal was no better than untreated meal when fed at a dietary CP of 20%, while slightly higher growth rate and feed efficiencies were observed in calves fed treated than untreated meal when the diets contained 13% CP. The relatively small response to protected peanut meal (Faichney and Lloyd Davis, 1972) might be explained by the low BV for peanut meal and the fact that it provided only 1/4 of the total protein in the diet.

Nimrick, et al., (1972) compared aldehyde-treated fishmeal, rich in S-containing amino acids, and soybean meal, for lambs. No metabolic difference was observed

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[2]Commonwealth Scientific and Industrial Research Organization, an Australian publication.

between the untreated proteins; but although treatment lowered the digestibility, N retention was greater for the treated fishmeal. A feedlot trial showed that treatment of both protein sources improved growth rate and feed efficiency in lambs fed ad libitum (Nimrick, et al., 1972). The authors point out that less response to treated protein might be expected at ad libitum feeding than at restricted feeding. Nevertheless, an improved pattern of absorbed amino acids due to protected protein would result in higher feed efficiencies.

Contrary to the preceding reports, Satter, et al., (1970) found formaldehyde-treated soybean meal inferior to untreated meal or urea in promoting tissue and wool growth in lambs. It may be speculated that too severe denaturation by an overdose of formaldehyde caused the poor results. The rate of treatment and the proportion of supplement in the diet was not given in the abstract of this work.

Peter, et al., (1971) found treatment with formaldehyde, glyoxal, and glutaraldehyde effectively protected soybean meal as evidenced by increased N balances in lambs. Nishimuta, et al., (1973) treated soybean meal with 1g formaldehyde per 100g air-dry meal, and showed depressed ruminal and postruminal digestion in lambs as indicated by a lowered N retention. On the other hand, heat-treated soybean meal increased N retention but depressed cellulose digestibility. Tannic acid treatment (9% w/w) did not alter digestibility or N retention compared to controls, but heat,

formaldehyde and tannic acid all increased the fraction of digested N that was retained. Whereas total amino acids in plasma did not differ compared to controls, all treatments shifted the molar ratios to less glycine and alanine and more of leucine, isoleucine, lysine and phenylalanine. Reis and Tunks (1969) likewise observed lower glycine and higher branched-chain amino acids levels in plasma of sheep fed formalinized casein or infused abomasally with casein compared to feeding the unaltered source.

Isaachs and Owens (1972) found casein treated with 1.2% (w/w) formaldehyde insoluble between pH 5 and 7 while solubilities of unprotected casein and other proteins were markedly influenced by pH. Insolubilization was suggested as the way that formaldehyde prevents ruminal degradation. Presumably, the effect of formaldehyde on digestion lies in formation of methylene bridges or other cross-linkages between chains of the proteins (Walker, 1964). For ruminant nutrition the key feature of formalin treatment is the stability of the formaldehyde-amino acid complex under close to neutral conditions in the rumen; yet susceptibility to a decreased pH and hydrolysing enzymes in the lower gut (Mills, et al., 1972).

Prolonged time intervals between treatment of casein with formaldehyde and feeding was found to increase the proportion of ^{14}C of formaldehyde recovered in feces (Mills, et al., 1972). Storing apparently rendered more ^{14}C-formaldehyde irreversibly linked to the protein such that

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the complex could not be degraded within the alimentary tract. The actual preparation under study consisted of equal parts of casein and safflower oil sprayed with formalin to a final formaldehyde concentration of 1.5% by weight. Large proportions (60-80%) of ingested formaldehyde was metabolized to CO_2 and CH_4. The appearance of ^{14}C in methane would imply that a portion of the formaldehyde was degraded by methanogenic bacteria in the rumen (Mills, et al., 1972).

Mills, et al., (1972) gave their feed to sheep and goats for several weeks before the tracer studies, and a very low content of ^{14}C was found in tissue and milk; indicating neglible break down of formaldehyde in the rumen. Around 5% of ^{14}C appeared in urine regardless of the amount passing in the feces (11 to 27%).

b. Extraruminal protein or amino acid supply and plasma amino acid patterns.--When Schelling and Hatfield (1968) infused casein into abomasum of lambs on purified diets containing urea as the sole N source, the feed intake went up, and N retention was improved. Abomasal casein infusion also resulted in higher N retention at controlled feed intake; suggesting an inadequate amino acid supply in the lambs on this purified diet. Isonitrogenous infusions of urea, acid hydrolysed casein or a mixture of essential amino acids gave lower N retention than casein infusion. Lysine and glutamate infusion improved N retention to the

same extent as a mixture containing arginine, histidine, lysine, phenylalanine and methionine. But neither phenylalanine nor methionine increased the N retention; and the absence of an effect from methionine contradicts other studies involving this amino acid (Schelling and Hatfield, 1969).

Postruminal urea infusion in order to achieve isonitrogenous treatments in this type of experiments (Schelling and Hatfield, 1969; Nimrick, et al., 1970a,b) can be criticized on the basis of adversely affecting the motility of the gut and the absorptive processes (Visek, 1966).

While Little and Mitchell (1968) found steers retained more N of casein,soybean and gelatin infused into abomasum than fed, digestibilities remained the same. Fujihara and Tasaki (1973) confirmed in goats that casein is equally well digested whether introduced into abomasum or rumen. Addition of starch by either route of adiministration had no influence on the digestibility of casein.

Besides being isonitrogenous, test diets should also be equal in energy. However, the utilization of energy can also be influenced by route of administration; with the metabolizable energy (ME) of postruminally administered casein higher than orally fed casein (Blaxter and Martin, 1962). These points should receive close attention if research is continued beyond the exploratory level.

By complete duodenal feeding of sheep and bottle-feeding of goats, Potter, et al., (1972) circumvented the ruminal influence on protein quality and related this to plasma levels of amino acids. The concentration of amino acids known to be inadequate in test feed showed the largest decrease relative to a reference pattern established with a high quality protein (egg and casein). Application of this approach to identify dietary amino acid imbalances would require an optimal reference pattern established for each species and physiological conditions (Potter, et al., 1972).

Potter, et al., (1968) and Eskeland, et al., (1971) showed i.v. glucose and VFA infusion depressed plasma amino acids in characteristic patterns which apparently related to the limiting amino acids for muscle protein synthesis. But Potter, et al., (1972) could not confirm that this approach identified the amino acid most deficient in the diet. Plasma amino acid data are generally difficult to interpret (Purser, 1970; Jacobson, et al., 1970), but can aid in explaining differences in animal performance in conditions of amino acid stress (Young, et al., 1973). Interactions in ruminants between amino acids, energy yielding metabolites (Potter, et al., 1968; Fenderson and Bergen, 1972) and hormones (Hertelendy, et al., 1969; McAtee and Trenkle, 1971; Davis, 1972) appear to be similar to reactions in simple stomached animals. Properties unique to ruminants may become evident as amino acid and hormone relations are further clarified.

Hatfield (1971) contended that a specific exogenous amino acid supplied for maximum ruminant productivity should fit the combined amino acid pattern of microbial and residual dietary protein. Experiments at Illinois (Nimrick, et al., 1970a,b, according to Hatfield, 1971) suggest the order of limiting of amino acids in rumen microbial protein produced on amino acid free diets is (1) methionine, (2) lysine and (3) threonine. Infusions of these amino acids increased N balance 60% over controls infused with isonitrogenous levels of urea. Most balances went up several times more than the amount of amino acid N infused, indicating a stimulated protein synthesis rather than merely storage of the infused amino nitrogen. Altered plasma amino acid levels reflected the infusates, but threonine was depressed by methionine infusion and still further by additional lysine (Nimrick, et al., 1970a). In another experiment most plasma amino acids decreased linearly with increasing methionine supplementation (Nimrick, et al., 1970b). Plasma concentrations of methionine, however, rose sharply with increasing methionine supplements beyond the level which promoted maximum nitrogen retention (Nimrick, et al., 1970b).

Scott, et al., (1972) showed abomasal methionine infusion in wethers elevated the plasma methionine concentrations while depressing threonine, with no influence on other plasma amino acid levels. These results paralleled those

of Wakeling, Annison and Lewis (1970)[3] showing that threonine followed methionine as the second limiting amino acid in lambs fed a barley straw diet. The lack of effect of dietary methionine on plasma amino acids, or on N balance is contrary to abomasal supplementation and clearly demonstrates ruminal degradation of orally supplied methionine (Scott, et al., 1972).

In steers, Steinacker, et al., (1970) found that abomasally infused methionine increased N retention about 20% on a 12% CP diet with 40% of total N from urea when compared to oral feeding of methionine or inorganic sulfur. Chalupa, et al., (1972), however, found inconsistent effects of abomasally infused methionine on N retention in growing steers. But retained N was doubled from 15g/day by infusion of casein + methionine, with no further increase when tryptophan was added to the infusate. An EAA-mixture gave results similar to casein + methionine.

In order to pass methionine through the rumen it has been encapsulated with kaolin as a protecting substance. Responses to coated methionine have also varied; and Mowat and Deelstra (1972) found the effect dependent upon dietary protein. Encapsulated methionine fed with soybean meal had no influence on gains or feed efficiencies, but these parameters as well as carcass quality were improved by feeding

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[3]As cited by Scott, et al., 1972, the source not available.

encapsulated methionine with formalized soybean meal or corn-urea. A growth trial revealed a cubic response in weight gain to increasing levels of encapsulated methionine, with indication of a toxic effect of the higher supplements of methionine (Mowat and Deelstra, 1972).

3.2 Studies in lactating cows.

Regardless of the ideal energy to protein ratio for milk production, high producing cows must absorb large quantities of amino acids; and milking cows require a higher ratio of protein to energy than finishing cattle. A sufficiently high ratio may be hard to achieve even in cows on high concentrate rations unless substantial amounts of feed protein bypasses the rumen (Chalupa, 1971). Hence, the favourable responses in wool and tissue growth to extra-ruminal amino acids suggest good possibilities for beneficial effects in lactating cows.

a. Methionine supplements in lactating cows.-- Although milk proteins are relatively low in methionine, the exogenous supply of this amino acid has been studied more extensively than any other in lactating cows as well as other ruminants. The versatile involvement of methionine in lipid and protein metabolism may render its availability crucial in times of metabolic stress, as in early lactation (McCarthy, et al., 1968).

As early as 1946 it was believed that methionine might be nutritionally limiting in ruminants (Loosli and Harris, 1946; Hungate, 1966); but Shaw (1946) did not show methionine beneficial in treating bovine ketosis when administered orally or intravenously. McCarthy, et al., (1968), however, found that intravenous methionine followed by oral doses of methionine hydroxy analog (MHA, 30g/day for three days) improved the health of ketotic cows. Further evidence for a key role of methionine in lipid metabolism was indicated by increased milk fat when methionine was supplied orally as MHA.

Other researchers have in later years tested methionine and its derivatives for ketosis with varying results. Only slow changes toward normal levels of blood metabolites and milk production were observed by Waterman and Schulz (1972) who treated six cases of clinical ketosis with 40g MHA per day. Pre-clinical conditions were not reached over the three-week examination period. Fisher and Erfle (1970) could not show that 40g methionine given intravenously over 24 hours alleviated symptoms of ketosis in three ketotic cows.

Griel, et al., (1968) found 40g MHA supplement per day from three weeks prepartum to eight weeks post-partum increased FCM production compared to the controls. Eighty g MHA per day had different effects in cows of different breeds but this was possibly due to an influence on feed intake rather than specific metabolic reactions.

In a study conducted by Polan, et al., (1970) high levels of MHA (90g/day) frequently reduced the intake of concentrate and corn silage, which offset any increase in milk production. But the fall in milk volume was not considered to account for a linear increase in milk fat percent with increasing levels of MHA. Altered composition of serum lipid fractions during the MHA treatment further strengthened the suggestion of an involvement of methionine in the cows' lipid metabolism.

Bishop (1971) found MHA had no effect on the fat test in heifers while there was a gradual increase with advancing age in milk fat for MHA-treated cows versus controls at a similar age. It appeared that MHA predominately affected protein metabolism in the younger cows with a progressive involvement in lipid metabolism associated with aging (Bishop, 1970). A trend towards a greater response with the maturity of the cows was again reported by Bishop and Murphy (1972) when the analog (2.2g/kg concentrate) was fed throughout a test year. The effect was evaluated by comparing DHI production data with the preceding year's records.

Kim, et al., (1971) observed higher milk fat production in cows while on MHA, but there was no effect on milk volume or SNF. Neither did the treatment affect energy or N digestibility, but cows on MHA lost more CH_4 and urinary N than did the controls. Neither Burgos and Olson (1970),

nor Whiting, et al., (1972) nor Begum and Jones (1972) observed any increase in milk production by feeding different levels of MHA. Digestibility of major feed constituents and N retention was not influenced by MHA (Begum and Jones, 1970).

Broderick, et al., (1970) found an increased methionine to valine ratio as well as higher absolute methionine levels in all of eight cows receiving 15g methionine per day in a kaolin-tristearate capsule. Milk production and composition was not significantly influenced by the supplement. An increase in the methionine/valine ratio has been suggested to indicate an oversupply of methionine, resulting in an imbalance to other EAA, particularly valine. Like other branch chained amino acids, valine is slowly metabolized by the liver (Kaplan and Pitot, 1970). Neudoerffer, et al., (1971) found about two-thirds of methionine available for intestinal absorption in cattle when the amino acid was encapsulated with kaolin and saturated fat. About 30% of the substrate was broken down in the rumen. The authors suggest that this method of nutrient supply allows for close to full availability for intestinal absorption.

b. Intravenous amino acid infusion studies in milking cows.--Since amino acids interact in a competitive fashion at absorption sites (Christensen, 1963) a gut overload of one or several amino acids may result in a distorted plasma

pattern (Hume, 1972). Therefore, intravenous (i.v.) amino acid administration may allow a more precise focusing on metabolic reactions than is possible by supplying the gut.

Naturally though, the i.v. approach requires special precautions and may still be hampered by complications. Infusion of enzyme-hydrolyzed casein or fibrin by the jugular vein (Yousef, et al., 1969) caused fever and depressed milk production. Nevertheless, the arterio-venous (AV) concentration difference over the mammary gland increased during infusion; 9% in three cows on a normal ration and 28% in three cows on a high-grain ration demonstrated to stimulate milk protein synthesis. Fifty g acid-hydrolyzed casein per day increased milk protein secretion 14%. Addition of glucose to the protein hydrolysate helped control fever, and combination of 50g glucose and 50g hydrolysate elevated milk protein production 10-15% through increases in milk volume and milk protein concentrations. When infusion of partially hydrolysed fibrin + glucose (1:1)[4] was compared to glucose, no fever or discomfort in the cows (2 per treatment) was seen during the first two days of the trial.[5] As the treatments were switched after two days break, however, severe fever developed in the cows that received the protein

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[4]Aminosol[R], Modified fibrin hydrolysate injection, Low sodium U.S.P., from Abbot Laboratories, North Chicago, Ill.

[5]Unpublished work for which the author held the main responsibility.

hydrolysate, while only slight fever was detected in one of the cows receiving glucose.

Since protein hydrolysates are used intravenously in clinical nutrition they ought to be suitable for experimental use in ruminants. However, the blood infusion technique seems more suitable for studying single amino acids, and several have conducted such experiments. Teichman, et al., (1969) infused three levels of methionine and saline continuously for four days into eight cows, with the highest rate of methionine equivalent to 20% of the expected output in milk protein. No effect was observed on milk production. Fisher (1969) infused as much as 26g of di-methionine per day, alone or together with 52g l-lysine hydrochloride, for four days and did not observe any production response, even though methionine and cystine in the plasma increased.

In later trials Fisher (1972) intravenously infused methionine, histidine, and lysine at two levels in lactating cows. Feed crude protein level was around 85% of theoretical requirement and urea supplied 85% of that N. Milk yield was about 16kg per day and was not affected by any infusion treatment. Milk protein concentrations tended to increase as level of methionine infusion was increased. At the lower rate of infusion, histidine increased feed consumption over the saline controls, while feed consumption was depressed by high histidine. Regardless of level of histidine infusion, it lowered milk protein production (Fisher, 1972).

Fisher (1972) implies that stimulation of feed intake by infusion of low levels of amino acids were due to sub-optimal feed protein levels. The trend toward an adverse effect at the higher levels of amino acid infusion might suggest an amino acid imbalance, which was most obvious for histidine. Valine, isoleucine and leucine in plasma fell as methionine infusion increased, which would indicate critical levels of these essential amino acids. Lysine did not alter plasma concentrations of other essential amino acids, but lysine itself was not determined in this study. From the plasma levels, Fisher (1972) suggested methionine as the most marginal amino acid in his experiment.

c. Feed proteins introduced postruminally.--Several recent experiments where whole proteins have been supplied postruminally have resulted in larger and more consistent responses in lactating cows than single amino acid supplementation.

By abomasal infusion of casein + methionine in three cows Broderick, et al., (1970) found an improvement in milk volume and production of all the main milk constituents. The only significant effects, however, were in crude protein (N%x6.38) concentration which increased about 6% ($P < .01$) and protein production which increased almost 12% ($P < .05$). The findings of altered EAA and NEAA levels agree with changes associated with a general improvement in protein status. A large increase in the plasma level of branched

chain amino acids during the abomasal infusion (Broderick, et al., 1970) paralleled observations in sheep which received a similar treatment (Hogan, et al., 1968).

In later studies Broderick, et al., (1972) fed formaldehyde treated casein to lactating cows on a corn-based diet which contained 9% CP. The protein content was raised by the additions of formalinized casein to give 12, 14, 16, and 18% CP. The casein supplements elevated milk yield and protein concentration of the milk, with a maximum protein production at 16% CP. Because increasing levels of treated protein resulted in continuously higher plasma concentrations of methionine, lysine, valine and isoleucine, the authors considered these as amino acids limiting for protein synthesis. Untreated protein fed to achieve a higher level of CP% would have strengthened this study.

Spechter (1972) compared blood amino acid concentrations in lactating cows during duodenal casein infusions with those during saline infusion. He reasoned that phenylalanine, histidine and methionine were most likely to be marginal since these amino acids showed the greatest concentration drop from control to casein treatment. He assumed that a marked increase in milk protein output taxed these amino acids harder than others. But Spechter (1972) finally concluded that a general shortage of protein rather than specific amino acids limited milk production on the basal, unsupplemented diet, in which 40 to 45% of the N was

urea. The investigation (Spechter, 1972) was done with six cows around the peak of lactation, producing between 20 and 35kg milk per day, and involved 2 week infusion periods. Yields of both milk and protein rose substantially during casein treatment compared to averaged pre- and post-casein infusions.

During casein infusion, DM intakes increased almost 50% (4.2kg, $P < .01$) for two cows on the lowest casein supplement while increases were less for the cows at the two higher casein levels. Quite evidently, the increased feed consumption might partially explain treatment responses. Cows on low casein, which had the dramatic increase in DM intake, showed the largest absolute and relative treatment response in milk yield. Average protein production, however, was increased most for the cows on medium level; and there was a quadratic response in SCM yield (Spechter, 1972).

A nitrogen balance trial, with sample collections in the latter week of each period, revealed a significant linear effect of level of casein infusion on N utilization (Spechter, 1972). There was marked difference in response to treatment levels between the first and second week; possibly related to stress during the balance trial. Reasons for the large responses (Spechter, 1972) observed in milk protein yield might be the early stage of lactation of the cows with a high milk yield potential; feeding of a ration

high in NPN, and the negative N balance in cows at the onset of the experiment.

After the casein study, Spechter (1972) infused glucose in a similar manner, but this had small and mostly negative effects on milk production. At this time, however, the cows were in positive N balance and feed intakes declined with glucose infusion which commenced at about the thirteenth week of lactation. The milk fat test dropped markedly during the two higher casein infusions, continued to fall thereafter, and fell still further with glucose infusion. Despite the diverse effects of glucose on feed intakes they were positively correlated to milk production, but could have been confounded with stage of lactation (Spechter, 1972).

Mugerwa (1969) found abomasal infusion of casein enhanced the intake of a urea-cellulose ration by 27%, and the intake of a urea-starch ration was increased 10%. On the other hand, the consumption of urea treated corn silage was not consistently affected by abomasal infusion of either casein or gelatin.

Derring, et al., (1972), however, did not find that abomasal or ruminal infusion of 440g casein per day altered the DM intake in milking cows; but the DM digestibility was higher, the plasma urea was lower and milk N higher for abomasal than ruminal infusions (Derring, et al., 1972). Milk yields did not differ significantly, but the fat

content of the milk was lower ($P < .05$) for the abomasal route of infusion.

Hale and Jacobson (1972) fed or abomasally infused casein, gelatin, partially delactosed whey (PDW) and zein to cows without influence of source of protein on milk production, but level of performance was quite low. Mugerwa (1969) reported higher N utilization for casein than gelatin when these proteins were infused into the abomasum; but milk production data were not presented.

Extensive balance studies were carried out by Tyrell, et al., (1972) during abomasal infusions of cows placed in a respiration chamber. Generally, the infused casein was utilized with low efficiency despite clear responses in milk production. When 860g casein was supplied to two cows producing about 24kg per day, milk yields increased 3kg/day, which was equivalent to 48% of the energy of the infusate. However, less than 25% of the casein N was recovered in milk, with 24% in feces, 21% in urine and 31% in positive tissue balance. Glucose infused at a rate of 3.6Mcal per day (≈ 900g) increased milk energy production equal to 16% of that supplied while 48% was lost in feces (Tyrell, et al., 1972). Spechter (1972) observed comparatively higher efficiencies in his balance experiments where increased milk protein could account for 75, 54, and 36% respectively of 27, 87, and 145g of casein N infused daily; but N balances were negative when the cows were on the basal diet.

4. Milk protein content and dietary protein

Non-genetic factors influencing milk protein production has been discussed in several reviews (Larson, 1958; Huber and Boman, 1966; Kirchgessner, et al., 1967). Total protein yield tends to be more constant from day to day than the yields of milk fat and lactose (Larson, 1958). While the need for dietary protein varies directly with level of milk production, it is also generally accepted that the content of protein in milk will not increase with excessive protein allowance. The content of NPN, however, may increase to some extent. High levels of energy and high energy concentrations in feeds, on the other hand, usually increase the concentration of protein in milk. This relationship is discussed further in section C.I.

Seasonal variations in milk protein concentrations have been related to energy supply. German workers (Kirchmeier, 1970) found that the amino acid composition of casein varied more than could be explained by shift in the ratio of the different caseins. The relative amound of non-essential to essential amino acids of casein increased as casein increased in seasons with ample nutritional supply. These findings conflict with the concept that an invariable mechanism is responsible for the biochemical replication of this protein (Kirchmeier, 1970; Larson, 1958; Jenness, 1970).

5. Summary of literature review which led to the experimental approach

The optimal level and quality of feed protein for milk production in general, and milk protein synthesis in particular, is obscured by the metabolism in the rumen. While ruminal synthesis of microbial protein is related to energy transactions and can be estimated with fairly good accuracy, the extent of rumen bypass of feed protein depends upon quality and quantity of the total ration. A relationship exists between absorbable amino acids and plasma free amino acids, but more information is needed before plasma concentrations can be used to identify an amino acid as rate limiting for milk protein synthesis.

Postruminal introduction of proteins and amino acids in non lactating animals have confirmed that ruminants depend on feed protein for maximum production performance. Responses to postrumen protein in lactating cows, however, have mostly been obtained with rations deficient in protein; and therefore the outcome has not been separated from the general need for crude protein. Experimentally, direct postrumen supply, as abomasal infusion, leaves out uncertainties about the extent of rumen bypass. For study of a general, nutritional effect, the intestinal route appears more proper than intravenous infusion.

C. RESEARCH SECTION

I. FIRST SERIES OF EXPERIMENTS, 1970

1. Methods and Materials

1.1 Rationale for treatments and design

The main objective of these experiments was to study the influence of postrumen supply of amino acids on production of milk and milk protein. No report on such investigations had appeared in the literature before 1970. Thus, the first trial was exploratory, although preceded by a pilot study with one cow (No. 480).

After this cow had recovered from surgical installment of an abomasal cannula 620g of bovine albumen hydrolysate[1] was infused over 50 hours. The cow was fed a common ration considered to be adequate in protein and energy according to NRC (1966) standards. Her daily milk yield was around 14kg. During the infusion, the volume of milk and its protein concentration were slightly increased compared to the days before and after, and milk protein production increased 11%.

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[1] Aminosol^{R} 5%, Modified fibrin hydrolysate injection, Low sodium, U.S.P., from Abbott Laboratories, North Chicago, Ill.

Since amino acids also have an energetic value, and may serve as a substrate for gluconeogenesis, it was decided to use an equicaloric infusion of glucose for control to the protein in the experiments that followed. Casein was chosen as the treatment protein as it is the major milk protein and has a high biological value for growth.

Still, it remained questionable how far the infused protein might alter the pattern of amino acids reaching the mammary gland, to improve conditions for milk protein synthesis. Nevertheless, a desire to challenge the cows' ability to produce milk proteins suggested a rather high treatment level. Partly based on the pilot study it was decided to infuse casein at a rate which would supply an equivalent to two-thirds of the daily milk protein output. A cow producing 20-22 kg milk per day should thus receive around 500g casein. Because of technical limitations it was possible to infuse only slightly over 300g casein per day in the first trial.

Only one infusion pump was available at the time trial I commenced; but reasoning the study would be more informative if the cows had fairly high production, the experiment was started promptly. Two cows were used in this trial; each was infused with casein before and after a glucose infusion, with control periods interspacing these treatments. During the first part of the trial

control observations were collected for one cow while the other was infused, and vice-versa (Table 1.1).

Table 1.1.--Trial I 1970. Treatment periods.

Period No.	Treatment	Cow No. 502	Cow No. 501
		Days	
1	Control	5/8-13	5/15-20
2	Casein infusion	5/14-19	5/21-26
3	Control	5/20-26[a]	5/27-6/1[a]
4	Glucose infusion	5/27-6[a]	6/2-7
5	Control	6/2-7	6/8-13[b,c]
6	Control	6/7-12[b]	
7	Casein infusion	6/13-18	6/14-19[d]
8	Control	6/19-24	6/20-25

[a]These periods served as post-casein as well as pre-glucose control.

[b]For cow No. 502, 6/2-7 was the post-glucose control, 6/7-12, was the pre-casein control. The delay was partly because the abomasal cannula tended to slip out of position.

[c]For cow No. 501, 6/8-13 served as post-glucose as well as pre-casein control.

[d]A new roller pump allowed simultaneous treatment of the two cows in period 7.

The period length of six days was thought a minimum for a production study although amino acids have a rapid turnover rate (Black, et al., 1968; Munro, 1970), and

altered substrate availability may change the composition of secreted milk within hours (Linzell, 1967). At any rate, the total experimental timespan was intentionally kept as short as possible since it was uncertain how durable the stomach cannulation would be.

A switchback type of design was preferred because it most efficiently removed the time trends which could bias within-cow comparisons of milk production when only few cows were receiving a sequence of treatments (Lucas, 1960). Since the same observations were post-treatment controls for one infusion and pre-treatment controls for a following infusion, treatment comparisons are not completely independent. This was not considred a major problem, because it was assumed that treatments would not have a carry-over effect; and two independent control periods between infusions would have prolonged the trial.

Even with satisfactory controls, only two cows give little power for statistical test. Therefore, with the intention of possible pooling of data, a second trial employing three cows was conducted similarly to trial I despite apparent weaknesses in design (Table 1.2).

1.2 Animals and abomasal cannulation

Three Holstein cows weighing 600-650kg were fitted with abomasal cannulas at the Michigan State University

Table 1.2.--Trial II 1970. Treatment periods.

Period No:	1	2	3	4	5	6	7	8
Treatment[a]:	O	K	O	G	O	O	K	O
Cow No.	days							
480	8/2-7	8/8-13	8/14-19	8/20-25	8/26-31	8/30-9/7[b,c]	9/8-13[c]	9/15-21[d]
501	"	"	"	"	"	8/30-9/4	9/5-10	9/11-16
502	"	"	"	"	"	"	9/5-11[e]	9/12-17

[a]Treatment abreviations: O = control observations; K = casein infusion; G = glucose infusion.

[b]Day 9/1 was discarded as urinals were fastened to the cows on that day. Days 8/30 and 31 together with 9/2-4 therefore served as pretreatment control for cows 501 and 502. Urine collection was incomplete due to leaking urinals.

[c]Cow No. 480 lost her fistula plug on 9/3, so days 9/3 and 9/4 had to be discarded and infusion in this cow could not start until 9/8.

[d]Day 9/14 was discarded as the infusion in No. 480 continued into that day.

[e]Cow No. 502 was without water on 9/8, the milk yield dropped severely, and 9/8 and 9/9 was discarded. Thus there are only 5 actual days of observations for this cow in period 7. A nitrogen balance test prevented infusion for another two days.

Veterinary Clinic[2] early in their second or third lactations.

Feed was withdrawn for 24 hours prior to surgery, which was performed with the cow laying on an elevated surgery table. A vertical incision on the lower part of abdomen was made. The abomasum was moved backwards and upwards from its normal position, and sutured to the abdominal muscle which surrounded the incision.

The first cow (No. 480) was given a tranquilizer and operated upon with local anesthesia. A silastic tubing[3] was run about 15 cm into the abomasum and the incision sutured close to the tubing. A 1mm silastic sheet reinforced with dacron mesh[4] about 5cm in diameter was glued to the tubing to hold it in place. After closure of the abdominal incision the tubing was run up on the side of the cow and fastened with branding cement[5] and surgical tape.

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[2]The surgical installation of abomasal cannula was performed by Drs. W. D. Oxender and C. L. Miller, aided by students in Large animal surgery and medicine at the College of Vet. Med., Michigan State University.

[3]Silastic[R] Medical-Grade Tubing, from Dow Corning Corp., Midland, Mich.

[4]Dacron mesh from Dow Corning Corp., Midland, Michigan.

[5]Branding cement[R] from Victor Business Forms Co., Lincoln, Neb.

Following surgery, the cow developed complete inappitance and dropped sharply in milk yield which never completely recovered. About a week following surgery it became apparent that the tubing would not stay in place despite considerable effort to keep it in position. After a few weeks the tubing came out from time to time and some leakage from the abomasum occurred, and this cow (No. 480) was not employed for the first trial.

In the other two cows (Nos. 501 and 502) a smaller tubing (PE 260,[6] 2.5mm outside diameter) was fitted through an abomasal stab wound. A purse string was fastened around the tubing to keep it in position at the entrance of the stomach. It was then attached to the cow's side with branding cement and surgical tape. The tubing ran about 20-30cm inside the stomach. A different anesthesia was used in these cows, which went back on feed the day after surgery. Likewise, milk production rapidly increased to presurgery level.

A few weeks after surgery one of the latter tubings broke at the entrance to the abdomen, and the other pulled out. New tubings were established, but they tended to leak. Before trial II, and about four months after the original surgery, all three cows were fitted with fistula plugs made by joining two Jarrett cannulas.[7] By the end of trial II

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[6]Intramedical[R] Polyethylene Tubing, PE 260 ID .070"/OD .110", From Clay Adams, Inc., New York.

[7]Jarrett cannula, from Australian Rubber Mill, Aberham, South Australia (Jarrett, I.G., 1948, J. Council Sci. Ind. Res. 21:311).

(periods 7 and 8) stomach contents did seep through the fistula openings and the tissue became red and swollen. This irritation was worst in cow No. 501, which was slaughtered shortly after the termination of trial II. Except for one occasion in trial II, however, when cow No. 480 lost her plug, leakage from the abomasum did not influence the feed intake or the health of the cows.

1.3 Feeding and feed sampling

Throughout both trials concentrate was fed in two equal portions twice daily (~7:30 AM and 4 PM). At the morning feeding the cows also received corn silage while hay was fed in the afternoon. All feed was weighed out and weighbacks were recorded daily. Feed refusals were minimal in both trials and were not sampled for analysis.

Feed in trial I.--The ration consisted of common feeds in their usual proportions (Appendix Table I.1) but was fairly high in urea (Table 1.3).

In addition to 4.5kg hay and 18kg corn silage, concentrate (6.4 and 7.2kg for No. 501 and 502, respectively) was fed to meet the cows' estimated requirement for energy and protein (NRC 1966) at the beginning of the trial. The level of feeding was not changed until the trial was terminated, at which time the cows were being overfed (Appendix Table I.5).

Table 1.3.--Trials I and II 1970. Feed composition.

	Trial I			Trial II		
	Dry Material (DM)	Protein in DM	Estimated NE	Dry Material (DM)	Protein in DM	Estimated NE
	%	%	Mcal/kg	%	%	Mcal/kg
Alfalfa hay	~90	~18	1.03	91.8	18.5	1.05
Corn silage	30.5	14.4[a]	0.50	33.1	10.2[c]	0.54
Concentrate[d]	87.6	13.0[b]	1.78	89.0	18.5	1.82

[a]Urea added, 0.5%.

[b]Urea added, 1.0%.

[c]Urea added, ~0.3%.

[d]Ingredients in the concentrates: Trial I: ground shelled corn, 88.6%, molasses, 7.4%, urea, 1%, limestone, 1.6%, salt, 1%, gypsum, 0.4%; Trial II: ground shelled corn, 68%, soybean meal (50% CP), 22.5%, molasses, 7.5%, dicalcium phosphate, 1%, salt, 1%.

During trial I feed samples were obtained at irregular intervals and since the hay quality changed, the nutritive value assigned to the hay (Table 1.3) was not always the same.

Feed in trial II.--In order to test the infusion treatments under nutritive conditions which optimized milk protein synthesis, the cows were changed to a high grain-low roughage ration; reported to increase milk protein content (Rook and Line, 1961; Huber and Boman, 1966; Kirchgessner, et al., 1967; Yousef, et al., 1970). The

ration (Appendix Table I.3) was compsoed of 2.3kg hay and 4.5kg corn silage and respectively, 10.0 (No. 480), 11.4 (No. 501), and 12.7kg (No. 502) of a concentrate mixture (Table 1.3). Crude protein greatly exceeded NRC (1966) standards (Appendix Table I.4) because the concentrate was higher in protein than anticipated.

The feeds were sampled twice in each period and samples were kept in sealed plastic bags until determination for DM and nitrogen.

1.4 Infusion treatments

A solution of casein was prepared by adding 2 to 2.5g NaOH to every 100g casein:[8] a 5% solution of NaOH was mixed with casein in a mortar to form a dough which was then placed in the appropriate amount of water in a bath at 60°C. The casein (2.5% w/v) gradually dissolved over several hours, hastened by occasional stirring. The glucose solution was made from cerelose[9] at the same strength as casein, and an equal content of ME in the two substrates was assumed. To be precise, however, the glucose solution should have been stronger (Table 1.4, and comments), but the correction required probably was smaller than errors due to irregularities in the infusions. Relative to the total

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[8]Casein from Nutritional Biochemicals, Cleveland, Ohio.

[9]Cerelose (methyl dextrose), obtained from Corn Industrial, CPC International, Englewood Cliffs, New Jersey.

Table 1.4.--Trials I and II 1970. Estimated net energy and crude protein supply by the infusates relative to total intakes (%).[a]

	Cow No.					
	501		502		480	
	CP	ENE	CP	ENE	CP	ENE
			%			
Trial I. Infusion						
First casein	11.5	4.7	11.5	4.1		
Glucose	--	4.3	--	4.3		
Second casein	11.7	4.7	11.9	4.4		
Trial II. Infusion						
First casein	12.3	5.1	11.1	4.6	10.2	4.2
Glucose	--	4.8	--	4.3	--	4.4
Second casein	11.6	5.1	10.6	4.3	10.0	4.3

[a](Infusate value/feed value) x 100%.

Comments: Approximately 300g (270-330g) casein or glucose was infused per 24 hours. DM content of the substrates was about 95%. Assumed energetic value of casein, 4.5kcal/g; of glucose 3.8kcal/g (Maynard and Loosli, 1962). Crude protein in casein was 88% (14.5% N in DM x 6.38). Estimated supply: by casein, 264g CP and 1.18Mcal, and by glucose, 1.08Mcal.

nutrients furnished, the infusates were quite minor, particularly for energy (Table 1.4).

In trial I, a double piston infusion withdrawal pump[10] was used for the continuous infusion. Because a

[10]Harvard continuous automatic infusion-withdrawal pump, series No. 950, from Harvard Apparatus Comp., Inc., 150 Dover Road, Millis, Massachusetts.

casein solution stronger than 2.5% (w/v) would block the pistons of the pump total infused casein was limited to 300g per day (Table 1.4).

For trial II, two roller pumps[11] were ready, but for valid comparisons and combining of data for statistical analysis, the rate of infusion was kept the same as in trial I (Table 1.4). The concentration of the substrate solutions, however, were 4.6% (w/v).

As the level of feeding differed between and within cows, the percent of total nutrients furnished by the infusates also varied (Table 1.4); but the design of the study allowed for each cow to serve as her own control for all treatments imposed; thus, changes in the relative level of infused nutrients were balanced among treatments.

The output by the pumps were recorded at frequent intervals and if a tube was leaking, the loss was estimated. These estimates admittedly were not exact. Thus, a detailed presentation of infusion rates would have little value. Small losses of infusate in the beginning of the experiments occurred because the cows disrupted the plastic tubings conveying the solutions.

1.5 Milking and milk sampling

In both trials the cows were milked shortly after 7:30 AM and again at 4 PM. The uneven milking intervals

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[11] Holter multi-channel roller pump, model 911^{R}, from Extracorporeal Medical Specialties, Inc., Mt. Laurel Township, New Jersey.

were necessary because of the working hours for the barn personnel. Milk weights were recorded at every milking and a milk sample (about 100ml) was collected into a bottle with 0.3ml formalin for preservation (Association of Official Agricultural Chemists, 1955). After warming these samples to 38°C aliquots to the milk weights were taken to make one composite sample representing two sequential days. Thus, for every control and treatment period three milk samples were analyzed for each cow.

1.6 Blood sampling

During both trials tail blood samples were obtained every other day before the morning feeding and nine hours later. Plasma was prepared by centrifuging at 2000xg for 20 min. During trial I several hours frequently elapsed before processing of samples commenced, and the blood was often clotted. Therefore, it was felt that data on blood composition in trial I would have low validity, so samples were not assayed.

1.7 Chemical analysis

a. Feed.--Dry matter was determined by drying at 100°C for at least 48 hours. Kjeldahl N was determined on composite samples for each trial by the macro-Kjeldahl procedure on wet silage and dried hay and grain. (AOAC, 1955). Net energy was estimated from NRC (1966) feed

tables by taking into account DM and N content and the ingredients of the concentrates.

b. Milk.--Milk fat was determined according to the Babcock method.[12] Milk protein: During the first part of trial I, a modification of the method of Lowry, et al., (1951) was used for quantification of milk protein. The milk was diluted 400 times with water and a casein solution was used as a standard. Protein concentration was calculated by a regression equation based on Kjeldahl N.

Apparently the Lowry method is not reliable if the milk is not fresh, so it was decided to use Kjeldahl N determinations (N x 6.38 = CP in milk) for trial II. This was done by placing 3 ml of milk in a glass-stoppered flask and quickly weighing on a balance. After pouring the milk into a Kjeldahl flask, the weighing flask was rinsed with distilled water and then with the 25ml sulfuric acid to be added for digestion. A mixture of 5g K_2SO_4 and 1g $CuSO_4$ was used as catalyst. If duplicates deviated more than 0.05% crude protein (CP) another determination was performed.

Total solids in milk were determined in duplicates by oven drying at 100°C for 4 hours. Two ml milk was pipetted for weighing in aluminum pans of 3cm diameter,

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[12]The fat test was performed at the center for the Dairy Herd Improvement Association, Forest Road, East Lansing.

which were used for the drying. The content of solids nonfat (SNF) was estimated as total solids % minus fat %.

Nonprotein N was assayed only in trial II, following the procedure of Shahani and Sommer (1951). Initially 10ml of milk was weighed into a 100ml volumetric flask and filled to the mark with 15% (w/v) trichloroacetic acid (TCA), and shaken. After standing at room temperature for 2-3 hours, the supernatant was filtered and stored frozen at -20°C until analyzed for N. The assay was done using a Technicon Auto Analyser[13] with urea in TCA solution as the standard. Due to low N concentrations in the diluted samples, the sensitivity of the analysis was less than desirable; but clear differences were discerned between samples, and duplicate samples agreed quite well.

c. Blood plasma.--α amino N was determined according to Palmer and Peters (1969) on the freshly prepared plasma.

Ammonia and urea were assayed in plasma stored frozen for two to three months. The micro-diffusion method of Conway (1960) was used, but there was not sufficient NH_3 present to be quantified.

1.8 Calculations and statistical analysis

Responses to infusion treatments were, as previously indicated, estimated by comparing performance

[13] Technicon[R] Auto Analyser[R], Methodology N-36, Kjeldahl Nitrogen, from Technicon Controls, Inc., Chauncey, New York.

during the infusion period to that of averaged pre- and post-infusion periods. For both trials the basic unit was the mean daily production during a period although determinations of milk constiuents were for two-day sub-periods. But the repeated measurements can not be considered independent observation and basis for estimated error mean square in the analyses of variance.

The following scheme shows how the treatment versus control differences (d_p) were calculated, Y_p indicating performance in any parameter during a period (p):

first casein infusion: $d_1 = Y_2 - (Y_1 + Y_3)/2$

glucose infusion: $d_2 = Y_4 - (Y_3 + Y_5)/2$

second casein infusion: $d_3 = Y_7 - (Y_6 + Y_8)/2$

d_1 and $d_3 = d_k$, $d_2 = d_G$.

As mentioned, data for period 3 in trial I and II was employed for two comparisons (d_1 and d_3). This was the case also with period 5 for cow No. 501 in trial I (Table 1.1). Besides a possible carry-over effect of glucose infusion on milk fat concentration (Appendix Tables I.5 and I.7) and plasma urea concentration (Appendix Table I.11), treatment effects were apparently not biased by the overlapping use of control periods.

The milk production results for these two trials were similar and had homogenous variances; thus, one statistical analysis of the data, involving all five cows,

was appropriate. Despite the time between the two trials and differences in feeding, it can be objected that the two cows re-used do not represent independent observations. This point was not considered serious enough to abandon the statistical evaluation.

Analysis of variance (AOV) (Appendix Table I.13) was done by means of standard programs on an Olivetti[14] desk computer, aided mainly by the textbooks of Sokal and Rohlf (1969) and Cochran and Cox (1957).

Since the mean values for α amino N and urea in blood plasma indicated distinct differences between AM and PM samples the data for these parameters were analyzed differently than those for milk production (Appendix Table I.14). The unit for the analysis, however, was the mean of two or three sampling days within a period, composited for the same reasons that prompted neglect of the sub-period observations in milk parameters. But the control periods before and after an infusion were not averaged for the blood parameters since it might be of interest whether a difference between these periods was significant or not; possibly influenced by the infusion treatment. Presumably, there should not be a time trend influencing the blood constituents in the way milk production is affected. Because the AM and PM samples were

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[14]Olivetti, Underwood Programma 101, electronic desk computer.

drawn repeatedly from any one cow on any one treatment the AOV had to follow a split plot pattern (Gill and Hafs, 1971).

2. Results

2.1 Feed, energy and protein intake

a. Feed composition.--Except for some variations in the hay, the major feed traits were quite constant based on random sampling (particularly in trial I). Only average values for the main feed characteristics were observed (Table 1.3); but consumed dry matter, protein and energy were calculated from the appropriate concentrations for each period and average daily intake for each feed.

b. Intakes.--The feeds offered in both of these trials were readily eaten, leading to equal feed consumption for all periods (Appendix Table I.1 and I.3). Since the feed quality except for the hay, varied little, the amounts of protein and energy consumed also remained quite constant from period to period (Appendix Table I.2 and I.4) and intakes of energy and protein stayed above standard requirements.

The percent of total energy and protein supplied by the infusates differed slightly between cows (Table 1.4). But the magnitude of these fractions were quite low and responses to the infusions should be attributable to the specific metabolites infused.

2.2 Milk production and composition

a. Trial I.--Observations for the two cows used in this trial are in Appendix Table I.5. These figures were pooled with the results for trial II (Table 1.5) for statistical analysis.

Appendix Table I.6 reveals that the milk production was consistently higher during infusions than during control periods, while composition was altered to a variable extent. When casein was infused, the milk protein yield was raised 5 to 11%; whereas the increase from glucose was 8% over the mean of controls before and after.

The response in protein production was greatest for the casein treatment in the highest yielding cow (No. 502); who responded both by a higher milk protein level and increased milk volume (Appendix Table I.6). However, an unusually steep fall after the last infusion period, which had no specific explanation, exaggerated the difference between the treatment and the control periods.

The fat test tended to drop during infusions, but the pattern was irregular. A decline in milk fat content during the last casein treatment and associated control periods might have been obscured by a rapid decline in milk volume for both cows. Increased ambient temperature (middle of June) might partially explain the fall in milk production. Up to the last period the

Table 1.5.--Trial I and II 1970. Production and composition of milk and the main milk constituents by periods.

Period	Treatment	Trial I and II (N=5)								Trial II (N=3)	
		Milk yield	Protein	Fat	SNF	Protein	Fat	SNF	FCM	Est. True Prot.	
		kg/day	%	%	%	g/day	g/day	g/day	kg/day	%	g/day
1	O m[a]	16.3	3.55	4.42	9.43	570	712	1540	17.2	3.51	468
	SE[b]	2.0	.12	.14	.11	57	80	205	2.0	.10	54
2	K m	17.3	3.60	4.05	9.47	617	693	1632	17.3	3.50	513
	SE	2.0	.10	.08	.07	65	66	183	1.8	.09	71
3	O m	16.0	3.56	4.21	9.44	563	663	1500	16.3	3.44	467
	SE	1.8	.09	.13	.13	52	59	151	1.6	.12	45
4	G m	16.3	3.62	3.71	9.51	588	598	1552	15.5	3.49	497
	SE	1.8	.09	.19	.13	55	49	159	1.4	.10	58
5	O m	15.0	3.74	3.92	9.59	558	573	1448	14.6	3.63	470
	SE	1.6	.09	.25	.15	53	27	180	1.0	.10	51
6[c]	O m	14.6	3.79	4.02	9.61	553	573	1413	14.5	3.64	463
	SE	1.5	.08	.26	.15	54	25	168	1.0	.11	56
7	K m	15.1	3.85	4.19	9.46	557	620	1419	15.3	3.72	476
	SE	1.6	.05	.16	.08	58	44	144	1.3	.03	50
8	O m	13.7	3.71	4.56	9.32	508	621	1274	14.8	3.56	426
	SE	1.3	.05	.10	.14	45	47	102	1.2	.08	40

[a] m = mean.

[b] SE = Standard error of the mean.

[c] Cow No. 501 had in trial I only 6 days between glucose and last casein infusion, and the same observations were used for period 5 as well as 6.

decline in milk production was 1.5 to 1.7% per week which is about normal (Hillman, et al., 1970).

Because fat content and milk yield changed in opposite directions during infusions, FCM was not consistently affected (Appendix Table I.5 and I.6).

While the estimated recovery of infused protein as milk protein was between 10 and 15%, recovery of infused energy in milk ranged from a negative value to a high of 75%.

The concentration of SNF did not show any consistent treatment trend (Appendix Tables I.5 and I.6), nor was there a significant correlation between SNF and milk protein concentrations ($r = 0.11$) (Table 1.7), although change in SNF generally evolves in the proteins (Huber and Boman, 1967). However, there was a significant negative correlation ($r = 0.67$, $P < .01$) between SNF and fat percentage.

b. Trial II.--Comparing yield data for trial I and II (Appendix Tables I.5 and I.7) readily shows that the milk production had fallen considerably before trial II commenced. Cow No. 501 reacted negatively to a change from liberal to restricted roughage feeding which took place over 10 to 12 days. Milk yield in this cow never recovered after the changeover period even though she consumed large amounts of feed.

The commonly observed depression in milk fat by high concentrate and low roughage feeding did not develop in these cows. Perhaps the late stage of lactation (6 to 8 months postpartum) and a declining milk production masked the fat decline (Loosli, et al., 1945).

The rapid consumption of allotted feed, the hungry appearance of the cows, in addition to the decreases in milk yield and high fat tests caused suspicion that mistakes were made in rationing of feed. Repeated checks of intakes and of the man during feeding did not reveal this. The high milk protein concentrations even from the beginning of trial II agrees with previously demonstrated effects of high energy rations (Rook and Line, 1961; Huber and Boman, 1966).

Changes in protein percent with the infusions, however, were small; but during casein infusion the trend was towards an increase rather than decrease (Appendix Tables I.7 and I.8). Thus, the production of milk protein was increased over controls by casein infusion more than by the glucose infusion (Appendix Table I.8). The high protein concentration of the milk prior to post-ruminal infusion might have limited stimuation of milk protein synthesis by the infusates. Again, the cow with the highest production (No. 502) showed the greatest increase in milk even though her response to the last casein infusion was less than for the two earlier infusions.

Fat tests consistently decreased during the infusion treatments without any obvious difference between the effects of casein and glucose. The changes in SNF concentration with the infusions were slight and less consistent than protein concentration. However, there was a significant correlation ($r = 0.71$, $P < .01$) between SNF and protein level which was much higher than that ($r = 0.11$, NS) in trial I (Table I.7). These correlation coefficients were too far apart to allow a pooled correlation for the two trials (Rohlf and Sokal, 1969).

Between SNF and fat content there was a low and insignificant correlation ($r = -0.29$); much weaker than between these parameters in trial I ($r = -0.69$, $P < .01$). A composite correlation coefficient for SNF and fat levels in the two trials, was low ($r = -0.36$) and not significant. As in the first trial, there was no significant relationship between the milk protein and fat ($r = -0.05$), and the coefficient for the combined trials was also insignificant ($r = -0.21$).

Data for NPN in milk (Appendix Table I.9) showed some variation with infusion treatments, but NPN concentrations during glucose infusion were significantly depressed ($P < .05$, Table I.6, row 9). Furthermore, the response in NPN to glucose was significantly different from the response to casein ($P < .05$). When NPN was expressed as a fraction of total N in the milk there was

no influence of casein infusion. On the other hand, the fractional expression confirmed that less of total N was NPN during glucose infusion than during the other periods of the trial (Appendix Table I.9).

Because of the different changes in NPN associated with glucose and casein infusions the difference between the two treatments in estimated true protein was less than for milk crude protein (Table 1.6, row 3 and 10). However, the influence of NPN on true protein production was minute since NPN makes up such a small part of the total milk N. Nevertheless, while the response in crude milk protein production was significantly larger ($P < .05$) for casein infusion than for glucose infusion (Table 1.6, row 6), there was little difference in true protein production response for the two treatments (Table 1.6, row 11). Although the increase in milk protein (N x 6.38) production with infusions were small, they were evidently not an artifact due to increased NPN content. Thus, the NPN data confirm greater milk protein synthesis during casein and glucose infusions than during the appropriate control periods.

The absolute differences in protein production between treatment and control periods were smaller in trial II than in trial I, probably because of the lower milk production. However, treatment responses, as percentages of control production, did not differ much for the two trials.

Table 1.6.--Trial I and II 1970. Combined data. Milk production parameters during infusion (T) versus control (O)[a] periods. Means and differences between means (d) ± SE_d.[b]

Parameter	Treat-ment	Col. No. 1	Col. No. 2	Col. No. 3	Col. No. 4			Col. No. 5
		1st Casein	Glucose	2nd Casein	Cas. (K)[c] vs. Gluc. (G)			d Cas.[d] vs. d Gluc.
Milk yield		kg	kg	kg		kg		kg
Row No. 1	T_2	17.3	16.4	15.1	K	16.2	d_K	1.01
	O	16.1	15.5	14.2	G	16.4	d_G	0.88
	d	1.2±.24*	0.9±.25	0.9±.19*		-.2	d	0.13±.19
	(d%)	(7.5)	(5.8)	(6.3)		(1.2)		
FCM								
Row No. 2	T	17.3	15.5	15.3	K	16.3	d_K	0.6
	O	16.4	15.5	14.7	G	15.5	d_G	0
	(d%)	.9±.4	0±.4	1.6±.2*	d	.8	d	0.6±.65
		(5.5)		(10.9)		(5.2)		
		%	%	%		%		%
Prot. conc.	T	3.60	3.62	3.85	K	3.72	d_K	.07
Row No. 3	O	3.55	3.65	3.75	G	3.62	d_G	-.03
	d	.05±.04	-.03±.04	.10±.02*	d	.10	d	.10±.05
	(d%)	(1.4)	(.8)	(2.8)		(2.8)		
Fat conc.	T	4.05	3.71	4.19	K	4.06	d_K	-.18
Row No. 4	O	4.31	4.06	4.29	G	3.65	d_G	-.35
	d	-.26±.06*	-.35±.09*	-.10±.05	d	.41	d	-.17±.13
	(d%)	(6.0)	(8.6)	(2.3)		(11.2)		
SNF conc.	T	9.47	9.51	9.46	K	9.44	d_K	.03
Row No. 5	O	9.43	9.51	9.47	G	9.50	d_G	.0
	d	.04±.06	0±.10	-.01±.08	d	-.06	d	.03±.09
	(d%)	(.4)	--	--		(.6)		

Table 1.6.--Continued.

Parameter	Treatment	Col. No. 1	Col. No. 2	Col. No. 3	Col. No. 4		Col. No. 5
		1st Casein	Glucose	2nd Casein	Cas. (K)[c] vs. Gluc. (G)		d Cas.[d] vs. d Gluc.
		g	g	g	g		g
Prot. prod. Row No. 6	T	617	588	577	K 597	d_K	49
	O	566	561	531	G 588	d_G	27
	d	51±11*	27±6*	46±7**	d 9	d	22±6*
	(d%)	(9.0)	(4.8)	(8.7)	(1.5)		
Fat prod. Row No. 7	T	693	598	620	K 656	d_K	14
	O	688	618	597	G 598	d_G	-20
	d	5±18	-20±13	23±8*	d 58	d	34±18
	(d%)	(0.7)	(-3.2)	(3.9)	(9.7)		
SNF prod. Row No. 8	T	1632	1552	1419	K 1526	d_K	94
	O	1520	1474	1343	G 1552	d_G	78
	d	112±19*	78±28	76±18*	d -26	d	16±18
	(d%)	(7.4)	(5.3)	(5.7)	(1.7)		
Est. True Prot. prod.[e] Row No. 11	T	513	502	477	K 495	d_K	38
	O	467	469	445	G 502	d_G	33
	d	46±10*	33±22	32±3**	d -7	d	5±6
	(d%)	(9.9)	(7.0)	(7.2)	(-1.4)		
NPN conc.[e] Row No. 9		mg%	mg%	mg%	mg%		mg%
	T	35.0	26.3	30.3	K 32.7	d_K	-0.67
	O	33.8	32.2	32.7	G 26.3	d_G	-5.85
	d	1.2 1.9	-5.9 1.3*	-2.6 3.7	d 6.4	d	-5.18 1.0*
	(d%)	(3.6)	(-18.2)	(-8.0)	(24.3)		

Table 1.6.--Continued.

Parameter	Treat-ment	Col. No. 1	Col. No. 2	Col. No. 3	Col. No. 4		Col. No. 5
		1st Casein	Glucose	2nd Casein	Cas. (K)[c] vs. Gluc. (G)		d Cas.[d] vs. d Gluc.
		mg%	mg%	mg%	mg%		mg%
Est. True	T	3.50	3.49	3.72	K 3.61	d_K	.07
Prot. conc.[e]	O	3.48	3.53	3.60	G 3.49	d_G	-.04
Row No. 10	d	.02±.03	-.04±.07	.12±.07	d .12±.06	d	.11±0.02

[a]The average control periods before (O_1) and after (O_2) the infusion treatment.

[b]Standard error of a difference between two means, SE = $\sqrt{2S^2/N}$, where S^2 = MS error in AOV and N = no. of replicates, here 5 (cows) generally, but 3 when trial II only is used. Asterisks indicate level of probability: * = .01 < P < .05; ** = .001 < P < .01.

[c]The average of the first casein (K_1) and the second casein (K_2) infusion period.

[d]The average estimated response to the first casein (d_1) and the second casein (d_3) infusion study.

[e]Only for trial II, N = 3 (cows).

Table 1.7.--Trials I and II 1970. Correlation coefficients between milk, blood and feed components.

Factors	Trial		
	I	II	I & II
milk fat (%) - milk SNF (%)	-.67**	-.29	-.36
milk CP (%) - milk SNF (%)	.11	.71**	[a]
milk CP (%) - milk fat (%)	.12	.05	.21
milk CP (%) - milk NPN (mg%)[b]		.25	

	Blood Sampling Time		
	AM	PM	Daily Mean
plasma urea N (mg%) - milk NPN (mg%)	--	--	.51**
plasma urea N (mg%) - pl. α am. N (μm/ml)	.84**	.59**	.76**
plasma urea N (mg%) - feed CP (kg/day)	.07	.57**	
plasma α am. N (μm/ml) - feed CP (kg/day)	.01	.24	

[a]The difference between trials significant ($P < .05$), pooling of data not permissible.

[b]NPN (mg%) observed only in trial II.

2.3 Evaluation of the compounded production data for trials I and II

As mentioned, it was found allowable to combine the production data in trials I and II. Results of the pooled AOV are in Appendix Table I.12. With the spread and changes in yield and composition of milk through the course of these trials, variances and standard errors naturally were large (Table 1.5). By taking out variations due to cows and time trend, however, the experimental errors were reduced so that some significant differences between infusion and control periods were detected (Table 1.6).

The statistical evaluation confirmed impressions from each of the trials that increased milk yield rather than altered protein concentration was the primary reason for increased protein production during the infusion treatments (Table 1.6). Although the increments in protein production due to casein infusion were not great, they were consistent and significant ($P < .05$). For the last casein infusion, protein content was also increased significantly ($P < .05$) over the controls. However, two of the five cows showed a marked fall in milk protein after the last infusion.

While the effect of the casein treatment on milk protein concentration was small, overall averages were higher than during glucose infusion (Table 1.6, row 3).

Statistical tests of this and similar comparisons were not carried out because of the unequal time span between the glucose infusion and the first and second casein infusions.

Differences in time after parturition might be the reason for the slightly higher milk production averaged for all glucose infusions compared to all casein infusions (Table 1.6, row 1). Still, only the casein treatments increased milk yields significantly ($P < .05$).

The infusion versus control differences in milk yield for the casein and glucose treatments (d_K vs. d_G), were almost identical (1.1 vs. 0.9kg, Table 1.6). Only for protein production (N x 6.38) did the differences between casein and glucose approach significance ($.05 < P < .10$). This difference ($d_K - d_G$), of only 22g, equalled about 4% of the protein production during the control periods, or about 7% of the infused casein, but its validity is strengthened by the observation that response to glucose infusion (d_G) never was larger than that for the averaged casein infusions ($d_1 + d_2/2 = d_K$) in any of the five cases studied.

Significant increments ($P < .05$) in true protein production were also noticed for the casein infusions but not for glucose; even though the casein increased and the glucose decreased the NPN content of the milk. With the few observations, however, the responses (d_K vs. d_G) were

not significantly different at the conventional level of probability ($P < .05$).

The influence of treatment on milk yield is also evident in SNF production. While differences in SNF concentrations between infusions and controls were nil, SNF production was increased significantly ($P < .05$ by casein, but not by glucose. The lack of a difference in SNF concentrations between infusion and control periods suggests that lactose and protein concentrations varied in opposite directions.

Although the fat depression during the glucose infusion was larger than for either of the casein infusions, it was not significant at the conventional level of probability (Table 1.6, row 4). This was probably due to large variations associated with the glucose treatment, for a smaller fat decrease during the first casein infusion was significant ($P < .05$). A small apparent fat depression during the second casein treatment, however, was largely due to an increase after the infusion, when milk volume fell off (Table 1.5).

During the glucose infusion (period 4) the fat percentage fell gradually for all five cows, and no other parameter showed such a gradual response to treatment (Appendix Tables I.5 and I.7). The most marked time trends were noticed for the highest yielding cows. In period 5 fat percentages for the two days immediately after glucose infusion were on an average lower than for the remaining

4 days (Appendix Tables I.5 and I.7). They continued to rise during period 6 which suggests a carry-over effect and the reason for the lack of nonsignificant treatment effect to the glucose treatment.

2.4 Blood parameters

Changes in blood parameters generally reflect the availability of metabolites for synthetic purposes. In these experiments it was also surmized that the fate of the infused substrates might be more completely explained by observing crucial blood parameters. Many factors, however, will influence the blood level of a metabolite; and the impact of a particular treatment may thus be obscured. For example, the diurnal variations evidently prevented some changes in plasma urea and α amino N from reflecting infusion treatments.

a. Plasma Urea N.--The levels of plasma urea N in these cows (Table 1.8, Appendix Table I.10) were in the normal range (e.g., Barry, 1964). Between days, within periods there was no particular concentration pattern. In several periods one or more observations were missing for a given day. Data from other cows were excluded for that same observation in order to give equal numbers for the period mean of all cows.

Period means (Table 1.8) reveal a distinct effect of treatment on plasma urea levels. There was a moderate increase during the first casein infusion, then a dramatic

Table 1.8.--Trial II 1970. Concentration of urea N and α amino N in blood plasma (period means and standard errors).

Per	Treat-ment	Urea N				α amino N			
		AM[a]	PM[a]	Day total[b]	(N)[c]	AM[a]	PM[a]	Day total[b]	(N)[c]
		mg/100ml				μM/ml			
1	O	15.9±1.1	19.9±1.2	17.9±0.6	(9)	2.17±.05	2.41±.08	2.29±.04	(6)
2	K	16.9±1.9	26.4±1.2	21.6±0.9	(9)	2.69±.20	2.51±.19	2.60±.19	(6)
3[d]	O	17.9±1.2	19.9±1.1	18.9±1.4	(6)	3.06±.23	2.31±.03	2.69±.11	(6)
4[e]	G	9.7±0.8	9.6±1.3	9.7±0.6	(9)	2.27±.06	2.10±.11	2.17±.09	(9)
5	O	12.6±1.1	12.8±0.9	12.7±0.5	(9)	2.37±.11	2.16±.09	2.29±.09	(6)
6	O	14.5±0.2	19.0±0.5	16.8±0.5	(6)	2.40±.02	1.97±.11	2.19±.05	(9)
7	K	13.7±0.6	20.7±1.9	17.2±0.5	(6)	2.39±.10	2.31±.13	2.35±.10	(9)
8	O	10.7±1.1	16.3±3.4	13.5±0.8	(6)	2.12±.15	2.03±.03	2.07±.09	(6)

[a]Sampling time (bleeding = B): AM = before morning feeding; PM = 9 hours after AM sampling.

[b]The mean ± SE for day total based on daily means for each cow (Appendix Tables I.10 and I.11).

[c]In case a cow lacked a sample or a sample was condemned, the data for other cows on that day were also discarded to get an equal number of observations for the cows within a period.

[d]The α amino N values possibly erroneously high because of improper assay.

[e]From period 4 on the α amino N assay was modified; thus the values for the three first periods cannot be compared to the values that follow.

decrease during the glucose infusion followed by a slow recovery. After the last casein infusion, when the level barely rose above the preceding control, there was again a drastic fall which has no explanation. The elevated level during casein infusion, however, can be regarded as an expected consequence of deamination of infused protein. The depressed urea level ($P < .01$), during glucose infusion suggests less amino acid deamination because the readily available glucose promoted protein synthesis (e.g., Potter, et al., 1968) or retarded protein mobilization. Alternatively, the augmented glucose available to the liver may have stimulated synthesis of nonessential amino acids. However, this probably would not alone have caused the large observed decrease in the plasma urea levels. In any event, it is puzzling that such a marked and lasting depression in plasma urea could result from the modest glucose infusion in cows fed an abundance of protein.

The higher plasma urea in the afternoon (PM bleeding) than before the morning feeding (AM bleeding) is probably a reflection of diurnal eating patterns because most of the feed was consumed between 8 AM and 5 PM. Thus, absorption of nutrients would be much lower in the early morning hours than in the afternoon. Changes in plasma urea are known to follow the deamination of feed amino acids, with peak levels occurring a few hours after a protein load (Knott, et al., 1972).

The interaction of casein infusion and bleeding time on plasma urea was most obvious for the first infusion study and probably resulted from a load of absorbed amino acids during the post-fed state. Before feeding, the infused amino acids were apparently handled without increasing the plasma urea levels, but the addition of infused protein in the postprandial state augmented the urea synthesis and increased plasma concentrations. With only the AM samples it would not have been possible to detect changes in plasma urea due to casein infusions; neither would the observations have inferred the fate of the infused amino acids. Regardless the presence of a treatment and bleeding time interaction, the PM samples clearly suggest deamination of infused amino acids.

b. Plasma α amino N.--While no quantitative estimate of infused amino acids was made, the concentration of α amino N in plasma (Appendix Table I.11) did increase during the casein infusion (Tables 1.8 and 1.9). This would mean that the availability of at least some amino acids for milk protein synthesis was increased. Generally, the levels obtained agree with those reported for sheep (Fenderson and Bergen, 1972) and lactating cows (Rook and Line, 1961).

For different reasons one or more observations were lacking for a sampling day in five of the eight periods; and similar to urea N data, these days were omitted to permit an equal sample size for all cows in each period.

Table 1.9.--Trial II 1970. Blood components: comparisons of mean values for different treatment periods and bleeding times (mean and difference between means ($d \pm SE_d$).[a]

Parameter	Treatment	Col. No. 1 1st Casein	Col. No. 2 Glucose	Col. No. 3 2nd Casein		Col. No. 4 Cas. vs. Glc.	Col. No. 5 Controls (O) only
		mg%	mg%	mg%		mg%	
Urea N	T_b	21.6	9.7	17.2	K[c]	19.4	
	O[b]	18.4	15.8	15.1	G	9.7	
	d	3.2±1.4[g]	-6.1±1.1**	2.1±2.5		9.7±3.1[h]	
	AM	16.9	13.4	13.0		14.0	14.3
	PM	22.1	14.1	18.7		18.1	17.6
	d	-5.2±0.8[i]	0.5±1.3	5.7±1.1**		4.1±0.6[i]	-3.3±.67[j]
		µm/ml	µm/ml	µm/ml		µm/ml	
α amino N	T_b	2.60	2.18	2.48	K[e]	2.47	
	O[b]	2.49	2.24	2.13	G	2.18	
		.11±.10	-.06±.06	.35±.19		.29±.02*	
	AM	2.85	2.35	2.31		2.46	2.43
	PM	2.38	2.07	2.11		2.22	2.16
	d	.47±.08[i]	.28±.04*	.20±.11*		.24±.13	.27±.06[j]

[a]Standard error of a difference between two means (see Table 1.6).

[b]The average of control before (O_1) and after (O_2) infusion.

[c]The average of first casein (K_1) and second casein (K_2) infusion.

[d]Only control after infusion (O_2) was considered because change in the assay.

[e]Only the second casein infusion (K_2) used for comparison because change in the assay.

[f]Asterisk indicates level of significance (see Table 1.6 ,and AOV, Appendix Table I.14).

[g]Significant, $P < .05$, but also significant treatment x bleeding interaction.

[h]Significant, $P < .01$, but also significant treatment x bleeding interaction.

[i]Significant, $P < .001$, but also significant treatment x bleeding interaction.

[j]Significant, $P < .01$, but also significant bleeding x period interaction.

The α amino N concentration for all cows varied considerably from day to day within a period (Appendix Table I.11), and period means for cows also differed in levels as well as diurnal trends. These diversities are expressed in an interaction between periods and bleeding times for the first casein infusion ($P < .01$) and for control periods ($P < .001$). For undiscovered reasons, the α amino N levels in period 1 increased from the morning to the afternoon, but in no other period was this trend noted.

Omitting the first sampling day for periods 4, 5 and 7, gave largely the same results for the treatment versus control comparisons as obtained with the complete set of observations; indicating no systematic carry-over effects.

For the second casein and the glucose infusions, the AM values were significantly ($P < .05$) higher than the PM values, and no interaction between sampling time and treatments was shown. The high concentrations in α amino N before feeding (Table 1.9) may have been due to a low availability of energy yielding metabolites, mainly volatile fatty acids (VFA). Diurnal patterns in plasma α amino N levels in sheep reflected the energy supply (Fenderson and Bergen, 1972). Still it seems contradictory that the α amino N level was high in the morning when plasma urea N was low, and vice versa. It might be that

mobilization of protein for gluconeogenesis in the pre-fed state resulted in an elevated amino acid level, but this possibility seems remote. Rook and Line (1961) generally found higher α amino N levels in cows 5 to 8 than 2 hours after feeding, with a peak at 5 hours. The difference between 2 and 8 hour samples were more marked in well-fed than under-fed cows (Rook and Line, 1961).

Although two samples a day is hardly indicative of an average daily value, the infusion of casein at least tended to raise α amino N concentration above control values, while glucose infusion failed to show this tendency.

3. Discussion

Although abomasal casein infusions in these experiments did consistently raise milk and protein production ($P < .05$ or lower, Table 1.6), the increments were not dramatic relative to control values. More data are needed to verify that improved amino acid supply to the mammary gland was the reason for observed responses. The validity of this hypothesis has been supported by more recent experiments in our own laboratory and elsewhere. Shortly after these experiments were completed, Broderick, et al., (1970) reported positive response to abomasal infusion of casein. They used three cows in a similar change over design. The cows averaged 31kg milk per day, and the

infusates furnished about 800g casein + 24g methionine daily for one week. Treatments increased milk protein production (N x 6.38) 110g/day, or 11.6% ($P < .05$); compared to an average of 50g, or a 9% increase ($P < .05$ or lower) for casein infusions of 300g/day in our experiments with cows averaging 16kg per day. They reported a nonsignificant increase in milk yield 1.2kg/day) compared to 1.2 ($P < .05$) and 0.9kg ($P < .05$) increments for casein infusions in our study. Milk protein in our experiments increased only 0.07 percent compared to 0.20 percent ($P < .10$) observed by Broderick, et al., (1970).

Although the Wisconsin workers noticed a 10% ($P < .5$) decrease in grain intake during casein infusion, consumption during control periods was not sufficient to meet the cows' energy requirements according to common standards.

On the other hand, Broderick, et al., (1970) noted no response in protein production or percent or in milk yield to infusion of glucose and urea designed to be isocaloric and isonitrogenous to the casein and methionine infusion. In the present experiments, however, the milk yield was increased about as much by glucose as by casein infusion while the influence of glucose treatment on protein content was nil (-0.3% change). Negative responses in milk protein content, milk yields, and protein production resulted from duodenal infusion of glucose in the studies of Spechter (1972). Increases of about 30% in

milk protein production were observed by Spechter (1972) when casein was infused into early lactation cows on a ration with 40-45% of total N as NPN. A negative N balance and deficit of natural protein in the ration may explain the greater responses obtained in that study.

As might be expected, the efficiency of conversion of postruminally infused protein to milk proteins have usually been inversely related to the level of treatment. Thus, for the lowest rate of casein infusion in Spechter's (1972) experiment, 74% was recovered as milk protein while the fractions for the medium and high treatments over 54 and 36%, respectively. Even the latter value is higher than apparent recovery of 17% in our experiment (supplying 300g/casein per day), and 13% in that of Broderick, et al., (1970); where 800g protein was infused.

Tyrell, et al., (1972) recovered 24% of 860g abomasally infused casein in two cows producing approximately 24kg milk per day. The treatment increased milk yield by about 3kg/day. Infusing 433g casein in one cow (Tyrell, et al., 1972) increased milk yield 2kg/day, but only 12% of the protein was recovered in the milk. Judging from these reports, the extent of recovery of the infused supplement depends on nutrient adequacy of the ration and physiological status of the animal, as well as level of protein infusion.

It should be noticed that the milk protein content in the studies discussed here refer to N x 6.38 with the exception of Spechter (1972), who applied an infrared spectrum analyser to determine true protein. While it is well documented that feed protein above accepted standards will not change SNF or protein content of milk (Huber and Boman, 1966), the NPN fraction may increase significantly by high levels of digestible protein (Storry and Rook, 1962; Senft and Klobasa, 1969).

Casein infusions slightly raised ($P < .05$) milk NPN; whereas, glucose infusion depressed this entity ($P < .05$). Others have not reported fractionation of N components in studies where postruminal infusions have enhanced milk protein secretion.[1]

The differential changes in milk yield and protein concentrations for the various studies suggest that the consistent increments in milk protein production originated by different routes. Thus, the infusates apparently elicited different metabolic or secretory mechanisms in the cows in different experiments. For example, cows in both Wisconsin (Broderick, et al., (1970) and Canadian (Spechter, 1972) studies responded to casein infusion with larger increases in milk protein concentration; perhaps because of a lower intake of dietary protein relative to needs, than in the current experiments.

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[1]After completing this manuscript it was learned that Broderick (1972) measured milk NPN when feeding formalinized casein.

Milk protein concentration per se apparently does not exert a strong feedback influence on its synthesis as Rook and Line (1965) found substantial increases in protein concentrations and unchanged protein yield when milk volume was lowered in insulin-treated cows with depressed plasma glucose levels. In vitro studies, on the other hand, suggested an end product inhibition of α lactalbumin synthesis in bovine mammary cells (Larson, 1969).

The depression in milk fat in our study was of a magnitude similar to that reported by Derring, et al., (1972) and Spechter (1972), but there was no fat decrease in the experiment of Broderick, et al., (1970). Apparently a lower threshold for dietary influences on milk fat content exists in cows producing large amounts of milk on a gluconeogenic metabolism (Orskov, et al., 1969).

The increased plasma urea level during casein infusion in our second trial suggests increased gluconeogenesis resulted from a greater absorption of amino acids. This seems very likely according to the scheme of Krebs (1963). The study of Derring, et al., (1972) also indicates that infused amino acids were deaminated for further catabolism. Plasma glucose, however, was not higher during abomasal than ruminal casein infusion in the experiment of Derring, et al., (1972), but this agrees with Wright, et al., (1966) who showed that 35kg sheep may handle 350g exogenous glucose per day without a raise in blood glucose concentrations.

In lowering milk fat content the abomasally infused casein resembled a high starch-low fiber diet. Such a ration characteristically yields a depressed acetate to propionate ratio in the rumen, enhances rumen bypass of starch, and probably increases glucose absorption (Van Soest, 1963; Wright, et al., 1966; Orskov, et al., 1969). Moreover, such a change in metabolism usually depresses milk fat and increases milk protein (Rook and Line, 1961; Huber and Boman, 1966). Ruminal additions of propionate have increased milk protein and depressed milk fat (Rook and Balch, 1961; Storry and Rook, 1962; Halfpenny, et al., 1969); but when ruminally infused propionate replaced 15% of ME for 6 weeks in a fat-depressing ration, milk protein content as well as milk yield were lowered compared to the basal ration (Orskov, et al., 1969).

An effect of energy form, generally starch versus cellulose, is often hard to separate from that of energy level since a high rate of energy supply usually is achieved by higher grain feeding (Huber and Boman, 1966). Rook and Balch (1961) imply that high energy levels will increase milk SNF and protein when "additional" energy from grain amounts to 4000kcal or more per cow. However, Yousef, et al., (1970), found increased milk protein concentration, particularly the α casein and β lactoglobulin fractions, resulted from increased energy concentration in the ration. They also demonstrated that ruminal VFA changes do not

always accompany the milk protein increments. Addition of sodium bicarbonate and magnesium oxide in the concentrate for cows on high-grain, low-roughage ration changed the ruminal acetate/propionate ratio toward that of normal feeding, and partly corrected a milk fat depression, but the milk protein content still remained as high as on the ration not supplemented with the salts.

These studies (Yousef, et al., 1970) suggested a greater capacity for protein synthesis by the mammary gland of cows on a high grain ration; apparently independent of a high rumen propionate. The swiftness of this reaction is not known; but Rook (1971), on the other hand, contended that increased protein secretion by propionate infusion has a lag phase of 2-3 weeks. Such a long term induction would indicate another mechanism than that seen in the experiments where postruminal protein infusion spontaneously increased the milk protein production. Still, the different experiments do not exclude an impact of a glucogenic type of metabolism as defined by Orskov, et al., (1969). Even though Yousef, et al., (1970) imply that propionic acid was not critical for raising milk protein, the relatively lower propionate to acetate ratio in the rumen after feeding $NaHCO_3$ and MgO may have been accompanied by a greater glucose absorption from the postruminal digestive tract (Wright, et al., 1966).

Armstrong and Prescott (1971) concluded in a review article that the stimulating effect of propionate on milk protein secretion probably is mediated through the sparing of amino acids for gluconeogenesis by the liver. The same authors (Armstrong and Prescott, 1971) also pointed out that glucose and propionate initiate different endocrine actions (Lindsay, 1970): whereas glucose stimulates insulin secretion; propionate stimulates both insulin and glucagon. The impact of route of introduction of these metabolites can also be extended from the work of Fisher and Elliot (1966) where intravenous infusion of propionate and glucose failed to increase milk protein; but both treatments lowered the milk fat content. On an equicaloric basis, glucose caused a more severe fat depression than did propionate. Since these infusions lasted four days, an effect on protein secretion might not be expected (Rook, 1971).

Although abomasal glucose infusion in our experiments increased the milk yield short of significance ($P > .05$), its effect resembles that of the intravenous infusions of glucose and propionate (Fisher and Elliot, 1966) which significantly ($P < .05$) increased milk yield and lactose concentrations ($P < .10$). Ruminal propionate, however, failed to raise the yield of milk while milk protein secretion was increased (Rook and Balch, 1961; Rook, et al., 1965).

Volatile fatty acids added to concentrate tend to depress intake, but Jones (1971) demonstrated that ME and feed protein were most efficiently used for milk production at maximal acetate levels. Although 25-30% of the glucose taken up by the lactating cows' mammary gland may be oxidized (Annison and Linzell, 1964; W-od, et al., 1965) acetate is apparently a more critical energy substrate (Rook and Hopwood, 1970). Propionate, on the other hand, inhibits acetate utilization by the sheep liver (Pennington, 1957); and may thus influence the mammary metabolism although hardly any propionate reaches the udder.

The specific effect of ruminal propionate in increasing milk protein secretion was independent of ration composition in one experiment by Rook, et al., (1965). In the two trials reported herein, response in protein yield to casein infusion was similar on a normal or a high-grain, low-forage ration. However, our increases in milk protein were due largely to higher milk yields and not to increased protein concentration as reported (Rook, et al., 1965) after infusion of propionate into the rumen.

In discussing changes in concentrations of the major milk components; Wiegner's law, according to Kirchgessner, et al., (1967), implies that the concentration stability of a milk component is inversely proportional to its degree of dispersion. Thus, the content of fat, the least dispersible component, is most easily altered,

followed by casein, the other milk proteins, lactose, and salts. Consequently, a metabolic change affecting synthesis of all milk components appears most easily in the fat secretion, and the lactose will be more stable than protein.

A more modern view on milk secretion, stated in biochemical terms, contends that the rate of milk-fat secretion may vary independently from that of the other constituents (Silcock and Patton, 1972). However, Silcock and Patton (1972) found closely related secretion rates for milk protein, lactose, and ionic potassium. Supported by related observations these authors (Silcock and Patton, 1972) suggest that lactose, protein, and K^+ are secreted together from the Golgi apparatus of the alveolar cell. This, however, seemingly would not need to exclude a different rate of synthesis of protein and lactose if the substrate, energy or hormonal exposure of the mammary cell were varied.

Rook (1971) has pointed out that the rate of uptake of nutrients by the gland may modify milk secretion not only through a specific precursor-product relationships, but also by altered supply of substrate for ATP production. German workers have found that pyruvate concentration of the mammary gland changes with the season in positive correlation with the content of casein in the milk (Waldschmidt, 1973) as influenced by nutrition (Kirchmeier, 1970).

Moreover, the content of nonessential amino acids increased with the casein content of the milk (Kirchmeier, 1970).

Regardless of metabolic mechanisms involved, an increased protein output means more amino acids lost from the animal, and, consequently, more amino acids were removed from the blood by the mammary gland. Yousef, et al., (1970), however, did not find increased AV differences of α amino N when milk protein production went up on a high-grain, low-roughage ration. But such differences are of limited value if not accompanied with blood flow data (Linzell, 1971). Since milk yield largely determines the mammary blood flow (Linzell, 1971), a substrate's AV concentration difference may stay fairly constant even though the gland's actual uptake differs due to changes in production.

In simple terms, an increase in plasma amino acid concentration should indicate improved conditions for protein synthesis (Munro, 1970). In the second trial here, when α amino N was measured, its concentration did tend to increase in tail blood plasma during casein infusion compared to the control; and it was significantly higher ($P < .05$) during casein than during glucose infusion. Likewise, Rook and Line (1961) found elevated levels of α amino N in jugular venous plasma when feeding a high energy ration that promoted increased milk protein production. It can be calculated from the data of Broderick, et al.,

(1970) and Spechter (1972) that abomasal infusion in both studies increased the level of total amino acids in plasma and whole blood. More noteworthy, though, was a higher ratio of essential to non-essential amino acids, a trend known to indicate an improved amino acid status in ruminants (Oltjen and Putnam, 1966) as well as single-stomached animals (Munro, 1970).

Dietary supply influences plasma free amino acid concentrations despite a high buffering capacity through continuous protein catabolism (Wannemacher and Allison, 1968) and hormonal regulations (Munro, 1970). Because of selective membrane transport mechanisms, absolute and relative concentrations of amino acids may vary widely between tissues and plasma (Wannemacher and Allison, 1968; Munro, 1970). As an augmented milk protein output causes a stronger drain on the amino acid pools, the plasma level of particular amino acids may be lowered, at least on a molar basis because those presented to the gland will not fit the pattern demanded for milk protein synthesis. Furthermore, diurnal variations in cows' plasma amino acid levels may be substantial (Halfpenny, et al., 1969) although less than in simple stomached animals (Champredon, et al., 1969).

Because milk secretion rates are quite constant over normal intervals and production levels (Linzell, 1960; Tucker, et al., 1961), and in view of the numerous factors affecting supply and demand of amino acids, there probably

is a regulated uptake by the mammary cell; as suggested by Rook (1971). As yet, amino acid transport into mammary cells has received little attention.

Regardless of complicated systems regulating amino acid availability, enhanced efficiency of protein nutrition by amino acid supplement requires that the limiting amino acids be introduced in a quantitatively tailored manner (Allison, 1963). Identification of critical amino acids therefore becomes a central part of this topic.

II. SECOND SERIES OF EXPERIMENTS (1971)

i. Trial I 1971

1. Methods and material

1.1 Rationale for treatments and design.

While the first series of experiments indicated the availability of amino acids was more critical for milk production than was glucose, a stimulating effect of improved glucose supply could not be excluded, and the estimated responses were possibly due to a combined effect of glucose and amino acids. Thus, the likelihood for an interaction effect of the two substrates was tested in an experiment aimed at verifying the results of the first series of experiments.

Intending to relate to our earlier findings, the mode of treatments were kept as previously, but the rate of supplementation was according to milk production. Thus, abomasal protein infusion equalled 75% of the milk protein output in one treatment, with equicaloric glucose infusion in another; and the third was a mixture of equicaloric amounts of protein and glucose. The mixture was infused at the same rate as the individual substrates, although for testing an interaction effect it might have

been more appropriate to keep the infusion rate of each substrate unchanged. The reciprocal influence on the availability of the two substrates, however, suggested the total supplement ought to be equal for all treatments. Originally six cows were available for placement of abomasal cannula. But because two cows were lost shortly after surgery and there was doubt as to the availability of a third cow, only three out of six cows were used initially. In order to include a control treatment and the three supplements described, the regular Latin square design was modified to that presented in Table 2.1.

Experimental periods lasted seven days, with the first day for transition and then two three-day sub-periods. When the fourth cow (No. 603) was recovered from surgery, she received the casein infusion because it was of primary interest. Although not included in means and statistical analyses, observations from this cow have been recorded in order to strengthen the overall conclusions from the data.

Casein is relatively poor in methionine and other S-containing amino acids; and methionine supplementation greatly improves the BV of casein for growth (Allison, et al., 1959). Moreover some experiments have shown that feeding methionine was beneficial to lactating cows (McCarthy, et al., 1968; Polan, et al., 1970; Bishop and

Table 2.1--Trial I 1971. Experimental design and timing of periods.

Period No.	Sub per. No.	Obsv. beg. end days	Cow No. 604	606	607	603[a]
			Infusion Treatment			
1	1	6/24-6/26	O	O	O	
	2	6/26-6/29				
2	1	7/2-7/4	G	K	M	
	2	7/5-7/7				
3	1	7/9-7/11	O	O	O	
	2	7/12-7/14				
4	1	7/16-7/18	M	G	K	
	2	7/19-7/20[b]				
5	1	7/24-7/26	O	O	O	O
	2	7/27-7/29				
6	1	7/31-8/2	K	M	G	K
	2	8/2-8/5[c]				
7	1	8/10-8/12	O	O	O	O
	2	8/13-8/15				

Symbols (infusion treatment):
O = saline control (volume as for the other infusions)
K = caseinate + 3% dl-methionine } equicaloric,
G = glucose } together symbolized T
M = mixture (50/50) of K and G }

[a]Results for cow No. 603 are not included in any mean or statistical analysis.

[b]Day 7/21 was discarded due to improper milking.

[c]A third 3-day infusion followed, but only 2 subperiods were used: No. 604 was obviously not well 8/1-2, infusion was faulty in 603 and 606 8/2-5, 607 was in heat 8/7-8; the affected subperiods thus discarded.

Murphy, 1970), but contrasting results were shown (Broderick, et al., 1970; Burgos and Olson, 1970; Williams, et al., 1970; Begum and Jones, 1972). Therefore the caseinate was enriched with 3% dl-methionine[2] which was similar to the supplement used for abomasal infusion by Broderick, et al., (1970).

For all the following experiments sodium caseinate[1] was dissolved in tap water by heating to 55-60°C and stirring occasionally. The glucose solution was made from cerelose.[3]

With the ME of casein protein being 4.6kcal/g and that of glucose 3.8kcal/g (Maynard and Loosli, 1962, p. 322), and sodium caseinate 85% protein, equal weights caseinate and cerelose yield that same ME.

In control periods saline (0.9% W/V NaCl) was infused at a volume similar to the substrates. The infusion rates were derived from milk protein production for three days preceding the trial, and are shown in Table 2.2.

The substrate concentration for each cow was regulated to fit a volume of 10-12 l for 24 hours. Infusion was achieved with multichannel pumps,[3] each serving two cows. Ruptured tubes and other malfunctions of the

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[1] Sodium caseinate, from Nutritional Biochemicals, Cleveland, Ohio. Typical analysis 5% moisture and 92.5% protein (N×6.38) in dry material.

[2] Obtained from Nutritional Biochemicals, Cleveland, Ohio.

[3] The same as in 1970, see section I, 1.1.

Table 2.2--Trial I 1971. Substrate infusion rates derived from pre-trial protein production.

Cow	Milk yield	Protein content	Protein yield (N×6.38)	Amount infused		
				Protein[a]	Total solution[b]	Energy (est.ME)
	kg	%	g	g	g	Mcal
604	25.9	2.7	700	525	650	2.37
606	22.2	2.7	600	450	550	2.09
607	23.6	3.0	700	525	650	2.37
603	18.1	3.1	580	435	550	2.09

[a]75% of daily protein production.

[b]The same for Na-casinate and cerelose, Na-caseinate being 85% crude protein.

infusion system occurred at times, particularly in the beginning,but the total infusion over 24 hours was usually very close to that intended.

1.2 Animals and abomasal cannulation.

Six cows were bought for these experiments, four within a month after calving. One was a first-calf heifer, the others were starting their fourth to eight lactations.

Because it was desirable to use the same cows for experiments requiring abomasal sampling, a cannula with 31 mm outer and 22 mm inner diameter was used.

The cannula was made from liquid plastic[4] which was heated to ~80°C before being poured into a form.

[4]Plastisol, liquid plastic material from U.S. Stoneware, Inc., P.O. Box 350, Akron, Ohio.

Air bubbles in the liquid material were removed by vacuum and gradual cooling was necessary for satisfactory quality. Consisting of one piece of pliable plastic, the cannula had an 8cm flange that prevented it from being forced out of the fistula opening.

The cows did not receive any feed for 24 hours preceding surgical insertion of the cannulas at the MSU Veterinary Clinc.[5] One attempt to operate on a cow (No. 603) while standing was abandoned because the abomasum could not be moved to a desirable position. Later this cow was cannulated as the other cows, but an infection in the abdominal wall after the first operation delayed the second incision.

Surgery was similar to the previous year, but the cows were laid down on a foam-covered floor using nitrous oxide as anesthesia. The first cow operated upon in this manner (No. 602) did not recover from the anesthesia until three hours after the operation; and she had a poor appetite for several days thereafter. A few weeks later this cow ruptured and lost her cannula to the interior of the abomasum; so she was slaughtered.

In operating on Cow No. 605 a second incision was required to locate the abomasum. Apparently this cow had not been deprived of food for the proper period before

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[5]Dr. D. J. Ellis supervised the surgery aided by staff and students in Large Animal Surgery and Medicine.

surgery. She was very weak and showed inappetance after the operation; still milk production stayed up quite well. However, she contracted severe mastitis and never became fit for experimental use.

The surgery in cows No. 604, 606 and 607 was without complication, lasting around two hours.

1.3 Feeding and feed sampling.

Both net energy and total protein intake were intended to be higher than NRC (1971) standards. Since the smallest cow (No. 604) produced more milk than the other two, the same amount of feed was offered to all three cows (Appendix Table II.1). The daily concentrate ration was devided in two feedings and fed at milking (at 8AM and 4 PM). Corn silage was fed around 10 AM and hay around 4 PM.

Samples of feeds were obtained on days 2, 4, and 6 of each period, and DM was determined immediately by oven drying. Period composites of corn silage were frozen at -20C; and hay and concentrate composites were dried, ground and stored for chemical analysis. Feed refusals were weighed and sampled as the feeds. However, the same DM values were used for the refused as for the fed corn silage, because this was always mixed with concentrate. A few DM values were obtained for concentrate refusals which were averaged for calculating intakes.

1.4 Milking and milk sampling.

Milk sampling and handling of the samples was similar to earlier experiments (section I, 1.5). Two composite milk samples were prepared, one for the first three days and the other for the last three days of each period.

1.5 Blood sampling.

On the last day of each period tail blood was sampled at three times; before the AM feeding ($B_0 \approx$ 7:30 AM), 3 to 3.5 hours after feeding ($B_3 \approx$ 10:30 - 11:00 AM), and 8.5 to 9 hours after the morning feeding ($B_9 \approx$ 4:30 - 5 PM). The blood was drawn into vacuum tubes without anticoagulants; but was immediately poured into 50ml centrifuge tubes containing 40mg potassium oxalate and 50mg sodium fluoride. The centrifuge tube was stoppered and placed on ice until further processing in the laboratory, which usually commenced within 30 minutes following the sampling. After whole blood was sampled for the urea determination, plasma was obtained by centrifuging at 5000×g for 10 minutes.

1.6 Chemical assays.

Chemical assays were generally as for the 1970 trials, and only additions or differences from previous practice will be mentioned.

(a) Feed analyses.--Crude fiber (CF) was determined on hay and corn silage only. This assay and N in hay and

corn silage were carried out by the Forage Analysis Laboratory in the MSU Department of Biochemistry.

(b) Milk analyses.--Total solids were determined gravimetrically by drying according to AOAC (1955). After weighing about 2ml milk in drying pans as previously (section I, 1.8b), the pans were placed on a steaming water bath for 30 minutes before drying in oven at 100°C for four hours.

Total nitrogen was determined by the macro Kjeldahl procedure (AOAC, 1955) using 3ml milk in triplicates for each sample.

Non protein nitrogen (NPN) was separated from the protein N by trichloro acetic acid (TCA) precipitation as described by Mahan, et al., (1971). Eight ml of milk was weighed in a 50ml centrifuge tube. After adding 24ml 15% (W/V) TCA, it stood at room temperature for 1 hour and was then centrifuged at 8000×g for 10 minutes. The supernatant was filtered over a Whatman No. 42 filter into a 50ml volumetric flask. Ten ml more TCA was added to the precipitate which was stirred, recentrifuged, and the supernatant filtered into the same flask. The volume was finally brought to 50ml with additional washings of 15% TCA.

The protein free filtrate was frozen until assayed on an auto analyser,[6] but the filtrate had to be concentrated

[6]Technicon[R] Auto Analyser,[R] see section I, 1.8.

4 times by evaporation on a steam water bath in order to reach a detectable N concentration. This inconvenience could have been avoided by using a more concentrated TCA solution to a larger amount of milk to achieve ~ 10% TCA in the final volume.

Lactose was determined by a method according to Hinton and Macara (1928), modified after Ling (1956). A lactose carrying filtrate was obtained by tungstic acid precipitation and stored frozen until the lactose content of the filtrate was quantified by iodometric titration. The lactose determination in this trial was not as precise as desired. Despite repeated determinations on the triplicates obtained from each milk sample the variations were large within sample (day) and between days in period for any cow, and results for period 1 was discarded.

(c) Assays of blood constituents.--Urea N was determined according to Coulombe and Favreau (1963). One ml oxalated blood was pipetted into 25ml centrifuge tubes with addition of 9ml of tungstic acid reagent, made from 8 volumes N/12 sulfuric acid + 1 volume 10% (W/V) sodium tungstate just before use. After standing 10 minutes upon shaking the tubes were centrifuged for 15 minutes at 8000×g, the supernatant decanted off and frozen until final assay.

Then, 0.4ml of the blood filtrate was taken out and mixed with 10ml of "reagent A" prepared just before use from 10 volume 60% (W/W) ortho phosphoric acid (H_3PO_4) and 2 volume DAM-TSC solution (0.6g diacetyl monoxide + 0.03g thiamine carbazide in 100ml water). Urea water standard solutions of appropriate concentration were prepared similarly. Sample, standard and reagent blank preparations were boiled together for 20 minutes as the tubes were sealed with glass beads. After a quick cooling in cold water the optical density was determined by spectrophotometry read at 540μm.[7] Urea N concentrations were calculated by regression formulas derived from the standards. Plasma glucose was determined enzymatically[7,8] in protein free filtrate of plasma prepared at time of sampling, frozen until the final assay 2-3 months later. Plasma free amino acid determination will be described in section III.

1.7 Calculations.

Estimated net energy (ENE_L) for corn silage and hay in each period was calculated from CP and CF in DM according to values in NRC (1971) feed tables. For the concentrate mixture, each ingredient was assigned a NE_L value according to NRC (1971) feed tables.

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[7]Using a Gilford Spectrophotometer, model 2000, Serial 650; Gilford Instrument Lab., Inc., Oberlin, Ohio.

[8]Glucostat^R, enzymatic glucose determination, from Wortington Biochm. Corp., Freehold,New Jersey.

Milk production parameters.--From the production of milk constituents for every sub-period a weighted mean was derived for the concentration in the milk for the whole period. For the concentrations of milk constituents as well as production, the averages for saline periods before and after casein, glucose and mixture were used as controls. Since this leads to repeated use of periods 3 and 5, the control periods as presented in Table 2.1 are not completely independent.

Because the fraction of NPN/total N in milk were all around 5%, these values were transformed by the arcsin function (Rohlf and Sokal, 1969, Table K) for statistical analyses.

1.8 Statistical analysis.

Since the order of the three infusion treatments were randomly allotted to three cows (Table 2.1), these treatements as well as the differences (d_T) between the treatments and averaged pre- and post-treatment controls form a 3×3 Latin square (e.g., Cochran and Cox, 1957). Thus, the estimated responses (d_T) were used for an analysis of variance (AOV) according to the Latin square design (Anova I-1, Appendix Table II.8) although this renders only two degrees of freedom (df) for the

error mean square (EMS) and a very low power of the F-test. Still the magnitudes of the F's for different parameters and the relative size of mean squares should indicate the impact of the different treatments as sources of variation (Appendix Tables II.11a and II.12).

The absolute production levels were used for two additional AOV, attempting to draw benefit from the several measurements in each animal to enhance the df for the EMS, thus strenghtening the power of the test. Furthermore, it was desirable to single out an effect of bleeding times. Hence, each of the three substrate treatments were compared to adjacent controls as outlined in Appendix Table II.9 (Anova I-2).

Although Anova I-2 yields the variance among controls before and after treatment as well as the variance among treatments and controls (orthogonal contrasts), the repeated use of control periods implies that the difference between controls has no relevance. Furthermore, because the cows received the specific infusions in different periods, this AOV assumes no effect of time and treatment sequence.

Direct comparison of the actual performance at each treatment and averaged adjacent controls was carried out according to the arrangement in Appendix Table II.10. Again it had to be assumed that the overlapping of control

periods had a neglible effect on the estimated variances. In addition it was assumed that there is no interaction between cows and sequence of treatment, neither that treatments and associated controls interacted with sequence or cows. The controls for different treatments naturally are not randomly distributed, but this objection was not considered serious since the difference between controls was of no interest in itself. Considering three separate controls, however, gave a balanced design.

Since all three bleedings occurred in each treatment and control period, a split plot pattern was used (Anova I-3, Appendix Table II.10b).

2. Results

2.1 Feed intakes.

Since the same amount of feeds were offered throughout the trial (Appendix Tables II.2 and II.3) variations in consumption of dry matter (DM) and nutrients reflect feed acceptability and composition (Appendix Table II.1).

The hay quality (Appendix Table II.1) varied from extremely poor in period 4 to very good in period 7. For the latter period the corn silage was also more acceptable than earlier as indicated by higher consumption. Thus, the intakes of CP and ENE were higher during period 7 than other periods (Table 2.3).

Table 2.3--Trial I 1971. Consumption of dry matter, crude protein and estimated net energy (mean for treatment periods).

Period	1	2	3	4	5	6	7
Control/Treatment	O[a]	T[b]	O	T	O	T	O
Dry matter, kg/day	16.2	15.5	16.3	16.2	15.8	16.1	17.0
Crude protein, kg/day	2.38	2.46	2.44	2.11	2.35	2.47	2.67
Est. NE_L, Mcal/day	28.8	26.8	28.8	28.0	27.6	28.8	30.5

[a]Saline. [b]Substrate infusion (see Table 2.1).

Generally, the cows refused much of their corn silage, but the intakes of CP and ENE (Table 2.4) still surpassed the NRC (1971) standards (Appendix Table II.4). Except for period 7 there was no real tendency towards greater overfeeding at the end of the trial and milk production remained stable.

Despite time trends and feed quality changes there was a small but consistent tendency for lower intakes during treatment than control periods (Table 2.4), and the F value for all treatments versus all controls approached significance (P ~.10).

Analyzing estimated treatment responses (d_T) by Latin square (Anova I-1, Appendix Table II.11a) showed a significant period effect (P<.05) on CP intake, evidently due to low intakes in period 4 when the hay quality was low. ENE was affected similarly but to a smaller degree. Adding the infused protein to CP eaten for cows No. 604

Table 2.4--Trial I 1971. Summary of feed intakes: mean comparisons between treatment and control periods.

Infusion Treatment	Dry matter kg/day[a]	Crude protein kg/day[a]	Est. NE_L Mcal/day[a]
(N=3)			
K	15.9	2.55	28.0
O_K	16.4	2.47	29.2
d	-.5±.57	-.08±.12	-1.2±1.22
G	15.3	2.29	27.1
O_G	15.8	2.41	28.2
d	-.5±.46	-.12±.12	-.9±.98
M	15.8	2.40	28.4
O_M	16.0	2.44	28.6
d	-.2±.78	.04±.13	-.2±1.03
(N=9)			
All T	15.7±.23	2.35±.06	27.8±.50
All O	16.1±.21	2.44±.03	28.7±.41
	-.4	-.09	-.9

[a]Mean values, and SE_d = standard error for the difference between two means for each infusion study, and SE = standard error of the composited means. See also Appendix Table II.11.

and 607 in period 4 elevated total protein supplied to above the standard requirements.

2.2 Milk production and composition.

Milk yield.--Milk yields were consistently increased by treatments, with the casein effect greater than in previous experiments. Because of the few replicates, however, responses (d_T) were not significant for any particular treatment (Table 2.5; Appendix Table II.12), but d_K and d_M exceeded d_G ($P<.05$, Table 2.5, Appendix Table II.12).

Table 2.5--Trial I 1971. Milk production parameters; treatment and control means (m±SE), differences between treatments and controls (d±SEd), and results of statistical analyses.[a]

Column:		1	2	3	4	5	6
ANOVA for F test	Trtm.	Milk yield m ± SE	FCM prod. m ± SE	Prot conc. m ± SE	Prot prod. m ± SE	Est. True Protein conc. m ± SE	Est. True Protein prod. m SE
	(N=3)	kg	kg	%	g	%	g
	K	26.03 ± 1.00	21.3 ± .83	3.19 ± .12	833 ± 60	3.02 ± .12	788 ± 56
	O_K	24.37 ± 1.39	20.8 ± 1.19	3.00 ± .11	731 ± 51	2.86 ± .10	697 ± 47
I-2	d	1.66 ± .80	.5 ± .98	.19 ± .051*	101 ± 22**	.16 ± .054*	91 ± 22**
	(d%)	7.0	2.5	6	14	6	13
	G	23.85 ± 1.29	18.8 ± 1.27	3.03 ± .13	722 ± 37	2.89 ± .12	686 ± 31
	O_G	23.28 ± .79	19.3 ± 1.61	2.95 ± .13	682 ± 22	2.81 ± .12	651 ± 21
	d	.57 ± .64	-.5 ± 1.03	.08 ± .052*)	40 ± 26	.08 ± .043*)	35 ± 24
I-2	(d%)	2.5	-2.5	3	6	3	5
	M	25.66 ± 1.50	19.9 ± .99	3.17	814 ± 59	3.01 ± .11	773 ± 59
	O_M	24.52 ± 1.41	20.4 ± 1.87	3.00	735 ± 47	2.85 ± .06	669 ± 47
I-2	d	1.14 ± .65	-.5 ± .97	.17 ± .069*	79 ± 37*)	.16 ± .044*	74 ± 38*)
	(d%)	4.5	-2.5	6	11	6	11
	(N=9)						
	T	25.18 ± .72	20.0 ± .69	3.13 ± .065	790 ± 31	2.97 ± .061	750 ± 30
	O	24.04 ± .71	20.2 ± .82	2.98 ± .050	716 ± 23	2.84 ± .047	682 ± 12
I-3	d	1.14 ± .69*	-.2 ± .83	.15 ± .041**	74 ± 29**	.13 ± .041**	68 ± 28**
	(d%)	4.5	-1	5	9	5	10
I-3	(Betw. T)	KM>G*		KM>G**	KM>G**	KM>G**	KM>G**
I - 1	(Betw. d_T)	KM>G*			KM>G*		KM>G*

[a]Symbols for probable significance: *)= P<.10
* = P<.05
** = P<.01
*** = P<.001

Table 2.5--Continued.

Column:		7	8	9	10	11	12
ANOVA for F test		NPN conc. m ± SE	NPN/Total N m ± SE	Fat conc. m ± SE	Lactose conc.[b] m ± SE	SNF conc. m ± SE	SNF prod. m ± SE
	(N=3)	mg/100ml	%	%	%	%	g
	K	27.4	5.5	2.80	4.87	8.47	2207
	O_K	21.6	4.6	3.03	5.07	8.42	2054
I-2	d	8.5 ± .29***	.9 ± .19***	-.23 ± .16	-.20 ± .11	.05 ± .11	153 ± 64*)
	(d%)	27		7.5	-4	<1	7.5
	G	22.9	5.1	2.58	4.85	8.43	2008
	O_G	21.1	4.7	2.85	5.01	8.34	1935
I-2	d	1.8 ± .96	.4 ± .46	-.27 ± .17	-.16 ± .14	.09 ± .06	73 ± 43
	(d%)	8.5	8.5	-9	2.5	1	4
	M	25.5	5.2	2.50	4.94	8.59	2208
	O_G	21.5	4.6	2.85	5.02	8.35	2050
I-2	d	4.0 ± 1.27*	.6 ± .29*)	-.35 ± .25	-.08 ± .04	.24 ± .16	158 ± 80*)
	(d%)	18.5	13	-12		3	7.5
	(N=9)						
	T	25.3 ± 1.16	5.3 ± .22	2.63 ± .07	4.88 ± .04	8.50 ± .10	2164 ± 71
	O	21.4 ± 0.62	4.6 ± .10	2.91 ± .09	5.03 ± .04	8.37 ± .10	2013 ± 66
I-3	d	3.9 ± 1.16***	.7 **	-.28 ± .15*	-.14 ± .11	.13 ± .09*)	151 ± 72*
	(d%)	18.5	15	-9.5	-3	1.5	7.5
I-3	(Betw.T)	KM>G**		K>M			KM>G**
I-1	(Betw. d_T)						

[b]The response in lactose production (d_T) was larger (P<.05) for K (33g/day) and M (39g/day) than G (-12g/day).

However, statistical analysis according to Anova I-3 (Appendix Table II.14) clearly shows differences between the substrate infusions and the controls. The overall difference of 1.1kg also was significant ($P<.05$), and protein infusions promoted higher ($P<.05$) milk output than glucose. Milk yields during full protein infusion were slightly higher than when mixture was infused. Moreover, responses for the three treatments above control tended to increase linearly as the level of protein infused increased. The largest increase in milk yield from casein infusion, 2.7kg for cow No. 607, however, was partially due to a steep decline in yield after infusion.

The extra cow (No. 603) that received casein infusion also produced more milk (+2kg) than during control periods.

Protein concentration (N×6.38).--The two infusions with protein (K and M) gave almost identical responses in crude protein concentration of milk compared to controls ($P<.05$). Individually, however, the cows responded somewhat differently; No. 604 showed a higher response to glucose than the mixture but greatest to the full protein infusion; No. 607 responded most to the mixture; and 606 responded equally to the mixture and full protein, but not to glucose. In no case was protein concentration lower during substrate infusion than during the appropriate

control periods, but the mean for control period 5 was equal to the preceding treatment period (Appendix Table II.5).

While the milk protein levels during infusion with protein were higher than during glucose infusion, responses (d_T) were not significantly different.

Protein (N×6.38) production.--The concentration of any milk constituent will be influenced by changes in other constituents as well as milk volume, and only the actual yield reflects the true rate of secretion. Since the infusions stimulated milk yield as well as protein concentration, crude protein production was higher during treatment than control periods. Greatest responses were shown for the casein infusion ($P<.01$) which also caused the largest increase in milk yields. A relatively large error attached to the estimated response to the mixture render this difference barely significant ($P<.10$). Responses (d_T) to protein were also higher ($P<.10$) than for the glucose treatment.

Estimated true protein (ETP).--The difference in true protein production between casein and the mixture was less than for crude protein and reflects the larger increase in NPN during casein infusion. Being such a small fraction of total N, however, changes in NPN must be large to have an impact on total protein.

For the glucose infusion no differences between treatment and controls were noted for CP and ETP production, suggesting no influence of glucose infusion on milk NPN even though total N went up. The reason for the lower milk NPN for cow No. 606 on glucose (period 4) may have been due to the low protein intake compared to her needs (Appendix Table II.4). With the addition of infused protein (K or M) the other cows received an adequate N intake also in period 4.

Statistical analyses of production of estimated true protein revealed essentially a pattern similar to crude protein; despite the significant changes in NPN concentration of milk.

Milk lactose.--All lactose values for period 1 were discarded as too high due to improper assay. Moreover, parallel determinations for lactose were often in poor agreement, so mean lactose levels had large experimental errors. Nevertheless, there was a trend towards slightly lower milk lactose during the treatment than control periods. Despite apparently depressed lactose concentrations during protein infusions the lactose output was increased due to increased milk volume. Increases in lactose yield due to protein infusions were different ($P<.05$) from the slightly negative responses to glucose (Table 2.5, col. 14).

Solid non fat (SNF).--Milk SNF concentration increased for the separate substrates compared to controls, but differences were small with rather large errors (Table 2.5, col. 11). Variance among treatments were larger than among controls (Appendix Table II.14). Similar to data for protein production, the multiple effect of milk volume and concentration made changes in SNF production more dramatic than those in concentration; but still the increments to protein infusion barely approached significance ($P<.10$). The overall treatment response was less significant than for protein production,but SNF yields during protein infusions were definitely higher than during glucose infusions.

Milk fat.--Although changes in milk fat content from control to substrate infusions were consistently negative (Appendix Table II.5, item (6)), the large errors attached to differences between treatment means explain why none were significant. The largest depression in fat content was noted with the mixture, but no physiological reason for this appears evident. While variances among treatments were larger than among controls (Anova I-3) Appendix Table II.14), the fat content for all treatments were significantly lower than for the controls ($P<.05$).

As fat percent dropped and milk yield increased due to treatments, responses in fat production and FCM were small and standard errors for the responses large.

Hence, none of the differences among infusion treatments approached significance and milk energy output was not systematically changed due to the substrate infusions. The energy supplied with these infusions should theoretically suffice for 1.5 - 1.8kg FCM (NRC 1971).

Supporting the findings in previous trials our data (Table 2.6) reveal that means and responses were similar for the full period and the last three-day subperiod indicating that treatment effects were instantaneous.

2.3 Blood components.

a. Blood urea nitrogen concentration (BUN).-- Blood urea nitrogen (BUN) was not determined the first control period (period 1). Thus comparison during substrate infusions to pre- and post-treatment controls is not possible. Using the control following any infusion as its particular control, statistical analyses were determined according to Anova I-3 (Appendix Table II.15).

Concentration at different bleedings within a day for individual cows varied considerably, as did the levels between days (Appendix Table II.6); thus, standard errors were relatively large (Table 2.7). Still, the BUN concentration during protein infusions were higher ($P<.05$) than during glucose infusion, which was slightly lower than for control periods.

Table 2.6--Trial I 1971. Comparison of production results for the full 6 day periods and adjacent controls to last 3 day of substrate infusion and closest 3 day controls.[a]

Treatment	Means for cows No. 604, 606 & 607 Milk Yield 6d	Milk Yield 3d	Prot. Conc. 6d	Prot. Conc. 3d	Prot. Prod. 6d	Prot. Prod. 3d	Fat Conc. 6d	Fat Conc. 3d
	kg		%		g		%	
K	26.03	26.28	3.19	3.17	833	834	2.80	2.87
O_K	24.37	24.50	3.00	3.00	732	732	3.03	3.10
d	1.66	1.78	.19	.17	101	102	-.23	-.23
G	23.85	23.20	3.03	3.00	722	694	2.58	2.57
O_G	23.28	22.85	2.95	2.93	682	666	2.85	2.88
d	.57	.35	.08	.07	40	28	-.27	-.31
M	25.66	25.69	3.17	3.18	814	818	2.50	2.37
O_M	24.52	24.76	3.00	2.99	735	742	2.85	2.83
d	1.14	.93	.17	.19	79	76	-.35	-.46

[a]Control period before and after infusion averaged as usual.

Including the control periods in the analysis (Anova I-3) showed significant ($P<.05$) decline in BUN throughtout the day (Table 2.7). This diurnal trend is opposite the usual (Knott, et al., 1972) but may reflect the cows' eating pattern. In no case was BUN influenced significantly by a treatment X bleeding hours interaction. But when the control periods were taken into account (Anova I-3) there was a highly significant ($P<.01$) interaction between treatment sequence (periods) and bleeding hour. No physiological explanation for this appears evident; but during sequence 2 (periods 4 and 5, Appendix Table II.6), the BUN was higher than before and after.

Table 2.7--Trial I 1971. Concentration of blood urea nitrogen (BUN) according to sampling times and infusion treatment.[a]

Anova for F test	Trtm.[b]	0 hr (B_1) m ± SE(N) mg/100ml	3 hr (B_2) m ± SE(N) mg/100ml	9 hr (B_3) m ± SE(N) mg/100ml	All hrs m ± SE(N) mg/100ml
	K	23.0 ± 3.1(3)	31.0 ± 4.6(3)	22.7 ± 8.4(3)	25.6 ± 3.2(9)
	O_K	26.7 ± 5.5(3)	25.0 ± 3.5(3)	13.0 ± 1.0(2)	22.6 ± 3.0(8)
	G	22.0 ± 1.5(3)	18.7 ± 4.3(3)	17.0 ± 1.7(3)	19.2 ± 1.6(9)
	O_G	25.3 ± 5.4(3)	17.3 ± 3.9(3)	18.0 ± 4.5(3)	20.2 ± 2.6(9)
	M	30.3 ± 2.0(3)	25.3 ± 3.4(3)	32.5 ± 4.5(2)	29.0 ± 1.9(8)
	O_M	20.7 ± 3.2(3)	21.0 ± 1.5(3)	16.0 ± 1.5(3)	19.2 ± 1.4(9)
	KM	26.7 ± 2.3(6)	28.2 ± 2.8(6)	26.6 ± 5.4(6)	27.2 ± 1.9(17)
	O_{KM}	23.7 ± 3.1(6)	23.0 ± 1.9(6)	17.4 ± 2.7(6)	21.6 ± 1.6(17)
	All O	23.9 ± 2.9(8)	21.1 ± 1.9(9)	15.9 ± 1.8(8)	20.3 ± 1.4(25)
I-3					T>O*)
I-3					KM>G*
					All infusions
I-3					$B_1>B_{2+3}$*[c]
I-3					$B_2>B_3$*[c]

[a] Missing values were stipulated for the statistical test (based on equations according to Cochran and Cox (1957, p. 125); but the derived values are not included in this table; used only for statistical analysis.

[b] Controls (O_T) are for periods following the respective treatments only because samples of period 1 were spoiled in storage.

[c] Significant interaction (P<.001) between bleeding times and treatment sequences obscure these differences.

Opposite to expected, BUN levels were highest before AM feeding and fell linearly throughout the day (Table 2.7). Only during full protein treatment (K) did the BUN concentrations increase after the AM feeding as often observed in this parameter. However, the prefeeding and late PM levels during the mixture treatment were as high as peak levels during casein infusion (Table 2.7). Influence of different eating patterns may be the reason for the different diurnal trends in BUN between treatments, as well as the deviation from the common pattern in control periods.

The overall increase in the BUN level during protein infusion confirms earlier results suggesting deamination of infused amino acids. A slight tendency to lower BUN level during glucose infusion than during controls also is in accordance with earlier findings; indicating improved N (amino acid) utilization or an amino acid sparing effect on the glucose supplement.

The BUN levels at 3 hr postfeeding were significantly correlated ($r = 0.57$, $P < .05$) with the NPN level in the milk. Although BUN was as high before feeding as 3 hrs later, prefeeding BUN and milk NPN were not significantly correlated ($r = 0.30$, NS) and BUN at 9 hrs postfeeding showed no relationship to milk NPN ($r = 0.06$).

b. Plasma glucose concentration (PG).-- Concentrations of PG for substrate treatments were significantly ($P < .05$) higher than for the controls

(Table 2.8). Variations among treatments also were larger than among controls; but the substrates were not significantly different in PG, although higher levels were noted during infusions with protein (K & M) than with glucose (G). Observations in PG are in Appendix Table II.7. Generally, the PG concentrations tended to be higher before than after feeding (Table 2.8).

ii. Trial II 1971

1. Material and Methods

1.1 Rationale and planning.--One of the main results of trial I 1971 was a higher response in milk protein production on casein + methionine (K) than on the mixture with glucose (M) which provided half as much protein as K. This difference suggested that graded treatments of protein infusion ought to be tested further. Although the recovery of infused protein as increased milk protein was lower for K than M (20 vs. 30%), still higher infusion levels of protein were desirable to fully challenge the milk protein synthesizing capacity. Thus, in a 4x4 Latin square experiment casein was infused at 50-, 100-, and 200% of daily milk protein production with saline infusion as the control treatment (Table 2.9).

Because responses in trial I 1971 were equal during both sub-periods (Table 2.6), infusions were for only 4 days with the first day as a change-over.

Table 2.8--Trial I 1971. Concentrations of glucose in blood plasma.

Anova for F test	Trtm.[a]	0 hr (B_1) m ± SE(N) mg/100ml	3 hr (B_2) m ± SE(N) mg/100ml	9 hr (B_3) m ± SE(N) mg/100ml	All hrs m ± SE(N) mg/100ml
	K	67.7 ±1.9(3)	64.9 ±1.6(3)	72.9 ±0.6(3)	68.4 ± 1.4(9)
	O_K	66.1 ±2.2	62.2 ±4.9(3)	63.2 ±2.8(3)	63.8 ± 1.8(9)
	G	71.4 ±1.8(3)	65.0 ±3.3(3)	63.8 ±5.1(3)	66.9 ± 2.2(9)
	O_G	67.9 ±2.1(3)	65.8 ±1.0(3)	64.5 ±3.7(3)	66.1 ± 1.4(9)
	M	69.5 ±2.1(3)	69.0 ±2.8(3)	69.6 ±2.0(3)	69.4 ± 1.2(9)
	O_M	65.6 ± .8(3)	68.1 ±1.5(3)	67.6 ±2.6(3)	67.1 ± 1.0(9)
	T	69.7 ±1.2(9)	66.3 ±1.5(9)	68.7 ±2.1(9)	68.2 ± 0.9(27)
	O	66.6 ±1.0(9)	65.3 ±1.7(9)	65.1 ±1.7(9)	65.7 ± 0.8(27)
I-3					T>O*

[a]Controls (O_T) are for periods following the respective treatments only because samples of period 1 were spoiled in storage.

Table 2.9.--Trial II 1971. Experimental design and timing of periods.

Per. No.[b]	Days	Bleeding hour No.[a] 1 2 3	1 2 3	1 2 3	1 2 3
		Cow 607	Cow 604	Cow 603	Cow 606
1	9/9-12	O	L	M	H
2	9/15-18	L	O	H	M
3	9/21-25[c]	M	H	O	L
4	9/29-10/2[d]	H	M	L	O

Symbols:

C = cows
T = Infusion treatments
O = saline control
K = caseinate + 3%dl-methionine
Level of K-relative to milk production
L = low = 1/2 X
M = medium = 1 X
H = high = 2 X

Number of factors
C: c=4
P: p=4 r=4
T: t=4
B: b=3

[a]Bleeding hours (B) relative to morning feeding: 1 = before feeding; 2 = 3 hours post-feeding; 3 = 9 hours post-feeding.

[b]Periods (P) lasted 4 days with day 1 used for transition. There were also 2 days of saline infusion between actual periods.

[c]Infusion lasted through 9/25 because cows 604 and 607 were in heat 9/24 and 9/22 respectively, which were omitted; and 9/25 was not included for cows 603 and 606.

[d]The start of the last period was delayed because No. 606 for unknown reasons apparently was not well (temperature 101°F) on 9/27 and 9/28.

Additionally, two days of saline infusion interspaced each period.

Levels of casein for infusion were derived from milk protein production just prior to the trial (Table 2.10), and the caseinate was fortified with 3% methionine as in trial I 1971.

1.2 Arrangements and procedures.--Infusion arrangements, sampling techniques and chemical assays were similar to trial I 1971. The cows were also the same as used in trial I 1971. They were in their 5th to 7th month of lactation so milk yields were lower than for the previous trial. Some difficulties were encountered in stabilizing the cows on their rations, so treatments had to be delayed 2-3 weeks. During treatments each cow received 6.8 kg hay per day and concentrate to furnish about 110% of NRC (1966) standards for energy and protein.

Feed samples were obtained twice during each period, on day 1 and 3, and they were handled as previously. Feed analyses are presented in Appendix Table II.17. Milking and milk sampling were also done as previously described but only one composite sample was taken for determination of milk constituents for each cow and period.

Blood was sampled three times on the last day of each period and handled as in trial I 1971. Chemical analyses were performed as in the previous trial with the

Table 2.10--Trial II 1971. The levels of infused protein.

Cow No.	Milk[d] Yield	Prot.[d] Cons.	Prot. prod. ± 5% range	Level of infusion[a] L	M	H
				protein[b,c]		
	kg	%	g	-------g/day-------		
603	16	3.2	480-540	255	510	1020
604	21	3.3	660-730	350	700	1400
606	18	3.1	530-590	280	560	1120
607	19	3.3	590-660	315	630	1260

[a]L = low level = 1/2 × milk protein
M = medium level = 1 × milk protein K (casein)
H = high level = 2 × milk protein

[b]The actual amount of sodium caseinate infused derived as protein (K) × 100/85; as the caseinate was 85% protein.

[c]DL-methionine added; 3% of the protein infused.

[d]Measured for a couple of days just prior to the trial.

following exceptions: the NPN content of the milk protein-free filtrate was assayed by semi-micro Kjeldahl rather than the autoanalyzer because of more precise duplication of results. Also, better agreement between duplicates in the lactose determination than for trial I 1971 apparently resulted from greater care with the assay.

The statistical analysis followed the outline for a Latin square design (e.g., Cochran and Cox, 1956), with additional splitting of the main plots for the bleeding

times (Appendix Table II.22). No interaction between factors related to the main plots was assumed, but the two-way interactions between bleeding times and the three factors of the main plots were estimated (Appendix Table II.22).

2. Results

2.1 Feed intakes.--Amounts for feed offered and consumed for each cow are in Appendix Table II.16. Cow No. 603 received 9.1kg concentrate per day, while 11.3kg was allotted to the other three cows. These amounts provided at least 15% more energy and protein than required according to NRC (1971) standards at the onset of the trial.

The intake of energy apparently was influenced by changing feed quality. Thus, the hay quality in period 2 was lower than for other periods (Appendix Table II.17). The CP content of concentrate also varied more than expected.

Increasing protein infusions in this trial depressed feed intakes almost linearly (Table 2.11), although differences between levels of treatments were not significant ($P > .05$, Appendix Table II.23). Similar trends appeared for CP and ENE intakes (Table 2.11), despite variations in feed quality. The similarity in DM intakes between control periods and days between treatments (Table

Table 2.11--Trial II 1971. Summary of feed consumption: intakes of dry material, crude protein, and estimated net energy for different treatments and periods, means per day.

	Dry material			Crude	Esti.
	Hay	Cons.	Total	protein	NE_L
Treatment	kg	kg	kg	kg	Mcal
O	5.1	8.4	13.5[a]	2.19[a]	23.2[a]
L	5.0	7.7	12.7	2.04	21.6
K M	5.5	6.9	12.4	1.99	20.5
H	5.3	6.5	11.8	1.91	19.6
Period					
1	5.1	7.4	12.5[b]	2.16[b]	21.4[b]
2	5.1	7.4	12.5	1.82	20.8
3	5.7	7.9	13.6	2.28	23.1
4	4.9	6.8	11.7	1.89	19.7
SE_d[c]			0.37	0.60	0.77
Days of change of trtm. (saline inf. between periods)			13.5 ± 0.4		

[a] O > K, P < .01

[b] Periods differed significantly, P < .05 or lower.

[c] SE_d = standard error of a difference between two means.

2.11) suggests no serious overlapping of infusion effects on feed intake.

2.2 Milk parameters.--Milk production data for individual cows are in Appendix Table II.18 and II.19, and results of AOV are in Table II.24.

Milk yields within cows varied much more than in earlier trials, probably because of fluctuating feed intakes. While milk yields did not increase as protein infusion increased, concentration of protein in milk was related to level of infusion; although there was no difference between the L- and M treatments (Table 2.12). Only cow No. 607 showed a linear trend of increased milk protein with level of infusion (Appendix Table II.19). Cow No. 606 had as low milk protein concentrations during the M and H treatments as during O, with the L causing a slight increase. Perhaps this was due to low DM intakes for No. 606 during infusion of the higher protein levels (Appendix Table II.16).

Because milk yields varied so much between treatments, protein production did not show any marked trend with level of infusion (Table 2.12).

Similar to earlier trials, concentrations of NPN in milk increased significantly ($P < .01$) with each level of protein infusion (Table 2.12). But these increments were not large enough to render the ranking between treatments in estimated true protein (ETP) different from that for crude protein.

The concentration of NPN in milk was again correlated with blood urea nitrogen with $r = 0.77$ ($P < .01$) at 3 hours postfeeding, and 0.48 ($P \approx .05$) at prefeeding. This relationship agrees with the concept that urea, the major constituent of milk NPN, diffuses readily into milk; and that high blood urea levels, as attained in this trial, substantially increases total milk NPN. Lactose concentrations were not affected by treatments and variations were low as demonstrated by small standard errors (Table 2.12).

Treatment means for SNF showed the same pattern as for milk protein. Statistical analysis of SNF data were not performed because loss of samples during period 1. Milk fat concentration did not show any trend with level of protein infusion, nor was there a significant difference between control and overall treatments; but the mean fat percent was slightly higher during the saline than other treatments.

Fat production as well as fat corrected milk were also highest during saline infusion. Thus, the energetic efficiency of milk production was decreased by the infused protein for total energy from feed consumed and infusate was not lower than the energy intake in control periods.

The adverse effect of high levels of protein infusion on feed intakes and the fluctuating milk yields in this trial greatly obscured the intended test of cows' ability to utilize high levels of infused amino acids for

Table 2.12.--Trial II 1971. Milk production and composition by treatments (mean ± SE).[e]

Treatment	Milk yield	FCM yield	Crude protein		Est. true protein	
			Conc.	Prod.	Conc.	Prod.
	kg	kg	%	g	%	g
O	18.09±1.07	15.54±1.08	3.25±.08[a]	588±50	3.07±.08[a]	558±48
L	17.85±0.95	14.18±1.47	3.34±.08[b]	594±35	3.14±.08[b]	561±31
M	17.07±1.60	14.29±1.51	3.38±.13[b]	579±68	3.16±.13[b]	542±64
H	17.98±1.32	14.99±1.16	3.53±.18[c]	637±69	3.28±.17[c]	593±65
SE_d	±0.59	±0.85	±0.05	±19	±0.05	±18

	NPN conc.	NPN/Total N	Lactose conc.	SNF[f] conc.	Fat	
					conc.	prod.
	mg/100ml	%	%	%	%	g
O	26.8±2.4[a]	5.3±.39[a]	4.78±.08	8.67	2.97±.12	555±50
L	30.7±1.0[b]	5.9±.15[b]	2.76±.08	8.84	2.58±.28	512±63
M	34.7±2.4[c]	6.6±.39[c]	4.80±.05	8.81	2.90±.10	520±56
H	38.3±1.4[a]	7.0±.11[d]	4.71±.06	8.99	2.84±.08	519±43
SE_d	±1.3	--	±0.06	--	±0.15	--

[e]Different superscripts in a column indicate significant differences; for protein concentrations $P < .05$, for NPN and NPN/Total N $P < .01$.

[f]Observations for period 1 are missing, thus N=3; and no statistical analysis performed due to unbalanced design.

milk protein synthesis. Just the effect on feed intake, however, may indicate that the amino acid load surpassed the cows' capacities for amino acid metabolism, possibly resulting in an amino acid toxicity (Harper, 1959).

2.3 Blood parameters.--Blood urea nitrogen (BUN) increased with level of protein infusion, but only between control and overall protein treatments were differences significant ($P < .01$). Observations are in Appendix Table II.20. Concentrations of BUN before feeding and 3 hrs, thereafter were essentially identical, but the diurnal trend differed for the different treatments (Table 2.13). Neither bleeding times and treatment, nor any other factors tended to have an interaction effect on the BUN levels (AOV in Appendix Table II.25).

Plasma glucose concentrations in this trial were slightly but significantly ($P < .05$) higher during the control than protein infusions (Table 2.14). This is contradictory to trial I 1971 (Table 2.8); and the discrepancy may reflect the more severe depression of feed intake by substrate infusions in trial II.

The medium level of infusion resulted in lowest blood glucose (Table 2.14), and this corresponded with minimum milk yields. However, the plasma glucose levels were all (Appendix Table II.21) above those (~40mg%) considered critical for milk secretion (Linzell, 1967). Diurnal trends in plasma glucose varied from period to period (Appendix Table II.21); but concentrations were

Table 2.13.--Trial II 1971. Concentrations of blood urea nitrogen.

Treatment	Bleeding times			Mean±SE(N)[b]
	1 (0 hr)	2 (3.5 hr)	3[a] (9 hr)	
	mg/100ml			
O	28.0	27.0	31.5	28.3±2.7 (10)[c]
L	30.8	39.3	39.0	35.8±2.1 (10)
M	38.0	37.3	47.6	37.6±3.4 (10)
H	47.5	43.0	42.7	44.6±3.8 (11)
m SE	36.1±3.1	36.6±2.3	41.2±4.0	36.6
(N)	(16)	(16)	(10)	(42)

[a]Not included in AOV because of missing samples.
[b]SE_d for treatments = 2.6.
[c]K > O, P < .01.

Table 2.14.--Trial II 1971. Concentrations of glucose in blood plasma.

Treatment	Bleeding times			Mean±SE(N=12)[a]
	1 (0 hr)	2 (3.5 hr)	3 (9 hr)	
	mg/100ml			
O	70.2	68.5	68.9	69.2±0.8[c]
L	70.3	67.4	68.3	68.6±1.1
M	68.7	64.4	67.0	66.7±0.9
H	70.0	68.3	67.5	68.6±1.2
m±SE (N=16)[b]	69.8±1.1	67.1±0.8	67.9±0.8	68.3

[a]SE_d for treatments = 1.1.
[b]SE_d for bleedings = 1.3. $B_1 > B_2 + B_3$, P < .05.
[c]O > K, P < .05; H > M, P < .05; $B_1 > B_{2+3}$, P < .01.

higher before the AM feeding than later in the day ($P < .01$, Table 2.14) although differences were only 2-3mg%. The interaction between bleeding times and periods (Appendix Table II.25) also was significant ($P < .05$), thus complicating the interpretation of diurnal changes.

iii. Trial III 1971

1. Methods and materials

1.1 Rationale for the experiment and design.-- Milk production may not reach its full potential when high-yielding cows receive rations with a large fraction of CP in the form of NPN. Crucial limits have been set to 1/3 of total N as NPN; but apparently a more appropriate limit is ~0.45g urea (= 0.2g NPN) per kg of body weight (Huber, et al., 1967; Conrad and Hibbs, 1968). In any event, high proportions of NPN demand an adequate supply of carbohydrates (starch). Improved performance observed when substituting NPN with plant portein may result from rumen by-pass of feed amino acids (Oltjen, 1967; Chalupa, 1972). Accordingly, a postrumen supply of amino acids, as with abomasal protein infusion, might increase milk protein synthesis in cows on high NPN rations.

By the time this experiment commenced, the cows were seven to nine months in lactation and produced less than 15kg/day. Thus, they were not as metabolically sensitive to high NPN and postrumen protein as higher producers. Nevertheless, it was decided to test the NPN affect and to accumulate more data on the infusion of

casein + methionine. An experiment was planned with two diets, supplying a low (~15%) and a high (~40%) fraction of the CP as NPN (Table 2.15). For a comparison of NPN levels in cows at similar production level and body weight it seems appropriate to express NPN as % of total N although the limit for NPN utilization is better defined in relation to body weight (Huber, et al., 1967). A crossover of the diets tested abomasal infusion of casein at both NPN levels in four cows. Infusing protein before and after the saline control in half of the cases would have been more correct statistically, but for practical reasons all infusions were conducted in parallel. More serious, it seems, for the statistical analysis, was the low power of the test resulting from fractionation of degrees of freedom. Nevertheless, the interaction between infusion treatments and level of NPN was of particular interest, and the design estimated this effect.

1.2 Arrangements and operations of the trial.--Animals. The four cows used in this trial were the same as employed in trials I 1971 and II 1971. No particular problem with their abomasal cannula were encountered, although scar tissue at the fistula was predominant in No. 606 toward termination of the experiment.

Infusion periods lasted 5 days with day 1 of each period omitted from the results for transition. Infused casein was intended to equal 20% of the feed CP; which again exceeded NRC standards. Dl-methionine was anew included at 3% of the caseinate, and the infusate was 76 to 109% of milk protein output (Table 2.16).

Table 2.15.--Trial III 1971. Experimental design and timing of periods.

Seqs. No.[a]	Per. No.[b]	Days	Inf. Tretm.	Bleeding Time No. 1 2 3 1 2 3 1 2 3 1 2 3			
				Cow No. 603	607	604	606
				Feeding			
1	1	11/28-12/2	O_1	H	H	L	L
	2	12/3-7	K	H	H	L	L
	3	12/8-12	O_2	H	H	L	L
2	4	1/10-14	O_1	L	L	H	H
	5	1/15-20	K	L	L	H	H
	6	1/21-25	O_2	L	L	H	H

[a]About 4 weeks elapsed between the two sequences with change over of feed rations.

[b]Periods lasted 5 days, but day 1 was for transition.

Symbols:

Infusion treatments (T, t=3):
O_1 = saline before casein + methionine.
O_2 = saline after casein + methionine.
K = caseinate + 3%dl-methionine, intentionally 20% of feed CP.

Feeding; i.e., NPN level in the feed (F, f=2):
H = high level.
L = low level.

Bleeding times relative to the AM feeding (B, b=2):
1 = just before feeding.
2 = 3-3.5 hours after feeding.
3 = ~9 hours after feeding.

Table 2.16.--Trial III 1971. Derivation of protein quantities infused and comparison to crude protein (CP) consumed and put out by the milk.

Cow	Column[a] No. 1	Column No. 2	Column No. 3	Column No. 4	Column No. 5	Column No. 6
	Est. CP fed	20% of CP fed	Na-cas. inf.	CP inf. / CP int.	CP inf. / Milk CP	E-ME of inf. CP
	g	g	g	%	%	Mcal
603	1970	350	400	22	109	1.62
604	2260	450	520	20	76	2.09
606	1860	370	425	23	81	1.71
607	1970	400	460	19	106	1.85

[a]Comment for each column follows as assigned:

(1) Estimated crude protein consumption at the onset of the trial.

(2) Infused protein (casein) was intended to be 20% of consumed protein, and protein fed (1) = potentially consumed. Additional dl-methionine was 3% of infused casein.

(3) Sodium caseinate was 85% casein.

(4) Crude protein infused in % of crude protein intake in control periods.

(5) Crude protein infused in % of milk crude protein production in control periods.

(6) Estimated metabolizable energy of the infused protein (4.6 kcal ME/g casein).

Feed, feed sampling and analysis. About three weeks adaptation to the high NPN ration was allowed before the trial started; but urea-treated corn silage was fed for several weeks before introduction of urea containing concentrate. Feed composition data are in Appendix Table II.27. Corn silage treated with urea ran out before the last sequence of infusions and the cows then received ProSil-treated[1] silage. The mean N content of the two silages, determined simultaneously for another experiment, was identical. The mean values for silage N and NPN were employed here since samplings did not follow experimental periods. The NPN content of an aqueous silage extract was determined after water soluble protein was precipitated with sulfosalicylic acid (SSA). Dry matter and crude fiber in corn silage were determined as previously described on samples obtained twice in each period. Hay and concentrate mixtures also were sampled and handled as described previously.

The formulas for the concentrate mixtures, with and without urea, are in Appendix Table II.26.

Milking, milk sampling, handling of the samples, and analyses were all done as previously mentioned. Milk constituents were determined on samples composited for two consecutive days, but a period mean weighted for the

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[1]ProSil, ammonia, minerals and molasses additive, manufactured by Ruminant Nitrogen Products Co., Adrian, Michigan.

amount of milk in the sub-periods was used for further calculations.

Since the preceding two trials showed that lactose concentrations were not particularly informative, lactose was not determined.

Blood samples were obtained and handled as earlier, and assays of blood components were analyzed by the same methods. However, plasma-free amino acids were not determined for the samples obtained 3 to 3.5 hrs post-feeding (B_2) (section B.III).

Statistical analysis followed the outline in Appendix Table II.33 (Anova III).

2. Results

2.1 Feed intakes.--Generally the urea-containing concentrate was readily accepted, but cow No. 603 had a low intake during the first control period (Appendix Table II.22). Concentrate consumption for this cow also dropped during protein infusion while on hte low NPN ration in sequence 2. But there was no overall trend towards lower feed intakes during protein infusion, nor any effect on intakes due to feed NPN levels (Table 2.17, Appendix Table II.28 and II.34).

Variable intakes of different feeds resulted in estimated NPN fractions below 40% for the high NPN ration (Table 2.17). The absolute amount of NPN (g/day, Table 2.17), however, was equivalent to a urea level considered maximum in lactating cows (Huber, et al., 1967; Conrad and Hibbs, 1968).

Table 2.17.--Trial III 1971. Summary of feed intake: daily consumption of dry matter, crude protein, NPN and estimated net energy.

Parameter Inf. Trtm.	NPN level fed		
	High	Low	Both feeds (N=8)
		kg	
Dry matter			
O_1	10.9	11.6	11.3 ± .69
K	11.4	11.1	11.3 ± .71
O_2	11.6	11.8	11.7 ± .51
All inf. (N=12)	11.3 ± .51	11.5 ± .52	
		kg	
Crude protein			
O_1	1.77	1.89	1.83 ± .112
K	1.88	1.84	1.86 ± .107
O_2	1.91	1.85	1.93 ± .077
All Inf. (N=12)	1.85 ± .082	1.89 ± .079	
NPN[a]		g	(L/H)%
O_1	109	44	40
K	116	41	35
O_2	118	44	37
All Inf. (N=12)	114.2	42.9	37.6
		%	(L/H)%
NPN/Total N			
O_1	38.3	14.3	37.3
K	38.0	13.8	36.3
O_2	38.5	14.0	36.4
All Inf. (N=12)	38.3	14.0	36.6
		Mcal	
Estimated Net energy			
O_1	19.5	21.0	20.25 ± 1.32
K	20.6	20.1	20.35 ± 1.26
O_2	21.0	21.5	21.23 ± .93
All Inf. (N=12)	20.37 ± .96	20.85 ± .93	

[a]Minimum values; NPN in silage + urea N in concentrate.

Intakes of crude protein (CP) and NE_L exceeded NRC (1971) standards, particularly for period 6 (Appendix Table II.29). For a test of nutritional stress imposed by high NPN fractions the total CP intakes probably were too high.

2.2 Milk production parameters.--Much as expected for reasons related to the CP intakes, feed NPN levels had no effect on any milk production parameter (Table 2.18, Appendix Table II.30). Neither was there a tendency for interaction between infusion treatments and feeds (T x F), (Appendix Table II.35).

Despite the low production levels and high level of feeding, the infusion of protein increased milk protein production significantly ($P < .05$, Table 2.18) as in earlier trials. Milk yield was only slightly higher during casein + methionine infusion than during the controls (+0.5kg), but the concentration of protein (N x 6.38), increased significantly ($P < .005$). Nominally, the estimated response of 0.3 per cent was even higher than in trial I 1971. The mean increases in crude protein concentration were identical for the two infusion sequences.

Estimated true protein increased slightly less on both feeding regimens than did crude protein, but the casein infusion response of 0.25% ETP was clearly significant ($P < .01$). NPN as a fraction of total N was slightly but not significantly higher during protein than saline infusion (5.68 vs. 5.28%), like noted earlier.

Table 2.18--Trial III 1971. Milk production parameters; summary of comparisons between treatments.

Item No.	(1) Milk yield	(2) FCM prod.	(3) CP conc.	(4) CP prod.	(5) ETP conc.	(6) ETP prod.
	kg	kg	%	g	%	g
Infusion trtm.						
K	13.31	11.62	3.692	491	3.483	463
O[a]	12.88	11.59	3.415	436	3.233	413
d[b]	.51±.42	<.1±.46	.277±.023**	55±11*	.250±.033**	50±8*
(d%)	(4.0)	--	(8.1)	(12.6)	(7.7)	(12.1)
Urea level fed						
H	13.00	11.52	3.539	459	3.346	434
L	13.27	11.69	3.567	468	3.370	442
d	-.27±.40	-.17±.60	<.1±.097	-9±14	.1±.025	-8±14

Item No.	(7) NPN conc.	(8) NPN/Total N	(9) SNF conc.	(10) SNF prod.	(11) Fat conc.	(12) Fat prod.
	mg/100ml	%	%	g	%	g
Infusion trtm.						
K	32.8	5.68±.16	9.03	1206	3.16	418
O[a]	28.3	5.28±.06	8.83	1134	3.35	429
d[b]	4.5±1.1*	.40	.20±.16	72±28*)	-.19±.12	-9±24
(d%)	(15.9)	(7.6)	(2.3)	(6.3)	(5.7)	(2.1)
Urea level fed						
H	30.2	5.44±.15	8.98	1173	3.30	421
L	31.0	5.53±.14	8.97	1167	3.21	425
d	-.8±.9	<.1	<.1±.10	6±27	.09±.10	-4±39

[a] O = averaged control before and after casein + methionine infusion (K).

[b] d = difference ± standard error of the difference between two means.

Statistical probabilities: P < .10 = *)
P < .05 = *
P < .01 = **

For both sequences there was a tendency for the CP as well as ETP content to be higher before than after protein infusion, but the difference was not great and is not readily explained.

Mean ETP yield increased significantly ($P < .025$), as shown in earlier trials, but recovery of infused protein was lower than in trial I 1971. This might be expected with a higher rate of infusion relative to milk production in this trial.

The difference in SNF% between protein and saline infusions in sequence 1 approached the difference in protein content, but with far larger standard errors. Moreover, in sequence 2 the apparent response (d_T) in SNF percent was nil (Appendix Table II.30, item 4). Variations within cow and periods also were larger in SNF than in protein. Although not estimated, it seems that analytical errors were larger for milk SNF than for nitrogen.

Milk fat also varied substantially within cows and periods, possibly due to different butterfat testers. The tendency for lower fat percent during protein infusion (Table 2.18) agrees with the 1970 experiments (Table 1.6) and trial I 1971 (Table 2.5).

Although milk NPN concentrations as well as BUN were increased significantly by protein infusions, these entities were not correlated for any of the three bleeding

times (r = 0.35 or lower). Contradictory to findings in our earlier experiments, this lack of correlation may merely reflect peculiar variations in these parameters.

2.3 Blood parameters.--Blood urea nitrogen concentrations observed in trial III are in Table II.31. Contrary to milk production, the BUN level was significantly ($P < .05$, Table 2.19) influenced by the NPN level of the feed. The higher BUN at high (H) than at low (L) NPN ration (36.1 vs. 32.5mg%) might be expected; but this relationship was not observed by Knott, et al. (1972).

BUN increased significantly ($P < .05$) during protein infusions compared to saline controls (37.3 vs. 32.8mg%), a trend similar to earlier experiments. The influence of infusions seemed more obvious in sequence 1 than sequence 2, although overall means for the two sequences were practically identical (Appendix Table II.31). The BUN concentrations before and after protein infusion also were similar, and there was no real interaction between infusion and factors of higher order (Appendix Table II.36). Infusion treatments and bleeding times, however, tended to interact ($P < .10$) on BUN and resulted in a higher BUN level 9 hrs post-feeding for protein than control infusions.

With the restrictions due to the interactions, the bleeding times had a significant ($P < .001$) influence on the BUN level. A lower concentration before feeding

Table 2.19.--Trial III 1971. Summary of blood urea N and plasma glucose concentrations.[a,b]

	NPN level in the feed (F)			Bleeding times (B) (N = 24)
	High	Low	Both feeds (N = 24)	
		mg/100ml		
Blood urea N Infusion (T)				
O_1	33.9	33.8	33.8a	B_1 30.3a
K	40.2	34.3	37.3b	B_2 35.9b
O_2	33.8	29.7	31.8a	B_3 36.6b
			SE_d-T=4.1	SE_d-B=4.2
All inf. (N=36)	36.1a	32.5b	SE_d-F=2.3	
		mg/100ml		
Plasma glucose Infusion (T)				
O_1	62.4	60.2	61.3a	B_1 66.4a
K	65.7	64.7	65.2b	B_2 60.4b
O_2	63.8	60.7	62.3a	B_3 61.9b,c
			SE_d-T=2.03	SE_d-B=2.04
All inf. (N=36)	64.0	61.8	SE_d-F=3.8	

[a]Means for the main factors and standard errors for a difference between two means (SE_d), as assigned: T = infusion treatments; F = level of NPN in feed; B = bleeding times relative to AM feeding.

[b]Figures with different superscript differ significantly, $P < .05$ or lower.

(30.3mg%) than later in the day (average 36.5mg%) was also observed in trial II 1971, while BUN in trial I was higher before feeding.

The high BUN levels generally fit the high N intakes and indicates that the capacity to clear urea from blood by excretion or recycling approached its upper limit (Mugerwa nad Conrad, 1971).

Plasma glucose (PG) observations for this trial are in Appendix Table II.32. The concentrations were somewhat higher during high than low NPN feeding (Table 2.19), but the difference wasnnot significant and has no obvious explanation. Markedly higher PG levels before than after the AM feeding ($P < .001$, Table 2.19) simulate the pattern of the previous trials. This diurnal trend occurred free of interaction between infusion treatment and level of NPN fed.

Protein infusions clearly elevated PG, but infusion treatments interacted ($P < .05$) with treatment sequences. During the second protein infusion the PG rose to a higher level than in the first and remained high during the control period that followed. While markedly influenced by the interaction (T x S), the mean PG level for sequence 2 tended to be higher than for sequence 1 ($P < .10$). At the time of the second protein infusion (period 5, January, 1972) ambient temperatures were lower, milk yield had fallen, and the cows were eating different silages. These changes might have influenced the

responses in PG to protein infusion, but the change of silage occurred before sequence 2 started. Elicited by the cold weather, endocrine mechanisms might have prompted the high PG concentrations during the last two periods of sequence 2. Hormonal involvements might also explain the decrease in PG levels in the post-fed state when an abundance of substrate is available for glucogeogenesis.

iv. Discussion of 1971 trials

Consistently positive responses, yet of variable magnitude, in milk protein production by abomasal protein infusion in trials I and III emerge as the main feature of this series of experiments. Despite different production levels in the two trials, the increments in protein production were identical; 12.3 $\pm$ 1.8% and 12.3 $\pm$ 1.1% for all protein infusions in trials I and III, respectively. In trial I, reponse to full protein treatment (K, 13.8 $\pm$ 2.1%) slightly exceeded that for the mixture (M, 10.7 $\pm$ 3.0%), which supplied half as much protein, while increments to glucose infusion were small and variable (5.8 $\pm$ 2.3%). These results support our earlier studies and those of Broderick, et al. (1970), Spechter (1972), Derring, et al. (1970), and Tyrrell, et al. (1972).

Because the response to full protein treatment (K) in trial I tended to be greater than to the mixture (M) (101g, $P < .01$ vs. 79g, $P < .10$), with an insignificant response to glucose alone (40g, NS), an improved

supply of amino acids again seems the reason for the increased milk protein yields.

While the cows in trial II failed to respond with an increased protein production, the content of protein in the milk increased with level of infused protein, although not linearly (Table 2.12). Fluctuating milk yields evidently prevented an augmented protein output in trial II as the protein infusion depressed feed intakes ($P < .01$).

By infusing casein + methionine at a rate similar to the higher levels in trial II, Broderick, et al. (1970) also observed lowered grain consumption than in control periods. Considering potential gluconeogenesis from amino acids, it is noticeable that intravenous infusion of glucose or propionate in lactating cows, at rates approaching the higher treatment levels in trial II, did not suppress appetite (Fisher and Elliott, 1966). And several different experiments in sheep and goats dismissed glucose as a mediator of intake regulation in ruminants in general (Baile and Mayer, 1970).

Apparently, the high loads of protein post-ruminally stressed the cows' capacity for amino acid metabolism. Treatment for only four days might have been too short for an adaptation, but intakes returned to normal during the two days between protein infusions, High protein diets lead to inappetance in rats, but this

was overcome as amino acid catabolic enzyme activities increased (Anderson, et al., 1968). Induction of these enzymes developed in two days after high protein consumption commenced.

The elevated levels of blood urea during protein infusions, particularly in trial II, suggest extensive catabolism of infused amino acids. High blood urea generally means increased N losses in the urine (Ford and Milligan, 1969), but there evidently prevails an upper limit for the cow's capacity to concentrate urea into the urine (Mugerwa and Conrad, 1971). This limitation was also linked to depressed appetite (Mugerwa and Conrad, 1971) as observed at high NPN feeding. Toxicity at extremely high NPN intakes relates to high levels of ammonia in body fluids (Visek, 1972), but blood ammonia was not determined in these experiments.

High BUN levels even in control periods and an excessive CP intake relative to NRC (1971) standards suggest excessive nitrogen intakes by these cows. A lowered efficiency of supplementary protein utilization through urinary N losses (Knott, et al., 1972), as indicated by high blood urea concentrations (Ford and Milligan, 1969), has been discussed earlier. However, blood urea in trial I tended to be higher on the mixture than on full casein (K) (29 vs. 26 mg%), although the latter provided twice as much infused protein. Nevertheless, the recovery of

infused protein in the form of increased milk protein production tended to be higher for the mixture (M) than the casein (K) treatment (22-46 vs. 16-24%).

Both protein infusions (K and M) in trial I as well as trial III (S_1 and S_2) raised the blood glucose level which indicated augmented gluconeogenesis from amino acids; but this was not observed in trial II. The crucial role of glucose as a precursor of lactose in milk secretion (Linzell, 1967) has been mentioned earlier. However, contradictory to earlier studies (Linzell, 1960), Linzell (1967) found a marked arteriovenous difference over the udder even at very low blood glucose concentrations (< 30mg%). From this it was concluded that the glucose uptake at low arterial levels is governed by the mammary blood flow.

Apparently plasma glucose in these experiments stayed above concentrations critical for milk secretion. Experimenting with insulin and other treatments, Kronfeld, et al. (1963) arrived at 60mg% and Rook and Hopwood (1970) at 40mg% as plasma glucose levels under which milk secretion rates decreased. Bartley and Black (1966) found as much as 1.5 kg glucose per day infused duodenally in lactating cows did not increase the rate of glucose oxidation. Following a peak a few hours after initiation of infusion, plasma glucose plateaued at 80mg%. The two cows in their experiment produced less than 10kg milk per day, and the

influence of the glucose load on volume and composition of milk was not reported. But Bartley and Black (1966) contended from their isotope dilution data that lactating cows encounter a relative glucose deficiency.

Fisher and Elliott (1966) found 1.8 to 3.3 Mcal glucose and propionate infused intravenously increased milk yields nad lactose concentrations of the milk, although only glucose increased blood glucose concentrations (from 40 to about 50mg%). Milk protein concentration was not affected by these treatments, while glucose was more effective than propionate in depressing milk fat percent, as discussed earlier. An association between increased plasma glucose and enhanced milk protein production in these trials seems plausible, and our data and those of others does not exclude the possibility that the protein infusion directly stimulates milk protein synthesis by improved glucose availability. Our consistently higher responses to protein than glucose, however, imply that amino acids were more crucial metabolites in this connection than was glucose.

The effect of the substrate infusions might in any event have involved altered hormonal status of the cows. Carstairs (1972) found growth hormone was increased in two cows abomasally infused with casein. Both cows had higher milk production during casein infusion than during the control or glucose infusions. The infusion

treatments did not consistently change milk composition but one cow also suffered large body weight losses.

A stimulatory effect of growth hormone on milk secretion has been documented (Meites, 1961). Hutton (1957) reported a highly significant linear relationship between the log weight of injected growth hormone and increases in milk yield. While the fat percent remained unchanged, there was a negative relationship between log level of growth hormone and solids nonfat of the milk. Radloff and Miyake (1969) reported STH significantly increased milk yields, while ACTH lowered milk yields and increased concentrations of fat and SNF.

Prolactin secretion in ruminants has also been stimulated by infusion of amino acids (Davis, 1972; McAtee and Trenkle, 1971), and one of two cows in the study of Carstairs (1972) showed elevated plasma prolactin from abomasal casein infusion. A galactopoietic action of prolactin in cows has not been fully established (Schmidt, 1971); but Keenan, et al. (1970) concluded that the present knowledge on the influence of prolactin on secretory events in the mammary cell indicate an all or none effect of this hormone in stimulating RNA and protein synthesis.

Due to lack of hormone data in these experiments, it can only be theorized that increased milk production by post-ruminal infusion of amino acids was mediated by an endocrine influence on metabolite interaction. An improved supply of essential amino acids to the mammary cell might, nevertheless, have been crucial for the observed increase in milk protein synthesis.

v. Protein production responses described by regression analysis

In trial I 1970 it was noticed that the cow with highest protein production in control periods showed the greatest response to abomasal casein infusion. With possible influence of repeated measurements on the same animals, the 1970 data combined showed responses in protein production significantly correlated ($r = 0.82$, $P < .01$, Table 2.20) to production in control periods.

This relationship was not seen in trial I 1970 where the amount of protein infused tended to show a graded response, even though the level of treatment was related to the level of production. However, combining all casein infusions in trials I and III 1971 ($n = 14$) revealed a significant correlation ($r = 0.70$, $P < .01$) between response and baseline production. The relationship ($r = 0.72$) was also significant ($P < .05$) for trial III alone. With the small number of observations, the correlation coefficients for the two trials in 1971 were

Table 2.20.--Correlations (r_{yx}) between response to abomasal infusion in milk protein (d)[a] and protein production in control periods (O)[b]

Year	Trial	Treatment[d]	(N)	d & O as CP (Nx6.38) g/day		d & O as ETP[c] g/day	
				r_{yx}	P	r_{yx}	P
1970	I & II	K_1	5	0.673			
	I & II	K_2	5	0.863	<.05		
	I & II	K_1 & K_2	10	0.824	<.01		
	II	K_1 & K_2	6	0.795			
	II	G_1	5	0.259			
1971	I	K & M	6	0.323		0.336	
	III	K	8	0.718	<.05	0.710	<.05
	I & III	K[e]	14	0.703	<.01	0.685	<.01
	I & III	K[e]	15[f]	0.658	<.01		
1970 & '71	All above	K[e]	24	0.711	<.01		
	All above	K[e]	25[f]	0.643	<.01		
	All above	G	8	0.396			
(1972)	Spechter[g]	K	6	0.547			
	All above[h]	K[e]	31	0.616			

[a] $d_T = Y$

[b] $O = X$

[c] Estimated true protein in milk; (N-NPN)x6.38.

[d] Infusion treatments K = casein or casein plus methionine, G = glucose and M = mixture K + G equicaloric to K and G.

[e] Also including M in trial I 1971.

[f] Including Cow No. 603 (1971) receiving K in an extra infusion (Appendix Table II.5).

[g] The cows were in negative N balance before the trial. With X = g casein infused r = 0.909, P < .05.

[h] With X = g casein infused r = 0.808, P < .01.

not significantly different, neither did the coefficients for the two years differ significantly.

A plot of responses to casein infusion (Y) versus control production (X) including both years' data (Figure 1) showed great spread. However, when control production (X_1) and level of casein infused (X_2) were included as linear and quadratic factors in a multiple regression analysis[1] (Table 2.21) a regression coefficient (R) of 0.79 ($P < .01$), $k = 4$, $n = 24$) was obtained. The corresponding R^2 (goodness of fit = 0.62) accordingly revealed that the factors included accounted for 62% of the variation around the mean (Table 2.21). Including cow No. 603, which showed great response to casein infusion at the end of trial I 1971 (Table II), resulted in an R^2 of 0.61 ($n = 25$).

Successive deletion of the least significant ($P < .10$) factor from the model ($n = 25$, Table 2.21) left only the linear components, and the fit remained essentially unchanged ($R^2 = 0.60$). With $n = 24$, the deletion of casein infused (X_2) as well as the quadratic effects showed $P = .169$ (Table 2.21). The β-weights for the two factors left in the regression (Table 2.22) confirm that the level of control production was relatively more important than the amount of casein infused for explanation of the responses shown here, but level of

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[1]LS routine program on CDC 3600 computer.

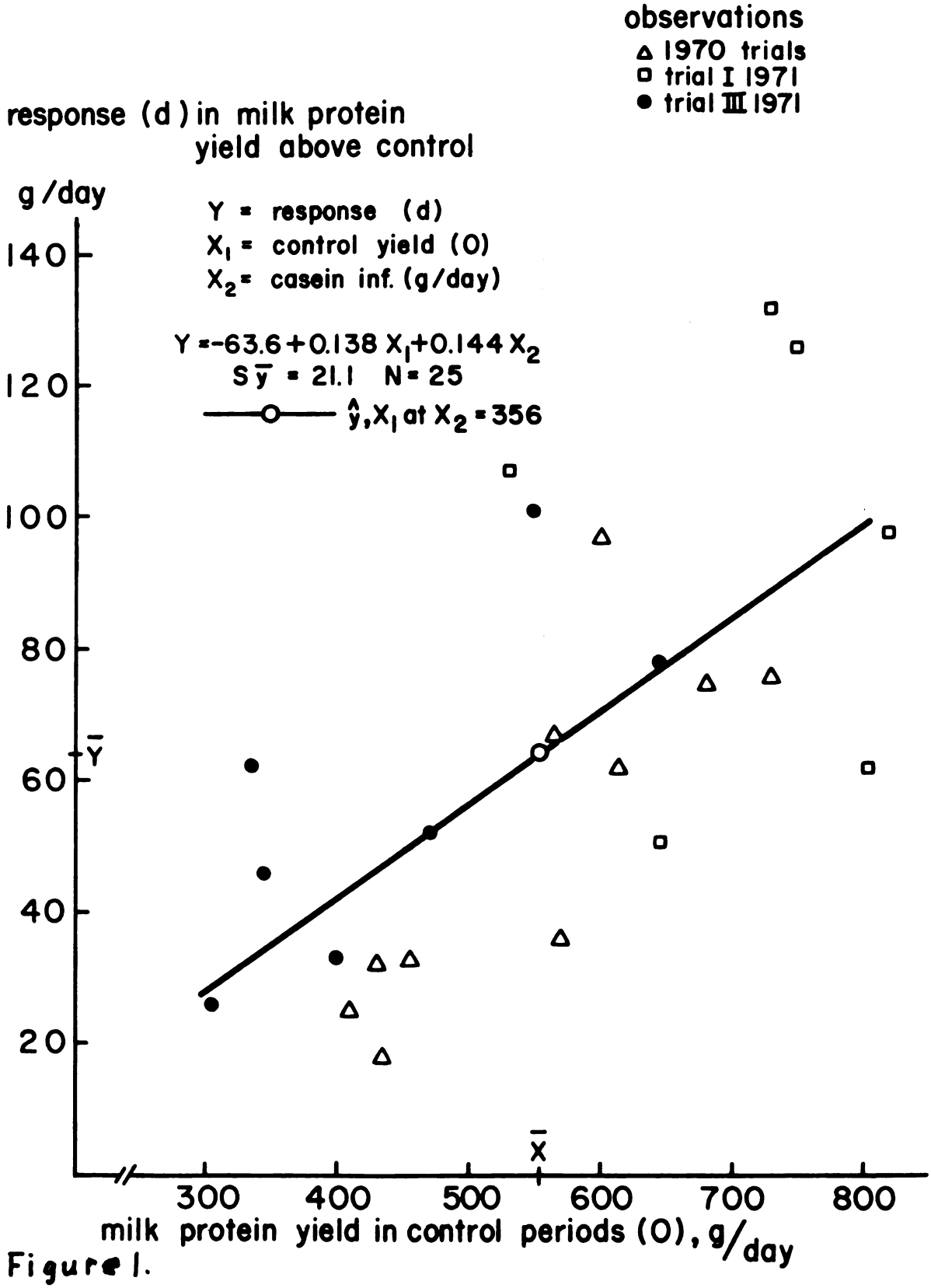

Figure 1.
The response in milk protein yield to abomasal protein infusion as related to yield in control periods

infusion was constant for the 1970 data (10 of 25 observations). This relationship as well as the regression coefficients changed slightly by excluding the extra observation in cow No. 603 (n = 24, Table 2.22).

Employing only the 1971 data (n = 15) when graded levels of casein were infused, the quadratic effect of infused casein was the second most important factor (Tables 2.21 and 2.22). However, its contribution to the fitting of the regression was far from significant in the small material.

An analysis of data from Spechter (1972) did not yield a regression equation with significant coefficients. Moreover, infusion level (X_2) alone showed almost as good fit as inclusion of yield level (X_1), the second most important factor (Table 2.21). The cows in the Canadian study were in early lactation and negative N balances, which might explain the difference in response functions compared to cows in our trials which were fed above standards.

Spechter (1972) contended that the large responses to abomasal casein in his study indicated an inadequate amino acid supply. Although the regression of responses to infused casein on protein yield failed to be significant in his study with only six observations, the higher R^2 than for our data suggests that in our experiments factors other than levels of production and treatment limited responses

Table 2.21.--Excerpts from multiple regression analysis of the relationship between protein production responses, control production and amount of casein infused.[a]

Trials Analyzed	Year	(N)	Factors deleted[b]		Regr. about mean		
			X_i	P	R^2	F	P
I & II	1970	24	none		.620	7.8	.001
and			X_3	.871	.620	10.9	.0005
I & III	1971		X_3X_4	.955	.619	17.1	.0005
			$X_3X_4X_2$	.160	.509	22.5	.0005
			$X_3X_4X_1^2$	.001	.400	4.1	.055
I & II	1970	25[c]	none		.610	7.8	.001
and			X	.857	.609	10.9	.0005
I & III	1971		X_4X_3	.812	.601	16.6	.0005
			$X_4X_3X_2$	.072	.451	18.9	.0005
			$X_4X_3X_1^2$	.002	.191	5.4	.029
I & III	1971	15[c]	none		.572	3.3	.056
			X_2	.812	.569	4.8	.022
			X_2X_3	.789	.552	7.3	.008
			$X_2X_3X_4$	.402	.443	9.9	.008
			$X_2X_3X_1$	.091	.205	3.4	.090
Spechter	(1972)	6	none		.984	14.9	.101
			X_4	.958	.984	39.7	.025
			X_4X_3	.316	.835	7.6	.067
			$X_4X_3X_1$	.386	.827	19.1	.012
			$X_4X_3X_2$	.194	.300	1.7	.261

[a]All data in g crude protein per day. Y = response (d) to abomasal infusion above control; X_1 = control production ($O_1 + .O_2/2$); X_2 = amount of casein infused; $X_3 = X_1^2$; $X_4 = X_2^2$.

[b]Successive deletions of the factor or factor combination least significant at each stage of factor numbers (k).

[c]Cow No. 603 (1971) included.

Table 2.22.--Regression equations for estimation of response to abomasal casein infusion from control production and amount infused.[a]

Trials Analyzed	Year	(N)	Regression	Factor sign. P	Factor sign. β wt.	Regression fit R^2	Regression fit R	Regression fit P
I & II and I & III	1970 1971	24	$y=bo+b_1X_1+b_2X_2$			.619	.787	<.01
			b_o -59.3040					
			b_1 0.13992	.001	.682			
			b_2 0.12350	.160	.338			
			$s_{\bar{y}}$ 20.27					
I & II and I & III	1970 1971	25	$y=b_o+b_1X_1+b_2X_2$			.610	.781	<.01
			b_o -63.6377					
			b_1 0.13718	.002	.643			
			b_2 0.14376	.072	.389			
			$s_{\bar{y}}$ 21.07					
I & III	1971	15	$y=b_o+b_1X_1+b_4X_2^2$			.620	.788	<.01
			b_o -18.8274					
			b_1 0.11458	.070	.589			
			b_4 0.00016155	.400	.350			
			$s_{\bar{y}}$ 24.19					
Spechter (1972)		6	$y=b_o+b_2X_2$			.827	.909	<.05
			b_o 35.3024					
			b_2 0.32956	.194	.909			
			$s_{\bar{y}}$ 56.33					

[a]Equations derived at by multiple regression as outlined in Table 2.21.

to postruminal amino acids. Including additional factors in the model probably could help explain more of the variability in our studies. Since the cows were fed above standards, metabolic data might be more informative than nutrient intake, although interactions between metabolites (e.g., amino acids) might tend to mask their importance.

Physiologically, the correlation between yield response as the dependent (Y) and control production as the independent (X) variables probably is an oversimplification. Rather, the magnitude of the response and the control production might depend on common causative factors and therefore they are correlated.

The difference in response functions in ours and the Canadian study did not warrant an analysis of combined data. Even a well-fitting mathematical expression may not be physiologically feasible (Kleiber, 1950). However, the importance of control production on responses in well-fed cows is supported by *in vitro* studies with mammary tissue. Emery, *et al.* (1970) reported the capacity for protein synthesis by mammary slices ranked according to milk production for the donor cows. Larson (1972) found mammary cell cultures from individual cows differed in total protein synthesis as well as in response to methionine. Schingoethe, *et al.* (1967) showed that the rate of synthesis of β-casein by a mammary cell culture could be augmented by amino acid concentrations above the normal

physiological level, abomasal protein infusion may have created such a situation *in vivo*.

In any event, the best fitting regression equations in Table 2.22 are presented to show the relative importance of factors in describing response variations in this material rather than for the purpose of prediction. If the latter was intended, pre-infusion production would be a more logical base line.

III. PLASMA FREE AMINO ACIDS (1971 TRIALS)

1. Introductory Remarks

Plasma free amino acids (PAA) are generally considered the currency for protein metabolism (Munro, 1970; Allison, 1964), but erythrocytes and proteins have been suggested (Elwyn, 1970) as additional means of amino acid transport. While the PAA pool is a small part (0.5%) of the body's total free amino acid, the PAA reflect the body's supply and demand for amino acid, being influenced by dynamic metabolic regulations (Munro, 1970). An essential amino acid (EAA) will not accumulate in plasma unless it is supplied in excess relative to other EAA or requirement (Almquist, 1954). In this study PAA were measured in an attempt to further explain the effect of postruminal protein on milk protein synthesis.

2. Materials and Methods

Plasma free amino acids (PAA) were determined on a protein free filtrate obtained by mixing 5ml plasma, 0.5ml 50% (w/w) sulfo salicylic acid (SSA), and 0.5ml of a norleucine (nle) solution containing 1μM nle/ml as an internal standard. The plasma reagent mixture was shaken and kept on ice for about 2hr before centrifugation at 15,000xg for

30 min. Supernatant thus obtained was decanted into glass tubes, sealed with a cork, and kept frozen until final assay.[1] For periods 1 through 5 of trial I, no nle was detected at assay, and nle had to be added.

Concentrations of individual amino acids were calculated by relating areas under traced curves to those for a standard, and the area of the nle curves related the sample areas to known concentrations for the standard. A standard curve was obtained for every 30-35 samples.

Generally, concentrations of total PAA were in the range found previously in this laboratory and by others (Jacobson, et al., 1970; Fisher, 1972) in lactating cows. An influence of the period of assay was evident; for a part of the samples in trial I which showed very low concentrations was assayed at a time when the traced areas were very small. This probably increased the error on the measurements. The reason for the low peaks is not known. Since all amino acids were low it was assumed that the molar ratios were unaffected; hence a molar % distribution was calculated for each sample (Table 3.1).

Since amino acids are incorporated into proteins in given molar ratios, and the metabolism of amino acids are interrelated, the molar % expression has merits as an expression for relative amino acid availability (Scott, et al.,

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[1]Assay was done on a Technicon TSM Amino Acid Analyzer in the Department of Animal Husbandry.

1972; Boling et al., 1972). Molar % ratios were also calculated for trials II and III, though concentrations for these trials did not fluctuate as in trial I.

Further calculations and statistical analysis followed the outline for blood urea and plasma glucose for each trial. However, because the molar percentages all were below 30, they were transformed by the arcsin function (Rohlf and Sokal, 1969) to achieve a normal distribution for the AOV.[2] For trial III, the samples obtained 3hr post-feeding (B_2) were not analyzed for amino acids. Diurnal variations and bleeding x infusion interactions generally were small and are not considered in the summarizing presentation of the PAA results.

3. Results and Discussion

Concentrations of amino acids (μM/l) in trials II and III (Tables 3.2 and 3.4) tended to increase with the protein infusion as observed by others in similar studies with lactating cows (Broderick, et al., 1970; Spechter, 1972) and sheep (Hogan, et al., 1968). Generally, this trend indicates an improved amino acid status, but can also suggest an imbalance (Young, et al., 1973). In trial II the levels of branched chain amino acids (BrAA = valine,

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[2]Calculations to molar %, transformations and statistical analysis were done on a CDC 3600 computer, programmed by Dr. R. R. Neitzel.

Table 3.1. Trial I 1971. Plasma free amino acids; molar % distribution at different infusion treatments.

Amino Acid	O_G[a]	G	P[b]	O_M[a]	M	P[b]	O_K[a]	K	P[b]
	----%----			----%----			----%----		
Lys[c]	3.9	4.1		3.7	4.1		3.6	4.0	
His	2.5	3.2		2.5	2.8	<.05	2.3	2.8	
Thr	6.0	5.4		6.2	5.4	<.05	6.1	4.7	<.05
Val	13.1	11.6		13.3	13.4		13.0	15.1	<.10
Gle	5.9	7.0		6.1	6.9		5.8	6.9	
Leu	8.9	7.8		8.5	8.5		8.5	9.4	
Met	2.1	3.0		2.0	3.5	<.10	2.0	3.9	<.10
Cys	1.5	1.3		1.6	1.1		1.5	1.1	
Phe	2.5	3.1		2.6	3.1	<.05	2.6	2.9	
Tyr	2.6	3.9	<.10	2.8	3.6		2.8	3.7	
Arg	3.7	3.5		3.6	4.1		3.5	3.5	
Asp	0.8	0.2		0.7	0.3	<.10	0.7	0.2	<.05
Glu	4.2	4.2		4.2	3.9		3.8	4.3	
Pro	6.7	6.0		7.1	5.8		7.2	6.2	
Ser	4.9	5.0		5.1	5.4		5.2	4.4	
Gly	18.2	17.6		17.5	15.2		18.6	13.3	<.10
Ala	12.4	13.1		12.2	13.0		12.9	13.4	
E/N ratio	0.82	0.83		0.82	0.91		0.78	0.99	<.10

[a]Average of pre- and post-treatment.

[b]Probability level in test of significance by Anova I-2 (Appendix Table II. 9d), O_T vs. T by ortogonal contrast. 9 observations behind T, 18 behind O_T.

[c]The names of the amino acids are given fully in Table 3.4, which also indicates essential (E) and non-essential amino acids (N) as conventional for rat growth. Tryptophan was not determined for these trials.

Table 3.2.--Trial II 1971. Plasma free amino acid concentrations.

Amino acid	Treatment				
	O	L	M	H	P[e]
	----μM/l----				
Lys[f]	89[a]	116[b]	97[c]	85[d]	<.01
His	64	70	60	60	
Thr	114[a]	116[b]	100[c]	114[c]	
Val	310[a]	332[b]	410[c]	486[c]	<.01
Ile	132[a]	139[b]	173[c]	174[c]	
Leu	191[a]	205[b]	252[c]	293[d]	<.01[g]
Met	31[a]	51[b]	66[c]	496[d]	<.01[g]
Cys	31	39	37	29	
Phe	56	57	58	54	
Tyr	58[a]	68[b]	63[a]	70[a]	
Arg	77[a]	84[b]	65[a]	51[b]	<.01
Asp	16	16	13[a]	19[b]	<.05
Glu	106[a]	117[b]	98[b]	88[c]	
Pro	116[a]	159[b]	173[b]	251[c]	<.05
Ser	95[a]	96[b]	79[c]	97[c]	
Gly	266[a]	239[b]	156[c]	160[c]	<.01
Ala	243	283	275	269	
EAA	987	1086	1216	1762	
NEAA	1008	1101	959	1034	
E/N	0.98[a]	0.99[b]	1.27[c]	1.70[d]	<.01

[a,b,c,d]The figures with different superscript are significantly different.

[e]Probability level in test of significance by Anova II (Appendix Table II.22) when comparing all treatments. The different superscripts indicate significant difference by ortogonal contrasts; O vs. LMH, L vs. MH and M vs. H. 12 observations per plot.

[f]The name of the amino acids are given fully in Table 3.4 which also indicates essential (E) and nonessential (N) amino acids.

[g]$P < .001$ for M vs. H.

Table 3.3.--Trial II 1971. Plasma free amino acids, molar % distribution.[a]

Amino acid		Treatment			
		O	L	M	H
		----%			
Lys[b]	E[c]	4.5	5.3	4.5	3.0
His	E	3.2	3.2	2.8	2.1
Thr	E	5.7	5.3	4.6	4.1
Val	E	15.5	15.2	18.9	17.4
Ile	E	6.6	6.4	8.0	6.2
Leu	E	9.6	9.4	11.6	10.5
Met	E	1.6	2.3	3.0	17.7
Cys	N	1.6	1.8	1.7	1.0
Phe	E	2.8	2.6	2.7	1.9
Tyr	N	2.9	3.1	2.9	2.5
Arg	N	3.9	3.8	3.0	1.8
Asp	N	0.8	0.7	0.6	0.7
Glu	N	5.3	5.4	4.5	3.1
Pro	N	5.8	7.3	8.0	9.0
Ser	N	4.8	4.4	3.6	3.5
Gly	N	13.3	10.9	7.2	5.7
Ala	N	12.2	12.9	12.6	9.6
E/N ratio		0.98	0.99	1.27	1.71

[a]Table 3.2 presents significance for difference between treatments in actual concentrations (μM/l).

[b]The name of the amino acids are spelled fully in Table 3.4.

[c]E = essential, N = nonessential amino acid.

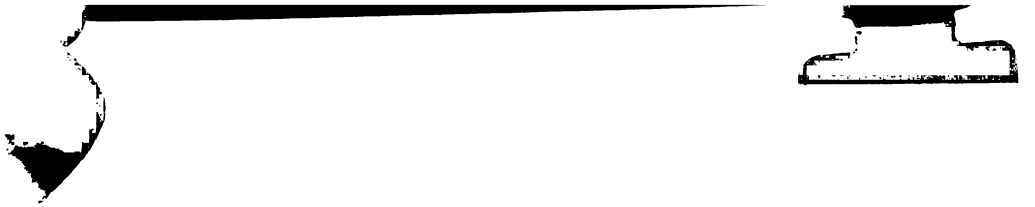


Table 3.4.--Trial III 1971. Plasma free amino acids at control and treatment infusions.

Amino acid		O[a]	K	O_1	K	O_2	P[b]
		----µM/l----		----%			
Lysine	E[c]	86	99	5.3	5.3	5.1	
Histidine	E	65	71	3.8	3.8	4.1	
Threonine	E	75	85	4.4	4.6	4.6	
Valine	E	193	271	11.6	14.5	12.0	<.05
Isoleucine	E	103	121	6.2	6.5	6.1	
Leucine	E	140	180	8.5	9.6	8.6	
Methionine	E	30	49	1.7	2.6	1.9	<.01
Cystine	N[d]	35	38	2.1	2.0	2.2	
Phenylalanine	E	42	41	2.6	2.2	2.5	<.05
Tyrosine	N	48	52	3.0	2.8	2.9	
Arginine	N	76	82	4.7	4.4	4.6	
Aspartic acid	N	13	12	0.7	0.6	0.7	
Glutaric acid	N	129	110	8.9	5.9	6.8	<.05
Proline	N	101	152	6.0	8.1	6.2	
Serine	N	72	72	4.2	3.9	4.5	
Glycine	N	224	193	12.8	10.3	14.3	<.01
Alanine	N	214	241	13.4	12.9	13.1	
EAA[c]		734	917	44.1	49.1	44.9	
NEAA[d]		912	952	55.8	50.9	55.3	
E/N ratio		0.80	0.96	0.80	0.97	0.81	<.05

[a]The control before (O_1) and after (O_2) casein + methionine infusion (K) averaged.

[b]Probability level in test of significance by Anova III (Appendix Table II.33) for O vs. K by ortogonal contrast. 16 observations per plot.

[c]E = essential amino acid.

[d]N = nonessential amino acid.

isoleucine and leucine) were high, even at the control, compared to trial III and other studies in lactating cows (Jacobson, et al., 1970; Fisher, 1972). Apparently the level did not decline to the normal between protein infusions which increased ($P < .05$) valine and isoleucine in particular. In rats on a high protein diet, the plasma BrAA remained high while there was an adaptation in catabolic capacity for other amino acids (Anderson, et al., 1968).

In all trials the casein and methionine infusions tended to increase the molar % of EAA relative to NEAA (Tables 3.1, 3.2 and 3.4), but the E/N ratio for the lowest level of infusion (L) in trial II was identical with the control. The glucose infusion in trial I did not change the E/N ratio. An increase in this ratio results from lower rate of catabolism of EAA relative to NEAA (Kaplan and Pitot, 1970), and generally it indicates an improved amino acid status (Munro, 1970).

In trial II the E/N ratio increased from treatment level L to M mainly because the BrAA increased, while the increase from M to H was due to a dramatic increase in methionine. The actual level of BrAA also increased from M to H, but in molar proportion this increase was offset by the large increase in methionine. Thus, the molar % comparison fails to show some changes which may have had metabolic significance.

Methionine and BrAA are metabolized largely by the muscles and at a slower rate than other EAA (Kaplan and Pitot, 1970). Inappetance has been imposed on rats by imbalances between leucine and isoleucine and by toxicity from high levels of methionine (Harper, 1958); and Broderick, et al., (1970) observed decreased grain intake during abomasal infusion of casein + methionine which increased BrAA and methionine. Because feed intakes were depressed by all protein treatments and did not differ significantly between levels of infusion, a direct relationship between the inappetance observed in trial II and a methionine toxicity was not apparent, despite the dramatic increase in methionine from M to H.

In trial II the molar % of certain EAA were depressed at the higher infusion levels compared to the control, due to increased BrAA and methionine. In trials I and III, however, the molar % increased or remained unchanged for most EAA, except threonine which was lowered ($P < .05$) by M and K in trial I, and phenylalanine which was decreased ($P < .05$) by the casein + methionine infusion in trial III. Moreover, phenylalanine was the only EAA which concentration (μM/l) did not tend to be higher during treatment than control.

As plasma concentration of the first limiting amino acid may decline when abundant supply of other EAA stimulate protein synthesis (Munro, 1970), threonine in trial I and phenylalanine in trial III might be considered the first

limiting when protein was infused in these two trials, respectively. In trial I the molar % of threonine, together with valine and leucine, tended to decline also by the glucose infusion; thus strengthening the impression of a relative threonine deficiency during the control as well as treatment period. Abomasal infusion of methionine, frequently considered the first limiting amino acid in ruminants, depressed plasma concentration of threonine in growing sheep (Scott, et al., 1972; Nimrick, et al., 1970a) while the level of methionine itself increased.

Different catabolic rates among amino acids, however, may obscure the significance of plasma concentration changes. Threonine dehydratase increased in rat liver with increased protein intakes (Anderson, et al., 1968). Thus, threonine may be lowered for reasons other than stressing demand by increased protein synthesis. But Harper (1968) implied that enzymes involved in metabolism of NEAA adapt according to intake while enzymes that catabolize EAA adapt to their intake as it relates to amino acid requirements.

Falling levels of threonine and phenylalanine and other amino acids by post-ruminal supply of protein in lactating cows were also observed by Spechter (1972) and Broderick (1972; Broderick, et al., 1972). However, their media, blood and plasma respectively, as well as their interpretations differed. Spechter (1972) concluded histidine, phenylalanine and methionine were limiting at the highest

level of infusion since the blood concentration of these amino acids then were lower than at the control. Broderick (1972) implicated methionine, valine and lysine as limiting amino acids because the plasma level of these were increased by formalinized casein supplements. Potter, et al., (1972) found that indices, as applied by Spechter (1972), did not identify the limiting amino acids in sheep as was earlier suggested (Potter, et al., 1968).

Spechter (1972) as well as Broderick (1972) supplied insufficient levels of crude protein during control periods which make search for a first limiting amino acid irrelevant in that dietary situation. By observing amino acid concentrations at graded levels of supplements, however, the limiting amino acids may be appropriately indicated at a total protein level which meets or exceeds suggested standards. In any event, blood concentrations no more than implicate the critical availability of specific amino acids. Separate experiments with appropriate additions of these amino acids are required in order to verify a limiting supply.

Young, et al., (1973) concluded that excessive plasma levels of amino acids may mask specific amino acid deficiencies. At low dietary protein levels, however, they found highly significant correlations between the plasma levels of most EAA and daily gains in steers. Thus, plasma amino acid levels during postruminal protein supply above standard feed requirements may be unsuitable to identify the

rate limiting amino acid. On the other hand, this treatment will express the potential for amino acid utilization more explicitly than supplementation of substandard diets.

Taking synthetic demands as well as availability of amino acids into account, Chandler and Polan (1972) related the concentration of EAA in blood serum to milk protein output by calculating a "minimum transfer efficiency" at an assumed blood flow rate (450l per l of milk produced). With a higher transfer efficiency than any other EAA at yields between 16 and 37kg milk per day, methionine was the most critical amino acid while the ranking among four other amino acids (lysine, phenylalanine, tyrosine and threonine) shifted with level of production. Transfer efficiency decreased with level of production (Chandler and Polan, 1972), as might be expected.

The data of trial III was evaluated similarly to the approach of Chandler and Polan (1972). The output of each amino acid by milk protein (Maa) was related to the content of this amino acid in plasma (Paa) (Table 3.5). The ratio, [Maa (g/day): Paa (g/l)] expresses how many l plasma must be "cleared" [C, (l/day)] to furnish the amino acid in milk protein but neglects differences in uptake efficiency by the mammary cells. Our data and that of Chandler and Polan (1972) ranked essentially the same amino acids as critical, although their order differs somewhat. Here phenylalanine was the EAA of lowest availability in plasma relative to

Table 3.5.--Trial III 1971. The relationship between output of amino acids in milk protein (Maa)[a] and plasma free amino acids (Paa).[b]

Amino acid	"Clearance" (C)[c]				(C/AVd[d])x100			
	O[e]		K		O[e]		K	
	l/day	(rank)[f]	l/day	(rank)	l/day	(rank)	l/day	(rank)
Lys	2792	(2)	2580	(2)	5369		4962	
His	1125	(8)	1130	(7)	4892		4913	
Thr	2226	(5)	2099	(3)	6953	(2)	6559	(2)
Val	1276	(7)	965	(8)	6079	(3)	4595	
Ile	1860	(6)	1742	(5)	5635		5279	(3)
Leu	2292	(4)	1922	(4)	4982		4178	
Met	2448	(3)	1653	(6)	4707		3179	
Phe	3102	(1)	3463	(1)	7754	(1)	8860	(1)
Tyr	2500		2596		5209		5408	

[a]Maa = the amount of any amino acid (aa) put out by estimated true protein (ETP, g/day) in milk (Appendix Table II.30). Maa = ETP x F, where F = the fraction (weight %) of each amino acid in total milk proteins derived from Porter, et al., (1968); i.e., Lys 7.60, his 2.53, thr 4.32, val 6.19, ile 5.54, leu 9.10, met 2.44, phe 4.60, tyr 4.78.

[b]Paa = the content of any amino acid in plasma (g/l); Paa = $\mu M/l \times MW \times 10^{-6}$, where MW is the molecular weight (Damm, et al., 1966).

[c]Clearance (C) = Maa/Paa (l/day) for any amino acid; i.e., the plasma volume that must be cleared to furnish the output of an amino acid by milk protein.

[d]AVd = the arterio-venous concentration difference over cows' udder as a fraction of arterial concentration, derived from Verbeke and Peeters (1964); i.e., in %, lys 52, his 23, arg 42, thr 32, val 21, ile 33, leu 46, met 52, phe 40, tyr 48.

[e]Average control before and after casein + methionine (K) infusion.

[f]Among EAA.

demand, followed by lysine, in the control (O) as well as the treatment (K) periods. While methionine was ranked the third least available in the control situation, casein + methionine infusion increased methionine concentrations and rendered it among the more abundant EAA. Threonine was the third most critical during protein infusion. Tyrosine, which was among the critical amino acids in the Chandler and Polan (1972) study, had a high clearance value, close to lysine. This high value for tyrosine emphasizes the low availability of phenylalanine. Moreover, phenylalanine was the only EAA for which molar % decreased ($P < .05$) from control to treatment periods. This amino acid stimulated milk protein synthesis more than other amino acids in an in vitro system (Emery, et al., 1970).

When dividing the clearance values by arteriovenous differences (AVd) observed in cows (Verbeke and Peeters, 1964) (Table 3.5), neglecting that a small part (~10%) of milk proteins are not synthesized by the udder, phenylalanine still appears as the least available amino acid, followed by threonine. Applying the AVd values obtained in goats (Mepham and Linzell, 1966) showed lysine as the least abundant EAA, followed by phenylalanine. Lysine was the only EAA that had lower AVd (%) in the goat study (Mepham and Linzell, 1966) than in the cow study (Verbeke and Peeters, 1964). However, it is a crucial question how far the efficiency of mammary amino acid uptake changes with the amino acid availability (Rook, 1971).

The high AVd observed in goats (Mepham and Linzell, 1966) for methionine, phenylalanine, threonine and leucine was suggested (Mepham, 1971) to indicate critical supply of these EAA. Experimental evidence shows transport into and out of cells generally provides one potent means for regulation of protein metabolism (Munro, 1970). And an extracellular excess of one amino acid may affect the entry of that or other amino acids into certain tissues (Munro, 1970). Possible amino acid transport to the udder by proteins and enythrocytes (Elwyn, 1970) have been neglected in this discussion, but variation in these sources might also influence the availability of amino acids for milk protein synthesis.

The irregularity in actual PAA levels in trial I and the fluctuating production in trial II discourage evaluation by clearance values for these experiments.

While the PAA data point to phenylalanine as the most critical amino acid in trial III and threonine in trial I, the dependency of response on treatment level (Table 2.22) may suggest that more than one EAA was responsible for the increase in milk protein production. The statement of Munro (1970) that "we know very little about the effect of lack of specific amino acids in the diet on synthesis of protein in tissues other than the liver" apparently is valid also for the cow's mammary gland.

BIBLIOGRAPHY

BIBLIOGRAPHY

Abidi, S. A., S. J. Gray and E. Menden. 1967. The kenetics of amino acid absorption and alteration of plasma composition of free amino acids after intestinal perfusion of amino acid mixtures. Amer. J. Clin. Nutr. 20:24.

Agricultural Research Council (ARC). 1965. The Nutrient Requirements of Farm Livestock. No. 2. Ruminants. Agric. Res. Council, London.

Allison, J. B. 1961. The ideal amiogram. Fed. Proc. 20:66.

Allison, J. B., R. W. Wannemacher, Jr., E. Middelton and T. Spoerlein. 1959. Dietary protein requirements and problems of supplementation. Food Technol. 12:597.

Allison, M. J. 1970. Nitrogen metabolism of rumen micro-organisms. In Physiology of Digestion and Metabolism in the Ruminant, p. 456. Ed. A. T. Phillipson, Oriel Press Ltd., Newcastle upon Tyne.

Almquist, H. J. 1954. Utilization of amino acids by chicks. Arch. Biochem. Biophys. 52:197.

Al-Rabbat, M. F., R. L. Baldwin and W. C. Weir. 1971a. In vitro 15-nitrogen tracer technique for some kinetic measures of ruminal ammonia. J. Dairy Sci. 55:1150.

Al-Rabbat, M. F., R. L. Baldwin and W. C. Weir. 1971b. Microbial growth dependence on ammonia nitrogen in the bovine rumen: a quantitative study. J. Dairy Sci. 55:1162.

Amos, H. E., C. O. Little, D. G. Ely and G. E. Mitchell, Jr. 1971. Abomasal protein and amino acids in steers fed different protein supplements. Can. J. Anim. Sci. 51:51.

Anderson, H. L., N. J. Benevenga and A. E. Harper. 1968. Associations among food and protein intake, serine dehydratase, and plasma amino acids. Amer. J. Physiol. 214:1008.

Annison, E. F. 1956. Nitrogen metabolism in the sheep. Protein digestion in the rumen. Biochem. J. 64:705.

Annison, E. F. and J. L. Linzell. 1964. The oxidation and utilization of glucose and acetate by the mammary gland of the goat in relation to their overall metabolism and to milk formation. J. Physiol. 175:372.

Annison, E. F., M. I. Chalmers, S. B. M. Marshall and R. L. M. Synge. 1954. Ruminal ammonia formation in relation to the protein requirement of sheep. III. Ruminal ammonia formation with various diets. J. Agric. Sci. 44:270.

Armstrong, D. G. and J. H. D. Prescott. 1971. Amount, physical form and composition of feed and milk secretion in the dairy cow. In Lactation, p. 349. Ed. I. R. Falconer. The Penn. State Univ. Press, College Park.

Association of Official Agricultural Chemists (A.O.A.C.). 1955. Official Methods of Analysis, 8th edit. Publ. by A.O.A.C., Washington.

Baile, C. A. and J. Mayer. 1970. Hypothalamic centers: feedbacks and receptor sites in the short-term control of feed intake. In Physiology of Digestion and Metabolism in the Ruminant, p. 254. Ed. A. T. Phillipson. Oriel Press Ltd., Newcastle upon Tyne.

Balch, C. C. 1967. Problems in predicting the value of non protein nitrogen as a substitute for protein in rations for farm ruminants. World Rev. Anim. Prod. 3:84.

Baldwin, R. L., H. L. Lucas and R. Cabrera. 1970. Energetic relationships in the formation and utilization of fermentation end-products. In Physiology of Digestion and Metabolism in the Ruminant, p. 319. Ed. A. T. Phillipson, Oriel Press Ltd., Newcastle upon Tyne.

Barry, J. M. 1964. A quantitative balance between substrates and metabolic products of the mammary gland. Biol. Rev. 39:194.

Bartley, J. C. and A. L. Black. 1966. Effect of exogenous glucose on glucose metabolism in dairy cows. J. Nutr. 89:317.

Bauchop, T. and S. R. Elsden. 1960. The growth of microorganisms in relation to their energy supply. J. Gen. Microbiol. 23:457.

Begum, M. and G. M. Jones. 1972. Effects of feeding MHA on nutrient digestibility or milk production by dairy cows. Can. J. Amin. Sci. 52:582. Abstract.

Bergen, W. G., D. B. Purser and J. H. Cline. 1967. Enzymatic determination of the protein quality of individual rumen bacteria. J. Nutr. 92:357.

Bigwood, I. 1964. Amino-acid balance studies in the ruminant during lactation: dietary lysine as an essential limiting factor in milk secretion. In The Role of the G. I. Tract in Protein Metabolism, p. 155. Ed. H. N. Munro. Blackwell Sci. Publ., Oxford.

Bishop, R. B. 1971b. Methionine hydroxy analogue supplementation in beef and dairy cattle. Feedstuffs 43(5):31.

Bishop, R. B. and W. D. Murphy, Jr. 1972. Effect of continuous methionine hydroxyanalog supplementation on complete lactations. J. Dairy Sci. 55:711. Abstract.

Black, A. L., A. R. Egan, R. S. Arnand and T. E. Chapman. 1968. The role of amino acids in gluconeogenesis in lactating ruminants. In Isotope Studies on the Nitrogen Chain, p. 247. Int. Atom. Energy Ag., Vienna.

Black, A. L., M. Kleiber and C. F. Baxter. 1955. Glucose as a precursor of amino acids in the intact cow. Biochem. Biophys. Acta. 17:346.

Black, A. L., M. Kleiber, A. M. Smith and D. N. Stewart. 1957. Acetate as a precursor of amino acids of casein in the intact cow. Biochem. Biophys. Acta, 23:54.

Black, A. L., E. Yeh and T. Chapman. 1972. Interconversion of amino acids in lactating dairy cows. Fed. Proc. 31:681. Abstract.

Blackburn, T. H. 1965. Nitrogen metabolism in the rumen. In Physiology of Digestion in the Ruminant, p. 322. Ed. R. W. Daugherty. Butterworths, Washington.

Blaxter, K. L. and A. K. Martin. 1962. The utilization of protein as a source of energy in fattening sheep. Brit. J. Nutr. 16:397.

Block, R. J. and H. H. Mitchell. 1946. The correlation of the amino acid composition of the proteins with their nutritive value. Nutr. Abstr. Revs. 16:249.

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

Broderick, G. A., T. Kowalczyk and L. D. Satter. 1970. Milk production response to supplementation with encapsulated methionine per os or casein per abomasum. J. Dairy Sci. 53:1714.

Broderick, G. A., A. E. Harper and L. D. Satter. 1972. Response of lactating cows to feeding formaldehyde-treated casein at graded levels. Fed. Proc. 31:681. Abstract.

Broderick, G. A. 1972. Protein and amino acid studies: 1. Effects of post-ruminal casein supplementation on lactating cows; 2. Gas-liquid chromatography of amino acids. Ph.D. thesis, The University of Wisconsin.

Bucholtz, H. F. 1972. Microbial protein synthesis in the rumen: Assessment of radioactive phosphorous as a marker for cellular growth. Ph.D. thesis, Michigan State University.

Burgos, A. and H. H. Olson. 1970. Effects of 40g of methionine hydroxyanalog on yield and composition of milk. J. Dairy Sci. 53:647. Abstract.

Carstairs, J. A. 1972. The influence of casein and glucose infusion on the blood levels of prolactin and growth hormone in the lactating dairy cow. B. Sci. thesis, University of Nottingham, School of Agriculture.

Chalmers, M. I. 1971. Nitrogen nutrition for lactation. In Lactation, p. 379. Ed. I. R. Falconer, Penn. State University Press, University Park.

Chalmers, M. I., D. P. Cuthbertson, and R. L. M. Synge. 1954. Ruminal ammonia formation in relation to the protein requirement of sheep. I. Duodenal administration and heat processing as factors influencing fate of casein supplements. J. Agric. Sci. 44:254.

Chalmers, M. I. and S. B. M. Marshall. 1964. Ruminal ammonia formation in relation to the utilization of groundnut meal and herring meal as protein sources for milk production. J. Agric. Sci. 63:277.

Chalmers, M. I. and R. L. M. Synge. 1954. Ruminal ammonia formation in relation to the protein requirement of sheep. II. Comparison of casein and herring-meal supplements. J. Agric. Sci. 44:263.

Chalupa, W. 1970. Urea as a component of ruminant diets. Proc. 1970 Cornell Nutr. Conf., p. 64.

Chalupa, W. 1972. Metabolic aspects of nonprotein nitrogen utilization in ruminant animals. Fed. Proc. 31:1152.

Chalupa, W., J. E. Chandler and R. E. Brown. 1972. Amino acid nutrition of grown cattle. Fed. Proc. 31:681. Abstract.

Chalupa, W., J. L. Evans and M. C. Stillions. 1963. Nitrogen source availability and activity of rumen nicroorganisms. J. Dairy Sci. 46:1431.

Chandler, P. T. and C. E. Polan. 1972. Considerations in interpretations of serum amino acids in lactating cows. J. Dairy Sci. 55:709. Abstract.

Champredon, C., R. Pion and G. Fauconneau. 1969. The free amino acid level of the bovine. Variations during the day [translated title]. C. R. Acad. Sci. Paris, t. 269D, 2029.

Christensen, H. N. 1962. Intestinal absorption with special reference to amino acids. Fed. Proc. 21:37.

Clarke, E. M. W., G. M. Ellinger and A. T. Phillipson. 1966. The influence of diet on the nitrogenous components passing to the duodenum and through the lower ileum of sheep. Proc. R. Soc. B. 166:63.

Cochran, W. G. and G. M. Cox. 1957. Experimental Designs. 2nd edit. Wiley, New York.

Coelho daSilva, J. F., R. C. Seeley, D. E. Beever, J. H. D. Prescott and D. G. Armstrong. 1972. The effect in sheep of physical form and stage of growth on the sites of digestion of a dried grass. 2. Sites of nitrogen digestion. Brit. J. Nutr. 28:357.

Commonwealth Scientific and Industrial Research Organization (CSIRO). 1971. What profit from protected protein? Rural Res. in CSIRO. 72:12.

Conrad, H. R. and J. W. Hibbs, 1968. Nitrogen utilization in the ruminant. Appreciation of its nutritive value. J. Dairy Sci. 51:276.

Conrad, H. R., R. C. Miles and J. Butdorf. 1967a. Estimation of methionine synthesis in intact cows after administering sulfide-35S. J. Nutr. 91:337.

Conrad, H. R., J. W. Hibbs and A. D. Pratt. 1967b. Effect of plane of nutrition and source of nitrogen on methionine synthesis in cows. J. Nutr. 91:343.

Conway, E. J., ed. 1969. Ammonia. Biological determinations. In Microdiffusion Analysis and Volumetric Error. Chemical Publishing Co., Inc., New York.

Cook, R. M., R. E. Brown and C. L. Davis. 1965. Protein metabolism in the rumen. I. Absorption of glycine and other amino acids. J. Dairy Sci. 48:475.

Coppock, C. E., H. F. Tyrell, W. G. Merrill and J. T. Reid. 1968. The significance of protein reserve to the lactating cow. Proc. 1968 Cornell Nutr. Conf., p. 86.

Coulombe, J. J. and L. Favreau. 1963. A new simple semimicro method for colorimetric determination of urea. Clinical Chem. 9:102.

Damm, H. C., P. K. Besch and A. J. Goldwyn. 1966. The Handbook of Biochemistry and Biophysics. The World Publ. Company, Cleveland.

Davis, S. L. 1972. Plasma levels of prolactin, growth hormone and insulin in sheep following the infusion of arginine, leucine and phenylalanine. Endocrinology 91:549.

Derring, R. G., J. H. Clark, and C. L. Davis. 1972. Abomasal and ruminal infusions of protein in lactating cows. J. Dairy Sci. 55:708. Abstract.

Downes, A. M. 1961. On the amino acids esential for the tissues of the sheep. Aust. J. Biol. Sci. 14:254.

Downes, A. M., P. J. Reis, L. F. Sharry and D. A. Tunks. 1970. Evaluation of modified [35S] methionine and [35S] casein preparations as supplements for sheep. Brit. J. Nutr. 24:1083.

Duncan, C. W., I. P. Agrawala, C. F. Huffman and R. W. Luecke. 1953. A quantitative study of rumen synthesis in the bovine on natural and purified rations. J. Nutr. 49:41.

Ellinger, G. M. and A. T. Phillipson. 1964. The nitrogenous compounds passing to the duodenum of sheep. In The Role of the G. I. Tract in Protein Metabolism, p. 137. Ed. H. N. Munro. Blackwell Sci. Publ., Oxford.

Ellis, W. C. and W. H. Pfander. 1965. Rumen microbial polynucleotide synthesis and its possible role in ruminant nitrogen utilization. Nature 205:974.

Elwyn, D. H. 1970. The role of the liver in regulation of amino acid and protein metabolism. In Mammalian Protein Metabolsim. IV., p. 523. Ed. H. N. Munro, Academic Press, New York.

Ely, D. G., C. O. Little, P. G. Woolfolk and G. E. Mitchell, Jr. 1967. Estimation of the extent of conversion of dietary zein to microbial protein in the rumen of lambs. J. Nutr. 91:314.

Emery, R. S. 1971. Disappearance of methionine from the rumen. J. Dairy Sci. 54:1090.

Emery, R. S., J. Boraas and J. D. Benson. 1970. Stimulation of milk protein synthesis by folic and amino acids. Fed. Proc. 29:691. Abstract.

Eskeland, B., W. H. Pfander, T. M. Badger and R. L. Preston. 1971. Intravenous energy sources and ovine N metabolism. J. Anim. Sci. 33:282. Abstract.

Faichney, G. J. 1971. The effect of formaldehyde-treated casein on the growth of ruminant lambs. Aust. J. Agric. Res. 22:453.

Faichney, G. J. and H. Lloyd Davies. 1972. The effect of formaldehyde treatment of peanut meal in concentrate diets on the performance of calves. Aust. J. Agric. Res. 23:167.

Faichney, G. J. and R. H. Weston. 1971. Digestion by ruminant lambs of a diet containing formaldehyde-treated casein. Aust. J. Agric. Res. 22:461.

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

Ferguson, K. A., J. A. Hemsley and P. J. Reis. 1967. Nutrition and wool growth. The effect of protecting dietary protein from microbial degradation in the rumen. Aust. J. Sci. 30:215.

Fisher, L. J. 1969. Effect of methionine infusion on milk production and plasma-free amino acids of lactating cows. J. Dairy Sci. 52:943. Abstract.

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

Fisher, L. J. and J. M. Elliot. 1966. Effect of intravenous infusion of propionate or glucose on bovine milk composition. J. Dairy Sci. 49:826.

Fisher, L. J. and J. D. Erfle. 1970. Effect of the intravenous infusion of either glucose or L-methionine on lactating cows exhibiting symptoms of ketosis. J. Dairy Sci. 53:664.

Ford, A. L. and L. P. Milligan. 1970. Tracer studies of urea recycling in sheep. Can. J. Anim. Sci. 50:129.

Fujihara, T. and I. Tasaki. 1973. Digestibility of casein and starch introduced into rumen and abomasum of goats. Jap. J. Zootech. Sci. 44:125.

Gill, J. L. and H. D. Hafs. 1971. Analysis of repeated measurements of animals. J. Anim. Sci. 33:331.

Gordon, F. J. and T. J. Forbes. 1970. The associative effect of level of energy and protein intake in the dairy cow. J. Dairy Res. 37:481.

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

Hale, G. D. and D. R. Jacobson. 1972. Feeding and abomasal administration of casein, gelatin, partially delactosed whey (PDW), or zein to lactating cows. J. Dairy Sci. 55:709. Abstract.

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

Harmeyer, J., B. Kurelec and H. Hill. 1968. The metabolic conversion of arginine in the rumen wall and its importance in ruminant nitrogen metabolism. In Isotope Studies on the Nitrogen Chain, p. 265. Int. Atom. En. Ag., Vienna.

Harper, A. E. 1958. Balance and imbalance of amino acids. Ann. N. Y. Acad. Sc. 69:1025.

Harper, A. E. 1968. Diet and plasma amino acids. Amer. J. Clin. Nutr. 21:358..

Hatfield, E. E. 1970. Selected topics related to the amino acid nutrition of the growing ruminant. Fed. Proc. 29:44.

Hatfield, E. E. 1971. Responses of ruminants to specific exogenous amino acids. 1971. Dist. Feed Res. Council, Proc. 26:80.

Helmer, L. G., E. E. Bartley, C. W. Deyoe, R. M. Meyer and H. B. Pfost. 1970a. Feed processing. V. Effect of grain and urea (Starea) on nitrogen utilization in vitro. J. Dairy Sci. 53:330.

Helmer, L. G., E. E. Bartley and C. W. Deyoe. 1970b. Feed processing. VI. Comparison of starea, urea, and soybean meal as protein sources for lactating dairy cows. J. Dairy Sci. 53:883.

Henderson, H. E., W. G. Bergen and C. M. Hansen. 1972. Corn silage additives compared. J. Anim. Sci. 35:229. Abstract.

Hertelendy, F., K. Takahashi, L. J. Machlin and D. M. Kipnis. 1970. Growth hormone and insulin secretory responses to arginine in the sheep, pig and cow. Gen. Comp. Endocr. 14:72.

Hillman, D., J. T. Huber, R. S. Emery, J. W. Thomas and R. M. Cook. 1970. Basic dairy cattle nutrition. Mimeograph D. 239, Dairy Dept., Michigan State University.

Hinton, C. L. and T. Macara. 1928. The determination of aldose sugars by means of chloramine T, with special reference to the analysis of milk products. Analyst 52:668.

Hogan, J. P., R. H. Weston and J. R. Lindsay. 1968. Influence of protein digestion on plasma amino acid levels in sheep. Aust. J. Biol. Sci. 21:1263.

Hogan, J. P. and R. H. Weston. 1970. Quantitative aspects of microbial protein synthesis in the rumen. In Physiology of Digestion and Metabolism in the Ruminant, p. 474. Ed. A. T. Phillipson, Oriel Press Ltd., Newcastle upon Tyne.

Hoogenroad, H. J. and F. J. R. Hird. 1970. The chemical composition of rumen bacteria and cell walls from rumen bacteria. Brit. J. Nutr. 24:119.

Houpt, T. R. 1959. Utilization of blood urea in ruminants. Amer. J. Physiol. 197:115.

Huber, J. T., D. F. Andrus, R. E. Erickson and C. E. Polan. 1972. Influence of production on response to high urea or low protein intakes. J. Dairy Sci. 55:708. Abstract.

Huber, J. T. and R. L. Boman. 1966. Nutritional factors affecting the solids-not-fat content of milk. J. Dairy Sci. 49:816.

Huber, J. T., R. A. Sandy, C. E. Polan, H. T. Bryant and R. E. Blaser. 1967. Varying levels of urea for dairy cows fed corn silage as the only forage. J. Dairy Sci. 50:1241.

Huber, J. T. and O. P. Santana. 1972. Ammonia-treated corn silage for dairy cattle. J. Dairy Sci. 55:489.

Hume, I. D. 1970. Synthesis of microbial protein in the rumen. II. A response to higher volatile fatty acids. Aust. J. Agric. Res. 21:297.

Hume, I. D. 1971a. Absorption of abomasally infused leucine in sheep. J. Anim. Sci. 33:287. Abstract.

Hume, I. D. 1971b. Amino acid absorption in sheep fed alfalfa. J. Anim. Sci. 33:287. Abstract.

Hungate, R. E. 1965. Quantitative aspects of the rumen fermentation. In Physiology of Digestion in the Ruminant, p. 311. Ed. R. W. Daugherty, Butterworths, Washington.

Hungate, R. E. 1966. The Rumen and Its Microbes. Academic Press, New York.

Hutton, J. B. 1957. The effect of growth hormone on the yield and composition of cows' milk. J. Endocrin. 16:115.

Hutton, K. and E. F. Annison. 1972. Control of nitrogen metabolism in the ruminant. Proc. Nutr. Soc. 31:151.

Hutton, K., F. J. Bailey and E. F. Annison. 1971. Measurement of the bacterial nitrogen entering the duodenum of the ruminant using diaminopimelic acid as a marker. Brit. J. Nutr. 25:165.

Isaacs, J. and F. N. Owens. 1972. Protein soluble in rumen fluid. J. Anim. Sci. 35:267. Abstract.

Ishaque, M., P. C. Thomas and J. A. F. Rook. 1971. Consequences to the host of changes in rumen microbial activity. Nature, New Biol. 231:253.

Jackson, P., J. A. F. Rook and K. G. Towers. 1971. Influence of the physical form of a barley grain and barley straw diet on nitrogen metabolisn in sheep. J. Dairy Res. 38:33.

Jacobson, D. R., H. H. Van Horn and C. J. Sniffen. Lactating ruminants. Fed. Proc. 29:35.

Jenness, R. 1970. Protein composition of milk. In Milk Proteins, Chemistry and Molecular Biology, p. 17. Ed. H. A. McKenzie. Academic Press, New York.

Jones, G. M. 1971. Volatile fatty acids in concentrates for lactating dairy cows. J. Dairy Sci. 54:1142.

Kaplan, J. H. and H. C. Pitot. 1970. The regulation of intermediary amino acid metabolism in animal tissue. In Mammalian Protein Metabolism. IV., p. 287. Ed. H. N. Munro, Academic Press, New York.

Keenan, T. W., R. G. Saacke and S. Patton. 1970. Prolactin, the Golgi apparatus, and milk secretion: Brief interpretive review. J. Dairy Sci. 54:295.

Kim, C. W., J. B. Holter, N. F. Colovos, W. E. Urban, Jr. 1971. Effect of methionine hydroxyanalog (MAH) on milk yield and ration utilization. J. Dairy Sci. 54:1240. Abstract.

Kirchgessner, M., H. Friesecke and G. Koch. 1967. Nutrition and the Composition of Milk. Crosby Lockwood and Son, Ltd., London.

Kirchmeier, O. 1970. Influence of nutrition on the composition of milk proteins. 8th Int. Congr. Nutr. Prague, 1969, p. 750. Excerpta Med. Int. Congr. Ser. 213.

Kleiber, M. 1950. Physiological meaning of regression equations. J. Appl. Physiol. 2:417.

Klopfenstein, T. J., D. B. Purser and W. J. Tyznik. 1965. Effects of defaunation of feed digestability, rumen metabolism and blood metabolites. J. Anim. Sci. 25:765.

Knott, F. N., C. E. Polan and J. T. Huber. 1972. Further observations on utilization of urea by lactating cows. J. Dairy Sci. 55:466.

Krebs, H. A. 1964. The metabolic fate of amino acids. In Mammalian Protein Metab. I., p. 125. Ed. H. N. Munro and J. B. Allison, Academic Press, New York.

Kronfeld, D. S., G. P. Meyer, J. McD. Robertson and F. Raggi. 1963. Depression of milk secretion during insulin administration. J. Dairy Sci. 46:559.

Land, H. and A. I. Virtanen. 1959. Ammonium salts as nitrogen source in the synthesis of protein by the ruminant. Acta Chem. Scand. 13:489.

Larson, B. L. 1958. Nongenetic factors affecting the production of nonfat milk solids by the bovine. J. Dairy Sci. 41:440.

Larson, B. L. 1969. Biosynthesis of milk. J. Dairy Sci. 52:737.

Larson, B. L. 1972. Methionine stimulation of milk protein synthesis in bovine mammary cell cultures. J. Dairy Sci. 55:629.

Larson, B. L. and D. C. Gillespie. 1957. Origin of the major specific proteins in milk. J. Biol. Chem. 227:565.

Lewis, D. 1955. Amino acid metabolism in the rumen of sheep. Brit. J. Nutr. 9:215.

Lewis, T. R. and R. S. Emery. 1962a. Relative deamination rates of amino acids by rumen microorganisms. J. Dairy Sci. 45:765.

Lewis, T. R. and R. S. Emery. 1962b. Intermediate products in the catabolism of amino acids by rumen microorganisms. J. Dairy Sci. 45:1363.

Lewis, T. R. and R. S. Emery. 1962c. Metabolism of amino acids in the bovine rumen. J. Dairy Sci. 45:1487.

Lindsay, D. B. 1970. Carbohydrate metabolism in ruminants. In Physiol. of Dig. and Metab. in the Ruminant, p. 438. Ed. A. T. Phillipson, Oriel Press Ltd., Newcastle upon Tyne.

Ling, E. R. 1956. A Textbook of Dairy Chemistry. Volume Two. Practical. Chapman and Hall, Ltd., London.

Linzell, J. L. 1960. Mammary-gland blood flow and oxygen, glucose and volatile fatty acid uptake in the conscious goat. J. Physiol. 153:492.

Linzell, J. L. 1967. The effect of infusion of glucose, acetate and amino acids on hourly milk yield in fed, fasted and insulin-treated goats. J. Physiol. 190:347.

Linzell, J. L. 1971. Techniques for measuring nutrient uptake by the mammary glands. In Lactation, p. 261. Ed. I. A. Falconer. The Penn. State Univ. Press, University Park.

Little, C. O., W. Burroughs and W. Woods. 1963. Nutritional significance of soluble nitrogen in dietary proteins for ruminants. J. Anim. Sci. 22:358.

Little, C. O. and G. E. Mitchell, Jr. 1967. Abomasal vs. oral administration of protein to wethers. J. Anim. Sci. 26:411.

Little, C. O., G. E. Mitchell, Jr., and G. D. Porter. 1968. Nitrogen in the abomasum of wethers fed different protein sources. J. Anim. Sci. 27:1722.

Loosli, J. K. and L. E. Harris. 1946. Methionine increases the value of urea for lambs. J. Anim. Sci. 4:435.

Loosli, J. K., H. L. Lucas and L. A. Maynard. 1945. The effect of roughage intake upon the fat content of milk. J. Dairy Sci. 28:147.

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

Lowry, O. H., N. J. Rosebrough, A. L. Farr and R. J. Randall. 1951. Protein measurement with folin phenol reagent. J. Biol. Chem. 193:265.

Lucas, H. L. 1960. Critical features of good dairy feeding experiments. J. Dairy Sci. 43:193.

McAtee, I. W. and Trenkle, A. 1971. Effects of feeding, fasting, glucose or arginine on plasma prolactin levels in the bovine. Endocrinology 89:730.

McCarthy, R. D., R. A. Patton and L. C. Griel, Jr. 1970. Amino acid nutrition of lactating ruminants. Fed. Proc. 29:41.

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

McDonald, I. W. 1948. The absorption of ammonia from the rumen of sheep. Biochem. J. 42:584.

McDonald, I. W. 1952. The sole of ammonia in ruminal digestion of protein. Biochem. J. 51:86.

McDonald, I. W. 1954. The extent of conversion of food protein to microbial protein in the rumen of the sheep. Biochem. J. 56:120.

McDonald, I. W. and R. J. Hall. 1957. The conversion of casein into microbial proteins in the rumen. Biochem. J. 67:400.

McDonald, I. W. 1968. Nutritional aspects of protein metabolism in ruminants. Aust. Vet. J. 44:145.

McLaughlin, D. R., F. D. Horney, and T. S. Neudoerffer. 1972. Formaldehyde protection of soybean protein against ruminal digestion. Can. J. Anim. Sci. 52:585. Abstract.

McNaught, M. L., E. G. Owen, K. M. Henry and S. K. Kon. 1954. The utilization of non-protein nitrogen in the bovine rumen. 8. The nitritive value of the proteins of preparations of dried rumen bacteria, rumen protozoa and brewer's yeast for rats. Biochem. J. 56:151.

Macrae, J. C., M. J. Wyatt, P. D. Pearce and J. Hendtlass. 1972. Quantitative intestinal digestion of nitrogen in sheep. Brit. J. Nutr. 27:39.

Mahan, D. C., D. E. Becker, B. G. Harmon and A. H. Jensen. 1969. Effect of protein levels and Opaque-2 corn on sow milk composition. J. Anim. Sci. 32:482.

Marston, H. R. 1935. Studies on the relationship between nutrition and wool production of Merino Sheep. II. The effect of the administration of cystine, cystein, sulphur and methionine on the growth of wool of a Merino ewe on a protein-poor ration. J. Agric. Sci. 25:113.

Mathison, G. W. and L. P. Milligan. 1971. Nitrogen metabolism in sheep. Brit. J. Nutr. 25:231.

Maynard, L. A. and J. K. Loosli. 1962. Animal Nutrition. Fifth edit. McGraw-Hill Book Co., New York.

Meites, J. 1961. Farm animals: Hormonal induction of lactation and galacto poresis. In Milk: The Mammary Gland and Its Secretion. I., p. 321. Eds. S. K. Kon and A. T. Cowie, Acadmeic Press, New York.

Mepham, T. B. 1971. Amino acid utilization by the lactating mammary gland. In Lactation, p. 297. Ed. I. R. Falconer. Penn. State Univ. Press, University Park.

Mepham, T. B. and J. L. Linzell. 1966. A quantitative assessment of the contribution of individual plasma amino acids to the synthesis of milk proteins by the goat mammary gland. Biochem. J. 101:76.

Meyer, R. M., E. E. Bartley, C. W. Deyoe and V. F. Colenbrander. 1967. Feed Processing. I. Ration effects on rumen microbial protein synthesis and amino acid composition. J. Dairy Sci. 50:1327.

Miller, E. L. 1972. The digestion of formaldehyde-treated groundnut meal before and after the abomasum of lambs. Proc. Nutr. Soc. 31:27A. Abstract.

Mills, S. C., L. F. Sharry, L. J. Cook and T. W. Scott. 1972. Metabolism of [14C] formaldehyde when fed to ruminants as an aldehyde-casein-oil complex. Aust. J. Biol. Sci. 25:807.

Mowat, D. N. and V. Deelstra. 1972. Encapsulated methionine supplement for growing finishing lambs. J. Anim. Sci. 34:332.

Mugerwa, J. S. 1969. The relationship of dietary nitrogen to urea kinetics and protein synthesis in dairy cows. Diss. Abstr. 30:2972-B.

Mugerwa, J. S. and H. R. Conrad. 1971. Relationship of dietary nonprotein nitrogen to urea kinetics in dairy cows. J. Nutr. 101:1331.

Munro, H. N., ed. 1970. Free amino acid pools and their role in regulation. In Mammalian Protein Metabolism. IV., p. 299. Academic Press, New York.

National Research Council (NRC). 1966. Nutrient Requirements of Domestic Animals. No. 3. Nutrient Requirements of Dairy Cattle. Third revised edit. Publ. 1349, Nat. Acad. Sci. -- Nat. Res. Coun., Washington, D.C.

National Research Council (NRC). 1971. Nutrient Requirements of Domestic Animals. No. 3. Nutrient Requirements of Dairy Cattle. Fourth revised edit. Nat. Acad. Sci., Washington, D.C.

Nimrick, K., E. E. Hatfield, J. Kaminski and F. N. Owens. 1970a. Qualitative assessment of supplemental amino acid needs for growing lambs fed urea as the sole nitrogen source. J. Nutr. 100:1293.

Nimrick, K., E. E. Hatfield, J. Kaminski and F. N. Owens. 1970b. Quantitative assessment of supplemental amino acid needs for growing lambs fed urea as the sole nitrogen source. J. Nutr. 100:1301.

Nimrick, K., A. P. Peter and E. E. Hatfield. 1972. Aldehyde treated fish and soybean meals as dietary supplements for growing lambs. J. Anim. Sci. 34:488.

Nishimuta, J. F., D. E. Ely, and J. A. Boling. 1973. Nitrogen metabolism in lambs fed soybean meal treated with heat, formalin and tannic acid. J. Nutr. 103:49.

Neudoerffer, T. S., D. B. Duncan and F. D. Horney. 1971. The extent of release of encapsulated methionine in the intestine of cattle. Brit. J. Nutr. 25:333.

Nolan, J. V. and R. A. Leng. 1972. Dynamic aspects of ammonia and urea metabolism in sheep. Brit. J. Nutr. 27:177.

Oltjen, R. R. 1967. NPN as the primary nitrogen source for cattle. Proc. 1967 Cornell Nutr. Conf., p. 48.

Oltjen, R. R. and P. A. Putnam. 1966. Plasma amino acids and nitrogen retention by steers fed purified diets containing urea or isolated soy protein. J. Nutr. 89:385.

Orskov, E. R., W. P. Flatt, P. W. Moe and A. W. Munson. 1969. The influence of ruminal infusion of volatile fatty acids on milk yield and composition and on energy utilization by lactating cows. Brit. J. Nutr. 23:443.

Palmer, D. W. and T. Peters, Jr. 1969. Automated determination of free amino groups in serum and plasma using 2,4,6-trinitrobenzene sulfonate. Clinical Chem. 15:891.

Paquay, R., R. deBaere, and A. Lousse. 1972. Influences of diet and body condition on the nitrogen utilization in the cow. J. Agric. Sci. 79:323.

Pennington, R. J. 1957. Effects of propionate upon acetate metabolism in animal tissue slices. Biochem. J. 65:534.

Perkins, A. E. 1957. The effect of rations excessively high and extremely low in protein content on dairy cows. Ohio Agr. Exp. Sta. Bull. 799.

Peter, A. P., E. E. Hatfield, F. N. Owens and U. S. Garrigus. 1971. Effects of aldehyde treatments of soybean meal on *in vitro* ammonia release, solubility and lamb performance. J. Nutr. 101:605.

Pilgrim, A. F., F. V. Gray, R. A. Weller and C. B. Belling. 1970. Synthesis of microbial protein from ammonia in the sheep's rumen and the proportion of dietary nitrogen converted into microbial nitrogen. Brit. J. Nutr. 24:589.

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

Porter, J. W. G., J. E. Ford, S. Y. Thompson and A. P. Williams. 1968. Composition of milk. In Metabolism, p. 7. Eds. P. L. Altman and D. S. Dittmer. Fed. Amer. Soc. Exp. Biol., Bethesda.

Potter, E. L., D. B. Purser and J. H. Cline. 1968. Effect of various energy sources upon plasma-free amino acids in sheep. J. Nutr. 95:655.

Potter, E. L., D. B. Purser and W. G. Bergen. 1972. A plasma reference index for predicting limiting amino acids of sheep and rats. J. Anim. Sci. 34:660.

Potter, G. D., C. O. Little and G. E. Mitchell, Jr. 1969. Abomasal nitrogen in steers fed soybean meal or urea. J. Anim. Sci. 28:711.

Purser, D. B. 1970. Nitrogen metabolism in the rumen: microorganisms as a source of protein for the ruminant animal. J. Anim. Sci. 30:988.

Radloff, H. D. and G. Miyake. 1969. Influence of exogenous hormones on milk and blood constituents in dairy cows. J. Dairy Sci. 52:914. Abstract.

Reed, R. H., R. J. Moir and E. J. Underwood. 1949. Ruminal flora studies in sheep. I. The nutritive value of rumen bacterial protein. Aust. J. Sci. Res. B2:304.

Reid, J. T., P. W. Moe and H. F. Tyrell. 1966. Energy and protein requirements of milk production. J. Dairy Sci. 49:215.

Reid, J. T., H. F. Tyrell and P. W. Moe. 1967. Digestible protein needs of milking cows. Proc. 1967 Cornell Nutr. Conf., p. 41.

Reis, P. J. and D. A. Tunks. 1969. Evaluation of formaldehyde-treated casein for wool growth and nitrogeneous retention. Aust. J. Agric. Res. 20:775.

Reis, P. J. and P. G. Schinckel. 1964. The growth and composition of wool. II. The effect of casein, gelatin and sulphur-containing amino acids given per abomasum. Aust. J. Biol. Sci. 17:532.

Rohlf, F. J. and R. R. Sokal. 1969. Statistical Tables. W. H. Freeman and Co., San Francisco.

Rook, J. A. F. 1971. Discussion on nutrient utilization by the lactating mammary gland. In Lactateion, p. 333. Ed. I. A. Falconer. Penn. State Univ. Press, College Park.

Rook, J. A. F. and C. C. Balch. 1961. The effects of intraruminal infusions of acetic, propionic and butyric acids on the yield and composition of the milk of the cow. Brit. J. Nutr. 15:361.

Rook, J. A. F., C. C. Balch and V. W. Johnson. 1965. Further observations on effects of intraruminal infusions of volatile fatty acids and of lactic acid on the yield and composition of the milk of the cow. Brit. J. Nutr. 19:93.

Rook, J. A. F. and J. B. Hopwood. 1970. The effects of intravenous infusions of insulin and of sodium succinate on milk secretion in the goat. J. Dairy Res. 37:193.

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

Rose, W. C. 1938. The nutritive significance of the amino acids. Physiol. Revs. 18:109.

Satter, L. D., G. P. Brooke and C. G. Schwab. 1970. Effect of formaldehyde-treated soybean oil meal on performance of growing lambs and lactating cows. J. Dairy Sci. 53:668. Abstract.

Schelling, G. T. and E. E. Hatfield. 1968. Effect of abomasally infused nitrogen sources on nitrogen retention of growing lambs. J. Nutr. 96:319.

Schelling, G. T., G. E. Mitchell, Jr. and R. E. Tucker. 1972. Prevention of free amino acids degradation in the rumen. Fedr. Proc. 31:681. Abstract.

Schingoethe, D. J., E. C. Hageman and B. L. Larson. 1967. Essential amino acids for protein synthesis in the in vitro secretory cell and stimulation by elevated levels. Biochem. Biophys. Acta. 148:469.

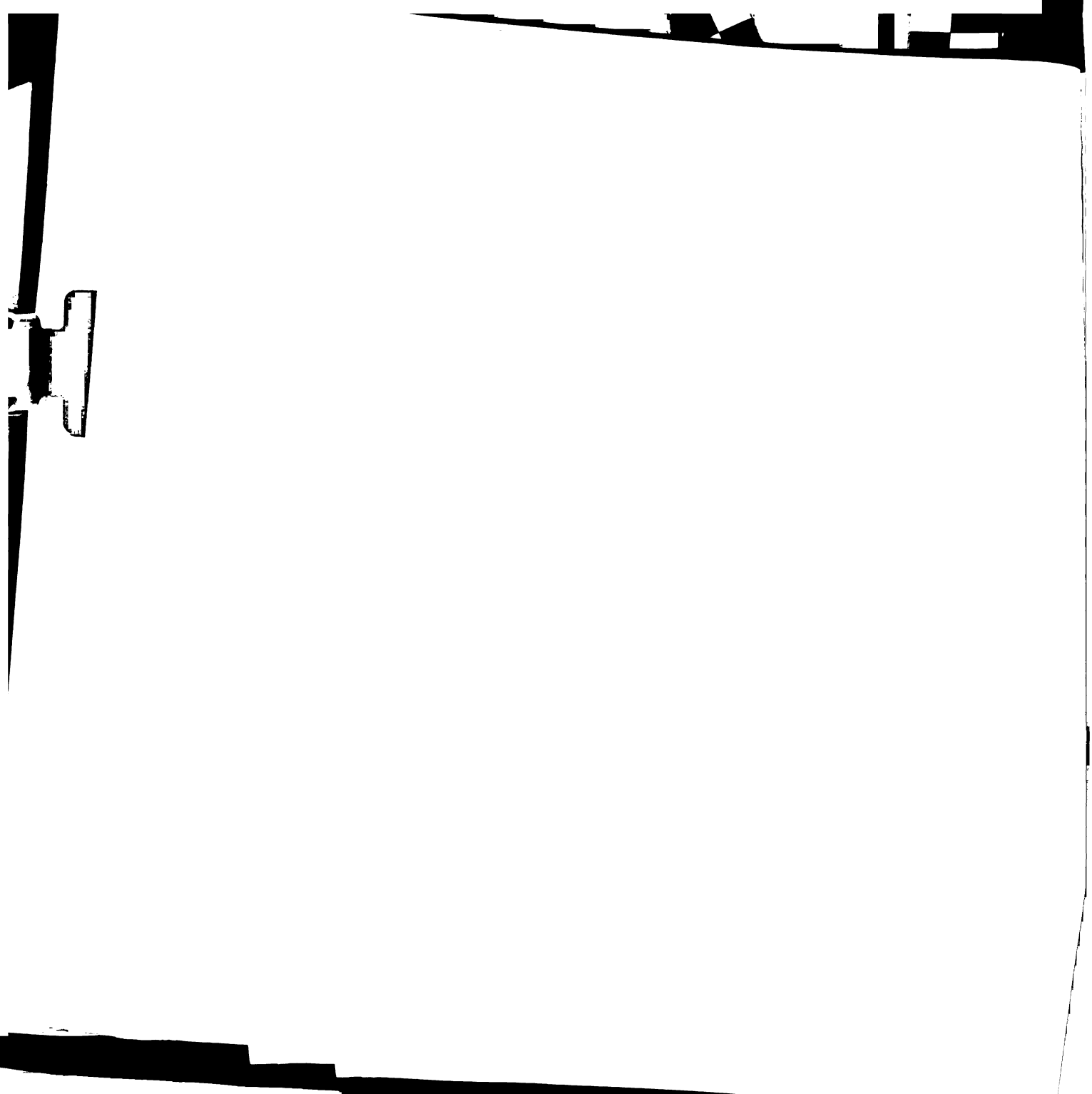
Schmidt, G. H. 1971. Biology of Lactation, W. H. Freeman and Co., San Francisco.

Scott, R. A., C. O. Little, H. E. Amos and G. E. Mitchell, Jr. 1972. Abomasal nitrogen, plasma amino acids and nitrogen balance responses of wethers to fed or abomasally infused methionine. J. Anim. Sci. 35:446.

Senft, B. and F. Klobaska. 1969. The influence of different environmental factors on the content of non-protein nitrogen (NPN) in milk [translated title]. Milch-wissenschaft 24:713.

Shaw, J. C. 1946. Studies on ketosis in dairy cattle. VII. The efficacy of B vitamins and methionine in the treatment of ketosis. J. Dairy Sci. 29:131.

Shahani, K. M. and H. H. Sommer. 1961. The protein and non-protein nitrogen fractions in milk. I. Methods of analysis. J. Dairy Sci. 34:1003.



Silcock, W. R. and S. Patton. 1972. Correlative secretion of protein, lactose and K^+ in milk of the goat. J. Cell. Physiol. 79:151.

Smith, R. H. 1969. Nitrogen metabolism and the rumen. J. Dairy Res. 36:313.

Sokal, R. R. and F. J. Rohlf. 1969. Biometry. W. H. Freeman and Co., San Francisco.

Spechter, H. H. 1972. Postruminal casein infusion of urea-fed lactating cows. Ph.D. thesis, University of Guelph.

Steinacker, G., T. J. Devlin and J. R. Ingalls. 1970. Effect of methionine supplementation posterior to the rumen on nitrogen utilization and sulfur balance of steers on a high roughage ration. Can. J. Anim. Sci. 50:319.

Storry, J. E. and J. A. F. Rook. 1962. Effects of large intraruminal additions of volatile fatty acids on the secretion of milk constituents. XVIth Intern. Dairy Congr., A:64.

Synge, R. L. M. 1953. Note on the occurrence of diaminopimelic acid in some intestinal microorganisms from farm animals. J. Gen. Microbiol. 9:407.

Teichman, R., E. V. Caruolo and R. D. Mochrie. 1969. Milk production and composition responses to intravenous infusion of L-methionine. J. Dairy Sci. 52:942. Abstract.

Thomas, J. W. 1966. Protein - kinds and amount to feed to dairy cattle. Feedstuffs 38(40):58.

Thomas, J. W. 1971. Dietary protein levels for milking cows. J. Dairy Sci. 52:944.

Thomas, J. W., Yu Yu, D. Hillman, J. T. Huber and R. Lichtenwalner. 1972. Unavailable nitrogen in haylage and hays. J. Anim. Sci. 35:1115. Abstract.

Thomas, P. C. and J. L. Clapperton. 1972. Significance to the host of changes in fermentation activity. Proc. Nutr. Soc. 31:165.

Tillman, A. D. and K. S. Sidu. 1969. Nitrogen metabolism in ruminants: Rate of ruminal ammonia production and nitrogen utilization by ruminants. J. Anim. Sci. 28:689.

Tyler, C. 1959. The historical development of feeding standards. In Scinet. Princip. of Feeding Farm Livestock, p. 8. Farmer and Stockbreeder Publ. Ltd., London.

Tyrrell, H. F., D. J. Bolt, P. W. Moe and H. Swan. 1972. Abomasal infusion of water, casein, or glucose in Holstein cows. J. Anim. Sci. 35:277. Abstract.

Tucker, H. A., R. P. Reece and R. E. Mathen. 1961. Udder capactiy estimates as effected by rate of milk secretion and intramammary pressure. J. Dairy Sci. 44:1725.

Van Soest, P. J. 1963. Ruminant fat metabolism with particular reference to factors affecting low milk fat and feed efficiency. A review. J. Dairy Sci. 46:204.

Verbeke, R. and G. Peeters. 1965. Uptake of free plasma amino acids by the lactating cow's udder and amino acid composition of udder lymph. Biochem. J. 94:183.

Virtanen, A. I. 1966. Milk production of cows on protein-free feed. Studies of the use of urea and ammonia salts as the sole nitrogen source open new important perspectives. Science 153:1609.

Virtanen, A. I. 1969. On nitrogen metabolism in milking cows. Fed. Proc. 28:232.

Virtanen, A. I. 1971. Protein requirement of dairy cattle--artificial nitrogen sources and milk-production. Milchwissenschaft 26:129.

Visek, W. J. 1966. Some effects of urea and bile acid feeding upon the gastro-intestinal tract. Proc. 1966 Cornell Nutr. Conf., p. 9.

Visek, W. J. 1972. Effects of urea hydrolysis on cell life-span and metabolism. Fed. Proc. 31:1178.

Waldo, D. R. 1968. Symposium: Nitrogen utilization by the ruminant. Nitrogen metabolism in the ruminant. J. Dairy Sci. 51:265.

Waldschmidt, M. 1973. Metabolite levels and enzyme activities in the bovine mammary gland at different stages of lactation. I. Metabolite levels related to energy production. J. Dairy Res. 40:7.

Walker, D. J. 1965. Energy metabolism and rumen micro-organisms. In Physiology of Digestion in the Ruminant, p. 296. Ed. R. W. Daugherty, Butterworths, Washington, D.C.

Wannemacher, R. W., Jr., and J. B. Allison. 1968. Plasma amino acid concentrations in relation to protein synthesis. In Protein Nutrition and Free Amino Acid Patterns, p. 205. Ed. J. H. Leatham, Rutgers Univ. Press, New Brunswick, New Jersey.

Waterman, R. and L. H. Schulz. 1972. Methionine hydroxy-analog treatment of bovine ketosis: effects on circulating metabolites and interrelationships. J. Dairy Sci. 55:1513.

Webb, K. E., Jr., Fontenot, J. P. and W. A. Phillips. 1972. Abomasal nitrogen fractions of sheep as affected by dietary urea and energy levels. J. Anim. Sci. 35:278. Abstract.

Weller, R. A. 1957. The amino acid composition of hydrolysates of microbial preparations from the rumen of sheep. Aust. J. Biol. Sci. 10:384.

Weller, R. A., F. V. Gray and A. F. Pilgrim. 1958. The conversion of plant nitrogen to microbial nitrogen in the rumen of the sheep. Brit. J. Nutr. 12:421.

Weller, R. A., A. F. Pilgrim and F. V. Gray. 1962. Digestion of foodstuffs in the rumen of the sheep and the passage of digesta through its compartments. 3. The progress of nitrogen digestion. Brit. J. Nutr. 16:83.

Weston, R. H. and J. P. Hogan. 1967. The digestion of chopped and ground roughages by sheep. I. The movement of digesta through the stomach. Aust. J. Agric. Res. 18:789.

Whiting, F. M., J. W. Stull, W. H. Brown and B. L. Reid. 1972. Free amino acid ratios in rumen fluid, blood plasma, milk, and feces during methionine and methionine hydroxyanalog supplementary feeding. J. Dairy Sci. 55:983.

Williams, L. R., F. A. Martz and E. S. Hildebrand. 1970. Feeding encapsulated methionine supplement to lactating cows. J. Dairy Sci. 53:1709.

Wood, H. G., G. J. Peeters, R. Verbeke, M. Lauryssens and B. Jacobson. 1965. Estimation of the pentose cycle in the perfused cow's udder. Biochem. J. 96:607.

Wright, P. L., R. B. Grainger and G. J. Marco. 1966. Post-ruminal degradation and absorption of carbo-hydrate by the mature ruminant. J. Nutr. 89:241.

Young, A. W., J. A. Boling and N. W. Bradley. 1973. Performance and plasma amino acids of steers fed soybean meal, urea or no supplemental nitrogen in finishing rations. J. Anim. Sci. 35:803.

Yousef, I. M., J. T. Huber and R. S. Emery. 1969. Action of high energy rations on milk protein synthesis. J. Dairy Sci. 52:943. Abstract.

Yousef, I. M., J. T. Huber and R. S. Emery. 1970. Milk protein synthesis as affected by high-grain, low-fiber rations. J. Dairy Sci. 53:734.

APPENDIX TABLES

Appendix Table I.1--Trial I 1970. Average daily intake of feed during each period.

Cow No.		501				502			
Period	Trtm.	Hay	Corn silage	Conc.	Dry Matter	Hay	Corn silage	Conc.	Dry Matter
			kg				kg		
1	O	4.10	18.2	6.4	14.8	4.3	18.2	7.1	15.6
2	K	4.5	18.2	6.4	15.1	4.3	18.2	7.0	15.6
3	O	4.4	18.2	6.3	15.1	4.5	18.2	6.5	15.2
4	G	4.3	18.2	6.4	15.0	4.5	18.2	6.5	15.2
5	O	4.3	18.2	6.4	15.0	4.5	18.0	6.8	15.4
6	O	--	--	--	--	4.5	18.2	6.9	15.6
7	K	4.5	18.2	6.4	15.2	4.5	18.2	5.9	14.8
8	O	3.5	18.2	6.4	14.2	4.0	18.2	6.4	14.8

Appendix Table I.2--Trial I 1970. Average daily consumption of crude protein and estimated net energy during each period; actual values and relative to NRC standards.

		Cow No. 501				Cow No. 502			
		CP		E-NE		CP		E-NE	
Period	Trtm.	Intake	Int/Reg	Intake	Int/Reg	Intake	Int/Reg	Intake	Int/Reg
		kg	%	Mcal	%	kg	%	Mcal	%
1	0	2.19	114	24.7	101	2.31	104	26.2	94
2	K	2.25	117	25.1	102	2.30	106	26.2	96
3	0	2.23	127	25.0	110	2.26	108	25.3	96
4	G	2.22	137	25.0	117	2.26	113	25.3	99
5	0	2.22	134	25.0	115	2.29	127	25.8	110
6	0	--		--		2.32	120	26.1	112
7	K	2.25	129	25.2	110	2.21	115	24.4	99
8	0	2.08	121	24.1	107	2.18	117	24.7	103

Appendix Table I.3--Trial II 1970. Average daily intake of feed during each period.

Cow No.		480				501				502			
Period	Trtm.	Hay	Corn sil.	Cons.	DM	Hay	Corn sil.	Cons.	DM	Hay	Corn sil.	Cons.	DM
		kg				kg				kg			
1	0	2.3	4.5	10.0	12.5	1.9	4.5	11.4	13.4	2.3	4.5	12.7	14.9
2	K	2.3	4.5	10.0	12.5	2.3	4.5	11.4	13.8	2.3	4.5	12.7	14.9
3	0	2.3	4.5	10.0	12.5	2.2	4.5	11.4	13.6	2.3	4.5	12.7	14.9
4	G	2.1	4.5	10.0	12.2	2.3	4.5	11.4	13.6	2.3	4.5	10.9	13.1
5	0	2.3	4.5	10.0	12.3	2.2	4.5	11.4	13.5	2.3	4.5	12.0	14.1
6	0	2.3	4.5	10.0	12.5	2.3	4.5	11.4	13.8	2.3	4.5	11.8	14.1
7	K	2.3	4.5	10.0	12.6	2.1	4.5	11.4	13.7	2.3	4.5	12.2	14.6
8	0	2.3	4.5	10.0	12.7	2.3	4.5	11.4	13.9	2.3	4.5	12.7	15.1

Appendix Table I.4--Trial II 1970. Average daily intake of crude protein and estimated net energy during each period; actual values and relative to NRC standards.

Cow No.		480				501				502			
		CP		E-NE		CP		E-NE		CP		E-NE	
Period	Trtm.	Int.	Int/Reg	Int.	Int/Reg	Int.	Int/Reg	Int.	Int/Reg	Int.	Int/Reg	Int.	Int/Reg
		kg	%	Mcal	%	kg	%	Mcal	%	kg	%	Mcal	%
1	0	2.18	143	23.1	114	2.34	159	25.2	127	2.61	151	28.0	124
2	K	2.15	143	23.1	115	2.37	157	25.6	127	2.58	139	28.0	117
3	0	2.14	139	23.0	112	2.35	157	25.5	127	2.57	150	27.9	125
4	G	2.08	139	22.6	114	2.33	161	25.3	129	2.35	132	24.4	109
5	0	2.12	145	22.8	110	2.33	163	25.2	130	2.44	149	26.4	122
6	0	2.76	161	23.2	121	2.50	167	25.8	128	2.57	155	26.5	122
7	K	2.27	156	23.3	119	2.48	171	25.7	131	2.65	158	27.4	124
8	0	2.28	155	23.4	122	2.52	179	26.0	136	2.66	165	28.4	132

Appendix Table I. 5--Trial I 1970. Observations in milk production parameters.

Per	Trtm	Sub-per	Milk yield 501	Milk yield 502	m	Prot. conc.[a] 501	Prot. conc.[a] 502	m	Fat conc.[a] 501	Fat conc.[a] 502	m	SNF conc.[a] 501	SNF conc.[a] 502	m
			kg/day			%			%			%		
1	0	1	19.10	22.60		3.22	3.29		4.1	--[b]		9.40	--	
		2	19.25	22.45		3.37	3.32		--[b]	4.8		--	9.65	
		3	18.95	21.90		3.19	3.26		4.0	4.1		9.05	10.00	
		m	19.10	22.32	20.71	3.262	3.291	3.277	4.07	4.44	4.26	9.22	9.81	9.54
2	K	1	19.00	22.60		3.32	3.41		4.2	3.8		8.80	9.60	
		2	20.25	22.95		3.22	3.58		--[b]	3.9		9.20	9.40	
		3	19.60	22.95		3.34	3.56		3.8	--[b]		9.55	--	
		m	19.62	22.83	21.25	3.293	3.518	3.406	3.96	3.83	3.89	9.19	9.50	9.35
3	0	1	17.65	21.47		3.26	3.35		4.0	3.9		9.45	9.10	
		2	16.80	21.60		3.46	3.37		3.6	4.2		9.55	8.70	
		3	17.35	22.25		3.82	3.22		3.9	4.0		9.65	9.30	
		m	17.27	21.77	19.50	3.512	3.311	3.412	3.84	4.03	3.95	9.55	9.04	9.27
4	G	1	17.25	21.35		3.75	3.07		4.2	3.9		9.20	9.10	
		2	16.55	21.65		3.81	3.43		2.9	3.4		10.30	9.10	
		3	17.25	22.85		3.78	3.50		2.7	3.4		10.20	9.20	
		m	17.02	21.95	19.49	3.781	3.338	3.560	3.27	3.56	3.44	9.90	9.13	9.47
5	0	1	16.00	21.45		3.82	3.52		3.2	3.5		10.00	9.55	
		2	16.00	20.35		3.77	3.54		4.3	2.4		9.30	10.70	
		3	14.80	20.50		3.66	3.34		4.4	--[b]		8.60	--	
		m	15.60	20.77	18.19	3.752	3.467	3.610	3.96	2.98	3.40	9.32	10.14	9.79
6	0	1	16.00	20.75		3.82	3.43		3.2	3.2		10.00	10.10	
		2	16.00	20.30		3.77	3.75		4.3	2.5		9.30	10.40	
		3	14.80	17.80		3.66	3.70		4.4	3.8		8.60	9.85	
		m	15.60	19.62	17.61	3.752	3.623	3.688	3.96	3.14	3.50	9.32	10.12	9.77
7	K	1	17.70	19.75		3.68	3.90		3.8	4.0		9.25	9.30	
		2	17.20	19.95		3.84	3.70		3.8	4.0		9.70	8.50	
		3	15.55	20.45		3.75	3.67		4.4	3.2		9.30	9.70	
		m	16.82	20.05	18.44	3.756	3.756	3.756	3.99	3.73	3.86	9.42	9.17	
8	0	1	15.30	16.75		3.73	3.68		4.5	4.8		9.40	8.65	
		2	14.95	18.60		3.62	3.60		4.8	4.5		9.10	8.80	
		3	14.95	17.70		3.50	3.69		4.4	3.6		8.80	9.25	
		m	15.07	17.68	16.38	3.617	3.655	3.636	4.57	4.29	4.42	9.10	8.90	

[a]Means (m) for each cow period weighted by the yield of milk.

[b]Fat test observation missing.

Appendix Table I.6--Trial I 1970. Differences in milk production parameters between treatment (infusion) and control periods.

Parameter		Cow No. 501 Infusion 1st CAS	GLC	2nd CAS	Cow No. 502 Infusion 1st CAS	GLC	2nd CAS
(1) Milk yield	Trt	19.62	17.02	16.82	22.83	21.95	20.05
kg/day	Ctr	18.19	16.44	15.34	22.05	21.27	18.65
	d	1.43	.58	1.48	.79	.68	1.40
(2) Prot cons	Trt	3.29	3.78	3.76	3.52	3.34	3.76
%	Ctr	3.39	3.63	3.68	3.30	3.39	3.64
	d	-.10	.15	.08	.22	-.05	.12
(3) Fat cons	Trt	3.96	3.27	3.99	3.83	3.56	3.73
%	Ctr	3.95	3.90	4.26	4.23	3.50	3.72
	d	.01	-.63	-.27	-.40	.06	.01
(4) SNF cons	Trt	9.19	9.90	9.42	9.50	9.13	9.17
%	Ctr	9.37	9.43	9.21	9.43	9.57	9.55
	d	-.18	.37	.21	.07	.07	-.38
(5) Prot prod	Trt	646	643	632	803	733	753
g/day	Ctr	614	596	565	727	721	679
	d	32	47	67	76	12	74
(6) Fat prod	Trt	778	557	670	875	782	747
g/day	Ctr	720	640	653	934	748	688
	d	58	-83	17	-59	34	59
(7) SNF prod	Trt	1802	1684	1584	2169	2005	1839
g/day	Ctr	1705	1552	1413	2079	2037	1780
	d	97	132	171	90	-32	59
(8) FCM prod	Trt	19.52	15.16	16.78	22.26	20.51	19.20
kg/day	Ctr	18.08	16.18	15.93	22.83	19.73	17.80
	d	1.44	-1.02	.85	-.57	.78	1.40

Appendix Table I.7--Trial II 1970. Milk production parameters; basic observations.

Per.	Trtm.	Sub-per.	Milk yield 480	501	502	m	Protein conc.[a] 480	501	502	m	Fat conc.[a] 480	501	502	m
			------kg/day-------				--------%-------				-------%-------			
1	0	1	12.03	12.00	17.13		3.64	4.02	3.76		5.1	4.9	4.0	
		2	12.65	11.38	15.60		3.50	3.91	3.82		4.6	4.6	4.0	
		3	12.10	11.47	15.60		3.46	3.80	3.77		4.5	4.7	4.4	
		m	12.26	11.62	16.11	13.33	3.526	3.887	3.780	3.731	4.73	4.74	4.13	4.49
2	K	1	12.77	13.16	19.03		3.58	3.77	3.77		4.1	4.3	4.0	
		2	12.66	13.29	18.30		3.59	3.89	3.79		4.4	3.8	4.0	
		3	12.82	11.84	17.76		3.49	3.82	3.81		4.2	4.7	3.9	
		m	12.75	12.76	18.36	14.62	3.553	3.826	3.790	3.723	4.23	4.25	3.97	4.13
3	0	1	12.53	11.65	15.99		3.45	3.82	3.73		4.4	4.9	4.1	
		2	12.97	12.40	17.12		3.46	3.79	3.65		4.5	4.6	4.1	
		3	12.77	11.90	14.76		3.38	3.92	3.71		4.6	4.1	4.2	
		m	12.76	11.98	15.96	13.57	3.431	3.844	3.691	3.655	4.50	4.53	4.13	4.36
4	G	1	12.86	12.73	17.92		3.41	3.83	3.46		4.4	4.2	3.8	
		2	13.07	12.50	17.97		3.41	3.79	3.52		4.1	3.8	3.4	
		3	11.95	12.02	17.54		3.68	3.97	3.85		4.3	4.2	3.0	
		m	12.63	12.48	17.81	14.28	3.498	3.860	3.608	3.655	4.27	4.07	3.40	3.85
5	0	1	10.77	11.84	16.12		3.65	3.80	3.95		4.4	4.2	3.6	
		2	12.40	12.25	14.38		3.60	3.92	3.95		4.4	3.6	4.6	
		3	11.98	11.64	14.77		3.60	3.95	4.00		4.7	4.6	4.0	
		m	11.72	11.91	15.10	12.91	3.613	3.890	3.967	3.823	4.50	4.12	4.05	4.21
6	0	1	11.75	11.64	14.77		3.68	3.95	4.00		4.2[a]	4.6	4.0	
		2	10.86	11.43	14.20		3.47	3.95	4.02		--	4.8	3.8	
		3	10.89	11.93	16.33		3.75	3.90	4.02		4.5	4.8	4.0	
		m	11.17	11.67	15.10	12.64	3.633	3.934	4.018	3.862	4.33	4.73	3.94	4.30
7	K	1	10.61	12.17	16.02		3.75	4.03	3.95		4.5	4.5[a]	3.8[a]	
		2	11.75	11.72	15.06		3.81	3.93	3.97		4.5	--	--	
		3	11.76	10.84	15.24		3.86	4.05	3.81		4.7	4.6	4.4	
		m	11.37	11.58	15.44	12.80	3.813	4.005	3.910	3.909	4.57	4.53	4.13	4.38
8	0	1	11.20	11.12	14.56		3.65	3.91	3.87		5.0	4.9	4.3	
		2	11.88	10.12	12.77		3.58	3.85	3.80		4.9	4.6	4.7	
		3	11.25	10.25	14.62		3.60	3.81	3.70		4.3	4.9	4.2	
		m	11.44	10.50	13.98	11.97	3.615	3.862	3.79	3.756	4.74	4.81	4.39	4.62

[a]Means (m) for each cow period weighted by yield of milk.

[b]Fat test observation missing.

Appendix Table I.7--Continued.

Per.	Trtm.	Subp.	SNF conc.[a] 480	501	502	m
			----------%---------			
1	0	1	9.04	9.20	9.40	
		2	9.24	9.50	9.62	
		3	9.37	9.44	9.55	
		m	9.22	9.38	9.52	9.39
2	K	1	9.66	9.34	9.48	
		2	9.38	9.95	9.40	
		3	9.46	9.50	9.76	
		m	9.50	9.60	9.54	9.55
3	0	1	9.26	9.40	9.70	
		2	9.21	9.58	9.59	
		3	9.18	10.22	9.66	
		m	9.22	9.72	9.65	9.54
4	G	1	9.25	9.70	9.55	
		]	9.37	9.75	9.40	
		2	9.37	9.60	9.50	
		m	9.33	9.69	9.48	9.50
5	0	1	9.15	9.40	9.26	
		2	9.37	--[a]	9.15	
		3	9.48	9.87	9.77	
		m	9.34	9.62	9.55	9.51
6	0	1	9.37	--[b]	10.10	
		2	9.48	9.03	9.70	
		3	9.17	9.61	9.50	
		m	9.34	9.51	9.75	9.56
7	K	1	9.50	9.50	9.70	
		2	9.55	--[b]	--[b]	
		3	9.72	9.60	9.37	
		m	9.59	9.55	9.56	9.56
8	0	1	9.42	9.68	9.60	
		2	9.40	9.97	9.43	
		3	9.46	9.39	9.45	
		m	9.43	9.68	9.50	9.53

Per.	Trtm.	Production of milk constituents 480	501	502	m
		---protein, g/day--			
1	0	432	450	609	497
2	K	453	488	696	546
3	0	438	461	590	496
4	G	441	479	643	521
5	0	423	463	599	495
6	0	406	459	606	490
7	K	434	464	604	501
8	0	413	405	530	449
		----fat, g/day-----			
1	0	580	550	665	598
2	K	539	542	729	603
3	0	574	543	659	592
4	G	539	505	606	550
5	0	528	491	611	543
6	0	484	552	595	544
7	K	520	525	637	561
8	0	542	504	613	553
		----SNF, g/day-----			
1	0	1130	1089	1534	1251
2	K	1212	1226	1752	1397
3	0	1176	1166	1540	1294
4	G	1178	1203	1689	1357
5	0	1094	1146	1441	1227
6	0	1043	1109	1474	1209
7	K	1091	1105	1476	1224
8	0	1079	1016	1328	1141
		----FCM, kg/day-----			
1	0	13.6	12.9	16.4	14.3
2	K	13.2	13.2	18.3	14.9
3	0	13.7	12.9	16.3	14.3
4	G	13.1	12.5	16.2	14.0
5	0	12.6	12.1	15.2	13.3
6	0	11.7	13.0	15.0	13.2
7	K	12.4	12.5	15.7	13.5
8	0	12.7	11.8	14.8	13.1

[a]Means (m) for each cow period weighted by the yield of milk.

[b]Fat test observation missing.

Appendix Table I.8--Trial II 1970. Differences between treatment (infusion) and control periods in milk production parameters.

(row)	Parameter		Cow No. 480 Infusion Study First Casein	Cow No. 480 Infusion Study First Glucose	Cow No. 480 Infusion Study Second Casein	Cow No. 501 Infusion Study First Casein	Cow No. 501 Infusion Study First Glucose	Cow No. 501 Infusion Study Second Casein	Cow No. 502 Infusion Study First Casein	Cow No. 502 Infusion Study First Glucose	Cow No. 502 Infusion Study Second Casein
(1)	Milk yield	Trt	12.75	12.63	11.37	12.76	12.42	11.58	18.36	17.81	15.44
	kg/day	Ctr	12.51	12.24	11.31	11.80	11.95	11.09	16.04	15.53	14.54
		d	.24	.39	.06	.96	.47	.49	2.32	2.29	.80
(2)	Prot. conc.	Trt	3.55	3.50	3.81	3.83	3.86	4.01	3.79	3.61	3.91
	%	Ctr	3.48	3.52	3.62	3.86	3.87	3.90	3.74	3.83	3.90
		d	.07	-.02	.19	-.03	-.01	.11	.05	-.22	.01
(3)	Est.True	Trt	3.33	3.35	3.66	3.61	3.69	3.78	3.56	3.43	3.71
	Prot.[a] conc.	Ctr	3.27	3.33	3.41	3.63	3.65	3.68	3.53	3.62	3.70
	%	d	.06	.02	.25	-.02	.04	.10	.03	-.19	.01
(4)	Fat conc.	Trt	4.23	4.27	4.57	4.25	4.07	4.53	3.97	3.40	4.13
	%	Ctr	4.62	4.50	4.53	4.63	4.33	4.77	4.13	4.09	4.16
		d	-.39	-.23	.04	-.38	-.26	-.24	-.16	-.69	-.03
(5)	SNF conc.	Trt	9.51	9.33	9.60	9.61	9.69	9.55	9.54	9.48	9.56
	%	Ctr	9.22	9.27	9.38	9.56	9.68	9.58	9.58	9.60	9.63
		d	.29	.06	.22	.05	.01	-.03	-.04	-.12	.07
(6)	Prot. prod.	Trt	453	441	434	488	479	464	696	643	604
	g/day	Ctr	435	431	410	455	462	432	599	595	568
		d	18	10	24	33	17	32	97	48	36
(7)	Est. True	Trt	424	422	416	461	458	437	654	611	574
	Prot. prod.	Ctr	408	407	386	428	436	409	567	633	539
	g/day	d	16	15	30	33	22	28	84	-22	35
(8)	Fat prod.	Trt	539	539	520	542	505	525	729	606	637
	g/day	Ctr	577	551	513	547	517	528	662	635	604
		d	-38	-12	7	-5	-12	-3	67	-29	33
(9)	SNF prod.	Trt	1212	1178	1091	1226	1203	1105	1752	1689	1476
	g/day	Ctr	1153	1135	1061	1128	1156	1063	1537	1491	1401
		d	59	43	31	98	47	42	115	198	75
(10)	FCM prod.	Trt	13.19	13.14	12.35	13.23	12.54	12.51	18.28	16.21	15.73
	kg/day	Ctr	13.66	13.16	12.22	12.92	12.54	12.36	16.35	15.74	14.88
		d	-.47	-.04	.13	.31	.00	.15	1.93	.47	.85

[a] (Total Kjeldahl N-NPN) x 6.38.

Appendix Table I.9--Trial II 1970. Non Protein Nitrogen (NPN) concentration in milk.

Parameter	Per	Trtm	Cow No. 480	501	502	Total mean±SE
			----------mg/100ml----------			
Non Protein Nitrogen (NPN)	1	0	34.1	37.7	30.5	34.1 ± 2.08
	2	K	35.0	34.1	35.9	35.0 ± 0.52
	3	0	33.3	35.0	32.4	33.6 ± 0.76
	4	G	23.4	26.9	28.7	26.3 ± 1.56
	5	0	28.7	32.3	31.4	30.8 ± 1.08
	6	0	31.4	35.0	36.7	34.1 ± 1.56
	7	K	23.4	35.9	31.5	30.3 ± 3.66
	8	0	35.0	32.2	26.1	31.1 ± 2.63
						C.V.=12.5
			----------%			
NPN as a fraction of total N	1	0	6.2	6.2	5.1	5.83 ± .36
	2	K	6.3	5.7	6.0	6.00 ± .17
	3	0	6.2	5.8	5.6	5.87 ± .17
	4	G	4.3	4.4	5.0	4.57 ± .22
	5	0	5.1	5.3	5.0	5.13 ± .08
	6	0	5.5	5.7	5.8	5.67 ± .08
	7	K	3.9	5.7	5.1	4.90 ± .53
	8	0	6.2	5.3	4.4	5.30 ± .52
						C.V.=12.4

Appendix Table I.10--Trial II 1970. Concentration of urea N in blood plasma, mg/100ml.

Per.	Trt.	Day	Cow No. 480 AM	PM	aver.	Cow No. 501 AM	PM	aver.	Cow No. 502 AM	PM	aver.
1	0	8-2	17.6	23.5	20.55	14.6	19.8	17.20	15.4	19.7	17.55
		8-4	19.3	20.6	19.95	15.5	17.4	16.45	14.6	19.0	16.80
		8-6	17.5	22.7	20.10	14.4	20.2	17.30	14.2	16.2	15.20
		m	18.13	22.27	20.20	14.83	19.13	16.98	14.73	18.30	16.52
2	K	8-9	18.9	24.6	21.75	12.8	20.3	16.55	16.8	24.9	20.85
		8-11	18.2	28.5	23.35	12.6	26.4	19.50	16.2	30.0	23.10
		8-13	22.2	30.0	26.10	14.5	25.6	20.05	19.6	27.2	23.40
		m	19.77	27.70	23.73	13.30	24.10	18.70	17.53	27.37	22.45
3	0	8-14	22.5	25.8	24.15	17.6	24.0	20.80	17.2	21.2	19.20
		8-18	18.1	18.4	18.25	15.8	14.8	15.30	15.9	15.3	15.60
		m	20.30	22.10	21.20	16.70	19.40	18.05	16.55	18.25	17.40
4	G	8-20	9.3	12.3	10.80	9.3	13.3	11.30	6.7	9.5	8.10
		8-22	13.8	9.2	11.50	10.8	13.0	11.90	8.9	5.3	7.10
		8-24	9.9	8.9	9.40	9.6	8.0	8.80	9.2	6.8	8.00
		m	11.00	10.13	10.57	9.90	11.43	10.67	8.27	7.20	7.73
5	0	8-26	11.6	14.6	13.10	13.9	8.7	11.30	6.8	20.0	13.40
		8-28	15.0	12.9	13.95	14.8	11.4	13.10	8.5	9.9	9.20
		8-30	15.1	14.0	14.55	11.9	13.0	12.45	16.0	10.7	13.35
		m	13.90	13.83	13.87	13.53	11.03	12.28	10.43	13.53	11.98
6	0	9-2	15.3	19.1	17.20	12.6	16.0	14.30	14.4	19.6	17.00
		9-6	14.4	18.8	16.60	15.8	20.6	18.20	14.5	20.1	17.30
		m	14.85	18.95	16.90	14.20	18.3	16.25	14.45	19.85	17.15
7	K	9-8	13.3	15.4	14.35	13.7	23.9	18.80	12.6	21.0	16.80
		9-10	12.2	18.7	15.45	15.9	23.3	19.60	14.5	21.8	18.15
		m	12.75	17.05	14.90	14.80	23.6	19.20	13.55	21.40	17.48
8	0	9-14	7.9	11.9	9.90	10.0	17.5	13.75	12.3	20.0	16.15
		9-16	9.0	7.5	8.25	13.2	18.9	16.05	11.7	22.0	16.85
		m	8.45	9.70	9.08	11.60	18.20	14.90	12.00	21.00	16.50

Appendix Table I.11--Trial II 1970. α amino N in blood plasma, μM/ml.

Per.	Trt.	Day	Cow No. 480 AM	PM	aver.	day	Cow No. 501 AM	PM	aver.	day	Cow No. 502 AM	PM	aver.
1	0	8-2	2.234	2.534	2.384		2.000	2.291	2.146		2.050	2.516	2.283
		8-4	2.238	2.437	2.338		2.181	2.565	2.373		2.315	2.083	2.199
		m	2.236	2.486	2.361		2.091	2.428	2.260		2.183	2.300	2.241
2	K	8-9	2.294	2.115	2.205		2.343	2.196	2.270		2.150	1.977	2.064
		8-11	3.190	2.872	3.031		3.010[a]	2.867	2.939		3.167[a]	3.045	3.106
		m	2.274	2.494	2.618		2.677	2.532	2.604		2.659	2.511	2.585
3	0	8-15	3.295	2.208	2.752		2.207	2.352	2.280		2.634	2.373	2.504
		8-18	2.990	2.260	2.625		3.771[a]	2.331	3.051		3.482[a]	2.188	2.835
		m	3.143	2.234	2.689		2.989	2.342	2.666		3.058	2.281	2.670
4	G	8-21	2.445	2.319	2.382		2.186	1.847	2.017		1.885	2.472	2.179
		8-23	2.310	2.450	2.380		2.394	1.853	2.124		2.050	1.505	1.778
		8-25	2.330	1.992	2.161		2.363	2.331	2.347		2.472	2.123	2.298
		m	2.362	2.254	2.308		2.314	2.010	2.162		2.136	2.035	2.086
5	0	8-27	2.092	2.195	2.144		2.321	2.043	2.182		2.687	2.594	2.641
		8-29	2.971	2.542	2.757		2.495	2.040	2.268		2.392	2.170	2.281
		8-31	2.470	2.053	2.262		2.078	2.048	2.063		1.828	1.728	1.919
		m	2.511	2.263	2.387		2.298	2.044	2.171		2.302	2.164	2.233
6	0	9-4	2.365	1.676	2.021	9-2	2.396	1.950	2.173	9-2	2.347	2.460	2.404
		9-7	2.432	1.773	2.103	9-4	2.464	1.946[b]	2.205	9-4	2.450	1.999[b]	2.225
		m	2.398	1.725	2.062		2.430	1.948	2.189		2.399	2.230	2.315
7	K	9-9	2.126	2.510	2.318	9-5	2.202	1.816	2.009	9-5	1.843	2.080	1.962
		9-11	2.843	2.862	2.853	9-7	2.470	2.110[b]	2.290	9-7	2.353	1.836[b]	2.095
		9-14	2.514	2.176	2.345	9-10	2.716[c]	2.654[b]	2.685	9-10	2.424	2.730[b]	2.577
		m	2.523	2.516	2.520		2.463	2.193	2.328		2.207	2.215	2.211
8	0	9-17	1.840	1.945	1.893	9-14	2.544	2.026	2.285	9-14	2.582	2.153	2.368
		9-20	1.934	1.999	1.967	9-17	2.163	2.073	2.118	9-17	1.624	1.955	1.790
		m	1.887	1.972	1.930		2.354	2.050	2.202		2.103	2.054	2.079

[a]Probably erronously high values due to incomplete deproteiniation in the assay.

[b]Average of two samples, one before and one after the AM value listed, but not parallel AM value for the two PM samples.

[c]Sampled morning after (12 hours) infusion stopped.

Appendix Table I.12--Trial I & II 1970. Analysis of variance for milk production parameters.

Infusion Study:		1st Casein			Glucose			2nd Casein			dCAS.vs.dGLC.		
Parameter	Source of Variance	SS	DF	Test of sign.	SS	DF	Test of sign.	SS	DF	Test of sign.	SS	DF	Test of sign.
Milk yield (kg)	Cows	149.5337	4		119.4019	4		92.4331	4		3.17814	4	
	Trtm	3.4164	1	P<.05	1.9492	1	P<.10	1.8879	1	P<0.5	.03969	1	NS
	Error	1.1866	4		1.2526	4		0.7243	4		.71441	4	
	Total	154.1367	9		122.6037	9		95.0453	9		3.93224	9	
Protein conc (%)	Cows	0.38665	4		.30719	4		.11579	4		.037267	4	
	Trtm	.00473	1	NS	.00237	1		.02406	1	P<.05	.025553	1	NS
	Error	.02806	4		.03475	4		.00892	4		.055022	4	
	Total	.41944	9		.34431	9		.14877	9		.117822	9	
Fat conc (5)	Cows	.43032	4		1.15841	4		1.11277	4		.143243	4	
	Trtm	.17450	1	P<.05	.30520	1	P<.10	.02520	1	(P<.25)	.069806	1	NS
	Error	.06728	4		.18817	4		.04232	4		.259507	4	
	Total	.67210	9		1.65178	9		1.18029	9		.472556	9	
Protein production (g)	Cows	141,901.1	4		114,415.6	4		115,786.8	4		2,369.00	4	
	Trtm	6,451.6	1	P<.05	1,849.6	1	P<.05	5,475.6	1	P<.01	1,166.40	1	P=.10
	Error	2,240.9	4		732.4	4		1,004.2	4		1,119.85	4	
	Total	150,593.6	9		116,997.6	9		122,266.6	9		4,665.25	9	
Fat production (g)	Cows	176,376.5	4		76,547.0	4		59,882.2	4		8,104.91	4	
	Trtm	55.2	1	NS	1,040.4	1	NS	1,292.6	1	P=.10	2,222.41	1	NS
	Error	6,343.9	4		3,546.6	4		1,197.2	4		2,915.56	4	
	Total	182,775.6	9		81,134.0	9		62,379.0	9		13,242.88	9	
FCM (kg)	Cows	125.606	4		71.633	4		55.345	4		1.3862	4	
	Trtm	0.702	1	NS	0.002	1	NS	1.056	1	P<.05	0.7840	1	NS
	Error	2.476	4		1.839	4		0.495	4		4.1768	4	
	Total	128.784	9		73.474	9		56.896	9		4.1768	9	

Appendix Table I.12--Continued.

Infusion Study:		1st Casein			Glucose			2nd Casein			dCAS.vs.dGLC.		
Parameter	Source of Variance	SS	DF	Test of sign.	SS	DF	Test of sign.	SS	DF	Test of sign.	SS	DF	Test of sign.
SNF cons. (%)	Cows	.133693	4		.245644	4		.124039	4			4	
	Trtm	.003055	1	NS	.000118	1	NS	.000513	1	NS		1	NS
	Error	.059439	4		.210075	4		.115959	4			4	
	Total	.196187	9		.455837	9		.240511	9			9	
SNF production (g)	Cows	1,301,503.4	4		1,025,523.6	4		764,333.4	4	[b]		4	
	Trtm	31,379.1	1	P<.025	15,173.4	1	(P<.25)	14,276.3	1			1	P<.05
	Error	7,233.6	4		15,793.9	4		6,284.2	4			4	
	Total	1,340,116.1	9		1,056,490.9	9		784,893.9	9			9	
NPN cons (mg%) (Tr. II only)	Cows	2.416	2		12.711	2		41.610	2		44.530	2	
	Trtm	2.042	1	NS	51.333	1	P<.05	9.127	1	NS	47.602	1	P<.05
	Error	11.235	2		5.448	2		41.543	2		4.923	2	
	Total	15.693	5		69.492	5		92.280	5		97.055	5	
NPN/T.N Tr.II only on transformed data	Cows	.5200	2		0.0850	2		1.006	2		0.5842	2	
	Trtm	.0520	1	NS	2.2571	1	P<.10	0.928	1	NS	2.0184	1	P<.10
	Error	.3500	2		0.5433	2		2.509	2		0.2548	2	
	Total	.9220	5		2.8854			4.444	5		2.8574	5	
True protein production (g) Trial II only	Cows	32,679.25	2		44,104.08	2		28,511.33	2	[a]	40,585.58	2	
	Trtm	1,683.38	1	P<.10	3,151.04	1	NS	1,536.00	1		77.04	1	NS
	Error	273.25	2		1,383.08	2		25.00	2		215.58	2	
	Total	34,635.88	5		48,638.20	5		30,511.33	5		40,878.20 and values	5	
	Cows	.11421	2		.11185	2		.04377	2		.035,536	2	
	Trtm	.00091	1	NS	.00322	1	NS	.02042	1	NS	.020,651	1	NS
	Error	.00189	2		.01772	2		.01556	2		.012,007	2	
	Total	.11701	5		.13279	5		.07975	5		.068,194	5	

[a] P<.05.

[b] P<.01.

Appendix Table I.13--Trial II 1970. Analysis of variance for blood plasma parameters.

Study:		1st Casein			Glucose			2nd Casein			Gluc. vs. Cas.		
	Source of Variance	SS	DF	Test sign.[a]	SS	DF	Test sign.[a]	SS	DF	Test sign.[a]	SS	DF	Test sign.[a]
Urea N (mg/100ml)	Cows	47.540	2		24.232	2		43.431	2		0.799	2	
	Trtm	44.836	2	(P<.05)	265.231	2	P≈.01	49.192	2		439.692	2	(P<.01)
	I vs. O	41.926	1	(P<.05)	150.839	1	P≈.01				380.640	1	(P<.01)
	O_1vs. O_2	2.911	1		114.392	1	P<.05				59.052	1	
	Error a	12.469	4		7.464	4		37.244	4		56.378	4	
	Bleed hr	121.576	1	(P<.001)	2.219	1		146.776	1	P .001	133.989	1	(P<.001)
	B×C	42.208	2		4.263	2		4.522	2		7.970	2	
	B×T	1.345	2	P<.01	0.722	2		14.630	2		75.231	2	P<.001
	Error b	3.944	4		9.561	4		7.300	4		1.884	4	
	Total	273.918	17		313.691	17		303.095	17		715.942	17	
α amino N (μM/ml)	Cows	.06200	2		.0909	2		.01455	2		.14443	2	
	Trtm	.44251	2	(P<.05)	.0185	1		.24211	1		.08433	1	P<.025
	I vs. O	.22142	1	(P<.05)									
	O_1vs. O_2	.22110	1	(P<.05)									
	Error a	.05739	4		.0094	2		.22094	4		.00369	2	
	Bleed hr	.98795	1	(P<.001)	.1108	1	P<.025	.19241	1	P<.05	.05095	1	(P<.01)
	B×C	.00383	2		.0129	2		.05979	2		.03688	2	P<.025
	B×T	.24302	2	P<.05	.0012	1		.12377	2		.00497	1	
	Error b	.03400	4		.0046	2		.06770	4		.00080	2	
	Total	1.82686	17		.2483	11		.92127	17		.32605	11	

[a]Brackets around the probability value means it is obscured by interaction between bleeding and treatment.

Appendix Table II.1--Trial I 1971. Feed composition and estimated net energy values.

Item	Period: 1	2	3	4	5	6	7	Average
	----------%----------							
Hay								
Dry matter	41.2	38.8	38.5	40.9	37.9	40.0	40.0	39.6
Crude prot in DM	9.9	11.7	11.0	10.3	11.6	10.1	9.8	10.6
Crude fiber in DM	19.7	17.5	19.3	18.3	18.0	19.7	17.0	18.5
Corn Silage[a]								
Dry matter	87.3	86.3	87.5	87.0	82.8	82.9	87.1	85.4
Crude prot in DM	14.7	14.9	16.9	12.5	17.0	18.8	20.4	16.5
Crude fiber in DM	30.1	36.5	26.7	37.3	35.3	26.2	25.5	31.1
Concentrate[b]								
Dry matter	88.6	89.6	89.6	89.7	89.7	90.4	89.2	89.5
Crude prot in DM	15.7	17.8	15.2	14.1	14.8	15.0	15.3	15.4
DM in feed refusals								
Hay	80.5	81.0	80.8	78.4	73.6	76.0	80.5	78.7
Corn Silage[c]			the same as fed					39.6
Concentrate[c]			one value used throughout					80.5
	----------Mcal/kgDM----------							
Est. Net En. Lact.								
Hay[d]	1.25	1.20	1.30	1.15	1.20	1.35	1.40	1.26
Corn Silage[d]			the same for all periods					1.70
Concentrate[e]			"					2.02

[a]Urea added at ensiling, 0.5%.

[b]Concentrate mixture; % ingredients: ground shelled corn 52.5, oats 23.9, soybean meal (50) 12.8, molasses 7.2, urea 1.0, TM salts 1.0, dical. phosph. 1.4, gypsum 0.2; added per kg: 6600 U vit A, 2200 U vit D.

[c]Refusals of concentrate and corn silage were usually mixed; only for concentrate was DM occasionally determined on clean material.

[d]Estimated on basis of CP and CF content from NRC (1971) feed tables.

[e]Calculated from values assigned to the ingredients (NRC 1971).

Appendix Table II.2--Trial I 1971. Feed offered, and consumed dry matter (DM), estimated net energy for lactation ($E\text{-}NE_L$) and crude protein (CP) for each cow in each period.

Item	Period 1	2	3	4	5	6	7
	Feed Offered, kg/day[a]						
Hay	5.4	5.4	5.4	5.4	5.4	5.4	5.4
Corn Silage	9.0	8.5	9.0	9.0	9.0	9.0	9.0
Concentrate	11.3	11.3	11.3	11.3	11.3	11.3	11.3
	Intakes, DM and CPkg/day, ENE_LMcal/day						
No. 604 Trtm.		G		M		K	
DM Hay	4.0	4.7	4.8	4.7	4.5	4.5	4.7
C.S.	1.5	1.4	1.6	2.0	1.8	2.0	3.3
C.tr.	10.0	9.7	10.1	10.1	10.1	10.2	10.1
DM Total	15.5	15.8	16.4	16.8	16.4	16.7	18.1
CP Total	2.31	2.40	2.41	2.15	2.39	2.46	2.80
$E\text{-}NE_L$ Total	27.8	26.0	28.5	28.6	28.3	29.2	32.4
No. 606 Trtm.		K		G		M	
DM Hay	4.4	4.6	4.6	4.6	4.5	4.4	4.6
C.S.	1.8	1.7	1.6	1.4	0.8	1.0	2.1
C.tr.	9.6	8.5	9.7	9.1	9.4	9.9	9.3
DM Total	15.8	14.8	15.9	15.1	14.7	15.3	16.0
CP Total	2.32	2.41	2.42	1.99	2.24	2.46	2.55
$E\text{-}NE_L$ Total	27.8	25.7	28.2	26.0	25.8	28.1	28.7
No. 607 Trtm.		M		K		G	
DM Hay	4.4	3.3	4.0	4.1	3.9	4.3	4.4
C.S.	2.9	2.5	2.4	2.3	2.4	2.1	2.6
C.tr.	10.0	10.2	10.2	10.2	9.9	9.8	9.9
DM Total	17.3	16.0	16.6	16.6	16.2	16.2	16.9
CP Total	2.51	2.58	2.48	2.19	2.41	2.49	2.66
$E\text{-}NE_L$ Total	30.7	28.6	29.7	29.3	28.7	29.2	30.5
No. 603 Trtm.[b]						K	
DM Hay					4.5	4.5	4.7
C.S.					3.2	3.0	3.4
C.tr.					8.1	8.2	8.1
DM Total					15.8	15.7	16.2
CP Total					2.34	2.38	2.53
$E\text{-}NE_L$ Total					27.2	27.7	28.7

[a]For cows No. 604, 606 and 607.

[b]Cow No. 603 received only the K treatment. Her daily feed was 5.4kg hay, 9.0kg corn silage and 9.1kg concentrate.

Appendix Table II.3--Trial I 1971. Feed intake in treatment periods and control periods averaged.

Trtm.	Cow No. 604	606	607	m[a]	Cow No. 604	606	607	m[a]	Cow No. 604	606	607	m[a]
	---DM, kg/day---			(N=3)	---CP, kg/day---			(N=3)	---E-NE_L, Mcal/day---			(N=3)
K	16.1	14.9	16.6	15.9	2.46	2.41	2.19	2.55	29.2	25.7	29.3	28.0
O_K	16.9	15.8	16.4	16.4	2.60	2.37	2.44	2.47	30.4	28.0	29.2	29.2
G	14.5	15.1	16.2	15.3	2.40	1.99	2.49	2.29	26.1	26.0	29.2	27.1
O_G	15.7	15.3	16.5	15.8	2.36	2.33	2.53	2.41	28.1	27.0	29.6	28.2
M	16.2	15.4	15.9	15.8	2.15	2.46	2.58	2.40	28.6	28.1	28.6	28.4
O_M	15.8	15.4	16.9	16.0	2.40	2.40	2.49	2.44	28.4	27.2	30.2	28.6
				(N=9)				(N=9)				(N=9)
T	15.6	15.1	16.2	15.7±.23	2.34	2.29	2.42	2.35±.06	28.0	26.6	29.0	27.8±.50
O	16.1	15.5	16.6	16.1±.21	2.45	2.37	2.49	2.44±.03	29.0	27.4	29.7	28.7±.41

	Cow No. 603[b]		
	DM, kg/day	CP, kg/day	E-NE_L, kg/day
K	15.7	2.38	27.7
O_K	16.0	2.44	28.0

[a]Mean includes cows No. 604, 606 and 607 only. Standard errors (±) are shown for the overall treatments (T) and controls (O) (N=9).

[b]Cow No. 603 received only treatment K.

Appendix Table II.4--Trial I 1971. Intakes of estimated net energy and crude protein relative (%) to NRC (1971) standards.

Trtm.	Cow No. 604	606	607	603	Mean[a]
	Crude Protein intake (%)				
K	101[d]	107	86	126	106
O_K	106	108	103	135	103
G	103	95	112		109
O_G	100	108	119		104
M	90[d]	117	105		98
O_M	98	119	101		106
	Estimated NE_L intake (%)				
K	117	108	110	137	117
O_K	120	121	116	142	119
G	108	118	124		119
O_G	114	118	129		120
M	119	126	111		112
O_M	112	127	115		118

Paramtr.	Period No.[a] 1	2	3	4	5	6	7	All	
	O[b]	T[c]	O	T	O	T	O	O	T
CP (%)	103	105	102	90	103	110	125	107	119
E-NE_L (%)	118	109	115	116	116	122	135	102	116

[a]Cow No. 603 (received only the K trtm.) not included.

[b]O = control; saline infusion.

[c]T = treatment; substrat infusion.

[d]In period 5 (after M, before K) the CP intake was 96 (%).

Appendix Table II.5--Trial I 1971. Observations in milk production parameters.

Item:		(1)					(2)				
Cow:		604	606	607	603	Mean[a]	604	606	607	603	Mean[a,b]
Per.	Subper.	Milk Yield					Protein (N×6.38) concentration				
1		----------kg/day----------					----------%----------				
	1	26.04	22.14	23.95			2.85	2.74	3.15		
	2	24.48	22.65	24.60			2.72	3.04	2.88		
	m	25.26	22.40	24.28		23.98	2.787	2.891	3.013		2.90
2		G	K	M			G	K	M		
	1	26.72	23.69	25.96			3.04	3.00	3.35		
	2	25.80	25.07	26.14			2.92	2.89	3.39		
	m	26.26	24.38	26.05		25.56	3.000	2.943	3.370		3.10
3											
	1	25.13	23.81	24.49			2.80	2.69	3.20		
	2	27.15	23.31	24.53			2.93	2.74	3.10		
	m	26.14	23.56	24.51		24.74	2.87	2.715	3.150		2.91
4		M	G	K			M	G	K		
	1	27.84	23.54	26.11			3.09	2.82	3.32		
	2	28.35	23.27	25.56			3.10	2.83	3.37		
	m	28.04	23.43	25.89		25.79	3.094	2.824	3.340		3.09
5	1	27.88	22.89	21.89	17.28		3.11	2.89	3.14	3.07	
	2	27.78	22.85	21.88	17.96		3.09	2.96	3.20	2.92	
	m	27.83	22.87	21.88	17.62	24.19	3.100	2.925	3.170	2.992	3.07
6		K	M	G	K		K	M	G	K	
	1	27.46	23.20	23.20	19.82		3.34	3.04	3.29	3.32	
	2	28.20	22.59	20.52	19.02		3.25	3.05	3.27	3.21	
	m	27.83	22.90	21.86	19.46	24.20	3.294	3.045	3.280	3.266	3.20
7											
	1	25.90	21.61	19.41	17.69		2.92	2.88	3.23	3.07	
	2	26.28	21.39	20.14	16.75		3.02	2.93	3.23	3.06	
	m	26.09	21.50	19.78	17.22	22.46	2.970	2.906	3.230	3.065	3.04

[a]Cow No. 603 not included in the means.

[b]Period mean for each cow weighted by the amount of milk in subperiods.

Appendix Table II.5--Continued.

Item		(3)					(4)				
Cow:		604	606	607	603	Mean[a,b]	604	606	607	603	Mean[a,b]
Per.	Subper.	NPN concentration[3]					Fat concentration				
1		----------mg/100ml----------					----------%----------				
	1						3.0	3.0	2.9		
	2	20.05	18.35	19.20			3.1	2.9	3.1		
	m	20.05	18.35	19.20		19.20	3.05	2.95	3.00		3.00
2		G	K	M			G	K	M		
	1	--	24.60	24.50			2.8	2.9	2.7		
	2	23.05	24.00	23.65			2.7	2.6	2.5		
	m	23.05	24.29	24.07		23.80	2.75	2.75	2.60		2.70
3	1	19.35	18.35	21.65			3.5	3.2	3.0		
	2	19.70	19.35	21.85			2.9	2.7	3.5		
	m	19.53	18.84	21.75		20.04	3.19	2.95	3.25		3.13
4		M	G	K			M	G	K		
	1	23.60	17.45	27.60			2.4	2.3	2.7		
	2	23.95	19.55	28.60			2.3	2.3	3.1		
	m	23.82	18.47	28.04		23.44	2.35	2.30	2.89		2.51
5	1	22.05	18.20	23.90	31.35		3.2	2.5	2.8	2.2	
	2	23.65	22.40	22.70	31.70		2.6	2.5	2.9	2.0	
	m	22.85	20.30	23.30		22.15	2.90	2.50	2.85	2.10	2.75
6		K	M	G	K		K	M	G	K	
	1	30.08	30.60	28.46	39.80		2.6	2.8	2.7	2.5	
	2	29.45	26.80	25.70	--		2.9	2.3	2.7	2.3	
	m	29.76	28.72	27.16	39.80	28.55	2.75	2.55	2.70	2.40	2.67
7	1	24.00	24.95	24.40	--		3.3	2.3	2.6	2.6	
	2	24.70	26.00	24.80	31.20		3.2	2.2q	2.5	2.5	
	m	24.35	25.47	24.60	31.20	24.81	3.25	2.25	2.55	2.55	2.68

Appendix II.5--Continued.

Item				(5)					(6)		
Cow:		604	606	607	603	Mean[a,b]	604	606	607	603	Mean[a,b]
Per.	Subper.	--------Lactose conc., %----------					---------SNF conc., %----------				
	1						8.32	8.30	8.65		
	2						8.40	7.93	8.20		
1	m[c]						8.36	8.11	8.42		8.30
							G	K	M		
	1	4.80	--[c]	5.01			8.50	8.20	9.15		
	2	4.56	4.82	5.03			8.38	8.24	8.89		
2	m	4.68	4.82	5.02		4.84	8.44	8.22	9.02		8.56
	1	5.16	5.35	4.57			8.25	7.90	8.73		
	2	5.04	5.08	5.62			8.25	7.88	8.70		8.29
3	m	5.10	5.22	5.10		5.14	8.25	7.89	8.72		
		M	G	K							
	1	5.14	4.62	5.11			8.45	8.10	8.74		
	2	4.99	5.24	4.80			8.56	8.04	8.35		
4	m	5.07	4.92	4.95		4.98	8.52	8.06	8.53		8.37
	1	5.12	4.86	4.57	5.24		8.42	8.02	8.62	8.37	
	2	5.00	4.80	5.03	5.06		8.64	8.18	8.63	8.56	
5	m	5.06	4.83	4.80	5.15	4.90	8.53	8.10	8.63	8.47	8.42
		K	M	G	K		K	M	G	K	
	1	4.79	4.76	4.86	4.88		8.61	8.21	8.93	8.60	
	2	4.87	4.67	5.03	4.86		8.70	8.26	8.96	8.83	
6	m	4.83	4.72	4.94	4.87	4.83	8.66	8.23	8.78	8.70	8.56
	1	5.08	4.93	4.95	5.03		8.78	8.14	8.94	8.72	
	2	4.89	4.93	5.02	5.13		8.50	8.02	8.70	8.70	
7	m	4.98	4.93	4.98	5.08	4.96	8.64	8.08	8.82	8.71	8.51

[c]Assayed values discarded, disparting unacceptably from the common values and wide variation between parallels.

Appendix Table II.5--Continued.

Item:				(7)					(8)		
Cow:		604	606	607	603	Mean[a]	604	606	607	603	Mean[a]
Per.	Subper.	Protein Production					Est. True Prot. Prod.				
1		----------g/day----------					----------g/day----------				
	1	742	607	754							
	2	666	689	709							
	m	704	648	732		695	672	621	702		665
2		G	K	M			G	K	M		
	1	812	711	870							
	2	753	725	886							
	m	788	718	878		795	744	680	839		754
3	1	704	641	784							
	2	796	639	760							
	m	750	640	772		721	718	609	734		687
4		M	G	K			M	G	K		
	1	860	664	867							
	2	878	659	861							
	m	869	662	865		799	826	637	822		762
5	1	867	662	687	531						
	2	858	676	700	524						
	m	863	669	694	528	742	823	639	661	492	708
6		K	M	G	K		K	M	G	K	
	1	917	705	763	658						
	2	917	689	671	611						
	m	917	697	717	634	777	864	655	680	586	733
7	1	756	622	627	543						
	2	794	627	654	513						
	m	775	625	639	528	680	734	583	608	494	642

[a]Cow No. 603 not included in the means.

Appendix Table II.6--Trial I 1971. Concentrations of urea nitrogen in blood (BUN).

Sequence	Period[a]	Bleeding (B)	Cow 604	606	607	603	Total period, Total seq. (the three first cows) m_p	m_s
			mg/100ml					
	Trtm		G	K	M			
1	2T	1	25	19	27		23.7	
		2	13	22	32		22.3	
		3	20	39	28		29.0	
		all	19.3	26.7	29.0		25.0	
			O_G	O_K	O_M			
	3O	1	15	16	17		16.0	
		2	15	21	19		18.3	
		3	13	14	15		14.0	
		all	14.3	17.0	17.0		16.1	20.6
			M	G	K			
2	4T	1	30	21	29		26.7	
		2	21	16	34		23.7	
		3	37	14	18		23.0	
		all	29.3	17.0	27.0		24.4	
			O_M	O_G	O_K	O		
	5O	1	27	33	34	30	31.3	
		2	24	25	32	32	27.0	
		3	14	27	[21][b]	31	(20.5)	
		all	21.7	28.3	(33.0)	31	26.3	25.4
			K	M	G	K		
3	6T	1	21	34	20	[29][b]	25.0	
		2	37	23	27	25	29.0	
		3	11	[23][b]	17	14	(14.0)	
		all	23.0	(28.5)	21.3	(19.5)	22.7	
	7O		O_K	O_M	O_G	O		
		1	30	18	28	35	25.3	
		2	22	20	12	31	18.0	
		3	12	19	14	16	15.0	
		all	21.3	19.0	18.0	27.3	19.4	21.6
Total for cow		m_c	21.1	22.4	23.7	26.7		

[a]Samples for period 1 lost in storage.

[b][ ]: stipulated according to Cochran and Cox (1957, p. 125), used for for statistical analyses, not included in mean (m).

Appendix Table II.7--Trial I 1971. Concentrations of glucose in blood plasma.

Trtm. Sequence	Period[a]	Bleeding (B)	Cow 604	606	607	603	Total period (the three first cows) m_p	Total seq. cows) m_s
			mg/100ml					
1	Trtm.		G	K	M			
	2T	1	68.7	66.0	65.3		66.7	
		2	69.4	66.8	65.3		67.2	
		3	74.0	72.7	73.2		73.3	
		all	70.7	68.5	67.9		69.0	
	3O		O_G	O_K	O_M			
		1	63.8	63.8	64.4		64.0	
		2	67.6	63.8	66.0		65.8	
		3	67.1	64.0	62.5		64.5	
		all	66.2	63.9	64.3		64.8	66.9
			M	G	K			
2	4T	1	72.2	74.8	65.5		70.0	
		2	74.5	67.2	61.7		67.8	
		3	66.5	58.5	73.7		66.2	
		all	69.3	68.6	58.4		68.3	
			O_M	O_G	O_K	O		
	5O	1	67.0	71.0	64.2	65.8	67.4	
		2	71.0	65.7	53.0	60.5	63.2	
		3	70.0	69.2	58.0	60.8	65.7	
		all	69.3	68.6	58.4	62.4	65.5	66.9
			K	M	G	K		
3	6T	1	71.5	71.1	72.3	68.4	71.6	
		2	66.2	67.2	58.5	55.0	64.0	
		3	71.5	69.0	58.8	62.6	66.4	
		all	69.7	69.1	63.2	62.0	67.3	
	7O		O_K	O_M	O_G	O		
		1	70.5	65.5	69.0	65.2	68.3	
		2	69.8	67.2	64.0	61.0	67.0	
		3	67.5	70.4	57.2	67.5	65.0	
		all	69.3	67.7	64.2	64.6	66.8	67.1
Total for cow		m_c	69.4	67.4	64.0			

[a]Samples for period 1 lost in storage.

Appendix Table II.8a--Trial I 1971. Layout of ANOVA I-1; Latin Square design applied to estimated treatment responses (d_T).

Cow:	604	606	607
Period	Treatments and estimated treatment responses (d_T)[a]		
2	G	K	M
1+3/2	O_G	O_K	O_M
(d_T)	d_G	d_K	d_M
4	M	G	K
2+5/2	O_M	O_G	O_K
(d_T)	d_M	d_G	d_K
6	K	M	G
5+7/2	O_K	O_M	O_K
(d_T)	d_K	d_M	d_G

Table II.8b--Anova I-1.

Source of Variance	Degrees of freedom (symbol)	No.	
Cows	(c-1)	2	
Periods	(p-1)	2	
Treatments	(t-1)	2	
Orthogonal contrasts			
d_G vs. $d_M d_K$			1
d_K vs. d_M			1
Error (rest)	(r-1)(r-2)	2	
Total	(r^2-1)	8	

[a]Symbols
c=3, for cows (c).
p=3, for period (P) sequences.
t=3, for treatment (T) vs. control (O) differences.
r=3, for the square side units.

Appendix Table II.9a--Trial I 1971. Layout for comparison of each infusion treatment to adjacent controls (ANOVA I-2).

Cow:	604			606			607		
				Bleeding hours					
Period	0	3	9	0	3	9	0	3	9
1		O_{G1}			O_{K1}			O_{M1}	
2		G			K			M	
3		O_{G2}			O_{K2}			O_{M2}	
3		O_{M1}			O_{G1}			O_{K1}	
4		M			G			K	
5		O_{M2}			O_{G2}			O_{K2}	
5		O_{K1}			O_{M1}			O_{G1}	
6		K			M			G	
7		O_{K2}			O_{M2}			O_{G2}	

Appendix Table II.9b--Periods for each cow employed in the AOV of the respective infusion treatment studies (ANOVA I-2).

Study:	K			G			M		
Cow									
604	5	6	7	1	2	3	3	4	5
606	1	2	3	3	4	5	5	6	7
607	3	4	5	5	6	7	1	2	3

Appendix Table II.9c--Example of a complete set of plots utilized in ANOVA I-2; the G (glucose) infusion study.

Cow	604			606			607		
				Bleeding hours					
	0	3	9	0	3	9	0	3	9
Infusion				period of infusion treatment					
O_{G1}		1			3			5	
G		2			4			6	
O_{G2}		3			5			7	

Appendix Table II.9d--Trial I 1971. Anova I-2: sources of variance and degree of freedom.

Source of Variation	Degrees of freedom symbols	No.	
a) Whole plot (between cows and infusions)	(ct-1)	8	
Cows	(c-1)	2	
Infusion trtm.	(t-1)	2	
Orthogonal contrasts			
T vs. O			1
O_1 vs. O_2			1
Error a (rest)	(c-1)(t-1)	4	
b) Split plot (bleeding times within cows and infusions)	(ctb-1)-(ct-1)	18	
Bleeding times orthogonal contrasts	(b-1)	2	
B_1 vs. B_2 & B_3			1
B_2 vs. B_3			1
interactions			
B × C	(b-1)(c-1)	4	
B × T	(b-1)(t-1)	4	
Error b (B×C×T, rest)	(b-1(c-1)(t-1)	8	
Total		26	

Appendix Table II.10a--Trial I 1971. Layout for ANOVA I-3.

Cow:		604			606			607		
		Bleeding hour No.								
Trtm. Sequence	Pers.	1	2	3	1	2	3	1	2	3
		Infusion Treatment								
1	2	G			K			M		
	1+3/2	O_G			O_K			O_M		
2	4	M			G			K		
	3+4/2	O_M			O_G			O_K		
3	6	K			M			G		
	5+7/2	O_K			O_M			O_G		

Appendix Table II.10b--ANOVA I-3.

Source of Variance	Degrees of freedom symbols	No.		F-test MS
a) Whole plots	(cst-1)	17		
Cows	(c-1		2	
Sequences	(s-1)		2	
Treatments	(t-1		5	
orth contrasts				
T vs. O			1	
Betw. O-s			2	
Betw. T-s			2	
KM vs. G				1
K vs. M				1
Error a (residual)			8	
b) Split plot	(bcst-1)-(cst-1)	36		
Bleedings	(b-1)		2	
orth contrasts				
B_1 vs. B_2B_3			1	
B_2 vs. B_3			1	
B×C		4		
B×S		4		
B×T		10		
Error b (residual)		16		

Appendix Table II.11a--Trial I 1971. Statistical analyses for feed intake parameters, Anova I-1.

Var. Source	DF	MS[a] DM Intake (Kg/day)	F	F sign.	MS CP Intake (kg/day)	F	F sign.	MS E-NE_L Intake (Mcal/day)	F	F sign.
Par Mt.										
Cows	2	.02781			.00166			.09023		
Pers.	2	1.03448	7.3		.08929	20.1	P< .05	2.99320	5.3	
Trtm.	2	.11443	<1		.00745			.96053	1.7	
K_M vs.G	1	.09386			.00445			.49999		
K vs.M	1	.13500			.01049			1.42107		
Error	2	.14117			.00445			.56250		
Total (SS)	8	(2.63576)			(.20573)			(9.21000)		

[a]Total SS in brackets to be discerned from MS for sources of variance.

Appendix Table II.11b--Trial I 1971. Statistical analysis for feed intake parameters, Anova I-2.[a]

Parameter Source of var.	DF	Casein Inf. (K) MS[b]	F	F sign.	Glucose inf. (G) MS	F	F sign.	Mixture inf. (M) MS	F	F sign.
DM Intake (kg/day)										
Cows		1.24000			1.44333			1.00811		
Trtm.		.58333	1.2		.30333	<1		.05445	<1	
T vs.O		.49999			.60499			.06722		
betw O's		.66664			.00016			.04167		
Error (rest)		.49334			.31667			.91552		
Total (SS)		(6.0200)			(4.76000)			(4.10223)		
CP Intake (kg/day)										
Cows		.03221			.06903			.03204		
Trtm.		.02974	1.4		.01563	<1		.00674	<1	
T vs.O		.02722			.02644			.00222		
betw O's		.03226			.00482			.01126		
Error (rest)		.02215			.02517			.02113		
Total (SS)		(.21249)			(.27000)			(.16209)		
ENE Intake (Mcal/day)										
Cows		6.004			6.281			3.431		
Trtm.		2.268	~1		1.401	~1		.275	<1	
T vs.O		2.494			2.801			.067		
betw O's		2.042			.001			.482		
Error (rest)		2.216			1.450			1.598		
Total (SS)		(25.409)			(21.163)			(13.803)		

[a]Only F for treatments presented for sake of simplification.

[b]Total SS in brackets to discern from MS for sources of variance.

Appendix Table II.12--Trial I 1971. Statistical analyses for milk production parameters, Anova I-1.[a]

Var. Source	DF	MS[b]	F	F sign.	MS	F	F sign.	MS	F	F sign.
Parmt.		Milk yield (kg)			FCM Prod. (kg)			Prot. cons. (%)		
Cows	2	.96160			2.53453			.020630		
Pers.	2	.16623			.88573			.003033		
Trtm.	2	.83223	6.6	<.25	.99023	<1		.011233	<1	
KM vs.G	1	1.26404	10.0	<.10	.52280			.022049		
K vs.M	1	.40042	3.2		1.46027			.000417		
Error	2	.12654			1.61561			.019904		
Total (SS)	8	(4.17320)			(12.05220)			(.109600)		
Parmt.		Prot. Prod. (g)			NPN Cons. (mg%)			NPN/Total N (%)		
Cows	2	2629.4			.3408			.02321		
Pers.	2	383.5			5.9404			.38148		
Trtm.	2	2935.5	9.1	<.10	12.0692	3.9		.19548	<1	
KM vs.G	1	5066.9	15.8	<.10	19.3857	6.3		.15494		
K vs.M	1	726.0	2.3		4.7526	1.5		.23602		
Error	2	320.8			3.0657			.33888		
Total (SS)	8	(12537.3)			(42.8318)			(1.87809)		
Parmt.		Est. True Prot. cons(%)			Est. true prot. prod. (g)			Fat cons. (%)		
Cows	2	.005621			2331.9			.07675		
Pers.	2	.004700			298.0			.12435		
Trtm.	2	.008122	1.6		2438.2	8.0		.01055	<1	
KM vs.G	1	.016140	3.2		4411.7	14.5		.00067		
K vs.M	1	.000104	<1		464.6	1.5		.02042		
Error	2	.005055			305.1			.07124		
Total (SS)	8	(.046955)			(10746.3)			(.56576)		

[a]For simplicity, only F for treatments are presented.

[b]SS for total in brackets to discern from MS for sources of variance.

Appendix Table II.13--Trial I 1971. Statistical analysis for milk parameters, Anova I-2.[a]

Parameter / Source of Var.	DF	Casein infusion (K) MS	F	F sign.	Glucose infusion (G) MS	F	F sign.	Mixture infusion (M) MS	F	F sign.
Milk Yield (kg)										
Cows	2	12.424			16.725			18.110		
Trtms.	2	3.598	2.8		.666	~1		1.329	2.2	
T vs. O	1	5.478			.724			2.607		
Betw. O's	1	1.717			.551			.050		
Error (rest)	4	1.276			.611			.611		
Total (SS)[b]	8	(37.146)			(37.214)			(41.522)		
FCM Prod. (kg)										
Cows	2	9.7655			19.5886			22.2642		
Trtms.	2	.8790	<1		1.2505	<1		.2544	<1	
T vs O	1	.5067			.5033			.4672		
Betw.O's	1	1.2512			2.0068			.0416		
Error (rest)	4	1.8848			1.5897			1.4181		
Total (SS)	8	(28.8278)			(48.0461)			(50.7095)		
Prot. Cons. (%)										
Cows	2	.11034			.14262			.03826		
Trtms.	2	.04406	11.4	<.025	.01755	4.3	<.10	.04130	11.4	<.025
T vs. O	1	.07450	16.9	<.025	.01458	3.6		.06207	16.9	<.025
Betw. O's	1	.01363	3.1		.02042	5.1	<.10	.02053	3.1	
Error (rest)	4	.00385			.00404			.00722		
Total (SS)	8	(.32419)			(.33652)			(.18800)		
Prot. Prod. (g)										
Cows	2	25941.2			6481.3			22295.2		
Trtms.	2	12786.6	17.0	<.025	1620.1	1.6		7296.9	3.5	
T vs. O	1	20550.4	27.3	<.01	3173.4	3.2		12624.6	6.0	<.10
Betw. O's	1	5022.8	6.7		66.7	<1		1969.3	<1	
Error (rest)	4	752.6			1000.9			2103.8		
Total (SS)	8	(79466.2)			(20206.3)			(67599.3)		

[a]Only F for treatments presented for sake of simplicity.

[b]SS for total in brackets to discern from MS for variance sources.

Appendix Table II.13--Continued.

Parameter / Source of Var.	DF	Casein infusion (K) MS	F	F sign.	Glucose infusion (G) MS	F	F sign.	Mixture infusion (M) MS	F	F sign.
Est. True Prot. Cons. (%)										
Cows	2	.03493			.12162			.04657		
Trtms.	2	.09626	8.1	<.05	.01547	5.7	<.10	.02979	3.4	
T vs. O	1	.05413	12.6	<.025	.01091	4.0		.05078	5.7	<.10
Betw. O's	1	.01571	<1		.02007	7.4	<.10	.00882	~1	
Error (rest)	4	.00430			.00273			.00890		
Total (SS)	8	(.27955)			(.28510)			(.18831)		
Est. True Prot. Prod. (g)										
Cows	2	22026.7			5558.5			22502.3		
Trtms.	2	10888.4	15.3	<.025	1324.2	1.6		5931.6	2.7	
T vs. O	1	16701.8	23.5	<.01	2563.3	3.0		10775.1	4.9	<.10
Betw O's	1	5075.0	7.1	<.10	85.1	<1		1008.1	<1	
Error (rest)	4	711.4			851.7			2191.9		
Total (SS)	8	(68675.8)			(17172.3)			(65635.5)		
NPN Cons. (%)										
Cows	2	21.642			25.989			51.710		
Trtms.	2	34.555	272.1	<.001	3.343	1.3		16.174	5.9	<.10
T vs. O	1	67.009	527.0	<.001	6.396	2.6		.195	<1	
Betw. O's	1	2.100	16.5	<.025	.789	<1		15.980	11.6	<.05
Error (rest)	4	.127			2.419			1.375		
Total (SS)	8	(112.901)			(68.338)			(73.384)		
NPN/Tot.N(%)[c]										
Cows	2	.41053			.89105			.70935		
Trtms.	2	1.03903	35.7	<.01	.25943	<1		.52528	4.1	≈.10
T vs. O	1	1.77347	61.0	≈.001	.41404	1.3		.48166	3.8	
Betw. O's	1	.30459	10.5	≈.025	.10482	<1		.56890	4.4	<.10
Error (rest)	4	.02907			.31091			.12840		
Total (SS)	8	(3.01560)			(3.54460)			(2.98283)		

[c]AOV on values obtained by aresin transformation (Rohlf & Sohal, 1969, p. 129).

Appendix Table II.14--Trial I. 1971. Statistical analysis for milk production parameters, Anova I-3.[a]

Source of Var.	DF	MS[b]	F	F sign	MS	F	F sign	MS	F	F sign	MS	F	F sign	MS	F	F sign
Parameter:		Milk Yield (kg)			Protein cons. (%)			Fat cons. (%)			NPN Cons. (mg%)			Est. Tru Prot.Cons. (%)		
Cows	2	25.226			.18684			.11441			7.999			.17161		
Seqs.	2	3.322			.02410			.06221			32.422			.01507		
Trtms.	5	3.387	4.7	<.05	.02344	11.4	<.005	.11227		<.10	19.658	9.7	<.005	.02309	9.2	<.005
T vs. O	1	5.813	8.1	<.025	.09828	38.2	<.001	.35843		<.025	67.590	33.3	<.001	.07894	31.4	<.001
Betw O's	2	1.464	2.0		.00250	~1		.03124			.187	<1		.00166	<1	
Betw T's	2	2.096	2.9		.02180	8.5	<.01	.07023			15.153	7.5	<.025	.01660	6.6	<.025
KMvs.G	1	7.987	11.1	~.01	.04300	16.7	<.005	.00845			25.300	12.5	<.01	.03294	13.1	<.01
K vs.M	1	.205	<1		.00600	<1		.13210		<.10	5.005	2.5		.00026	<1	
Error(rest)	8	.717			.00257			.03467			2.029			.00251		
Total (SS)	17	(79.751)			(.58936)			(1.19171)			(195.355)			(.50893)		
		FCM Production (kg)			Protein Prod. (g)			Fat Prod. (g)			NPN/Total N (%)[c]			Est. True Prot.Prod.(g)		
Cows	2	24.118			35025.7			34152.5			.07038			31494.4		
Seqs.	2	4.165			670.1			8554.1			2.40722			987.1		
Trtms.	5	2.709	2.5		10148.5	8.1	<.01	4486.4			.73543	3.0		8528.0	7.1	<.01
T vs. O	1				24346.9	19.4	<.005	7560.4			3.22540	13.0	<.01	20207.2	16.8	<.005
Betw O's	2				2624.8	2.1		3906.7			.19818	<1		2213.3	1.8	
Betw T's	2				10573.0	8.4	<.025	10258.6		<.10	.02858	<1		9003.0	7.5	<.025
KM vs.G	1				20604.5	16.4	<.005	9076.5						17659.5	14.7	<.005
K vs.M	1				541.5	<1		11440.7						346.6	<1	
Error(rest)	8	1.026			1255.9			2906.2		<.10	.24844			1202.4		
Total (SS)	17	(78.320)			(132181.1)			(154553.4)			(10.72185)			(117221.6)		

[a]Only F for treatment presented for sake of simplification.

[b]Total SS in brackets so as to discern from MS for sources of variance.

[c]ANOVA on values obtained by arcsin transformation (Rolf and Sokal, 1969, p. 129).

Appendix Table II.15--Trial I 1971. Statistical analysis for blood parameters, Anova I-3.

Parameter:	Blood urea N (mg/100ml)				Plasmaglucose (mg/100ml)			
Source of Variance	DF	MS	F	F sign.	DF	MS	F	F sign.
a) Whole plots	17				17			
Cows	2	17.055			2	131.760		
Seqs.	2	117.166			2	.019		
Infs.	5	126.077	2.7	~.01	5	33.111	2.5	
Contrasts								
T vs. O	1	188.907	4.1	<.10	1	87.911	6.7	<.05
amg.O	2	24.481	<1		2	13.775	~1	
amg.T	2	196.259	4.3	<.10	2	25.055	~2	
KM vs.G	1	357.796	7.8	<.05	1			
K vs.M	1	34.722	<1		1			
Error a(rest)	8	46.084			8	13.111		
b) Split plots	34				36			
Bleedgs.	2	111.722	6.0	<.025	2	24.221	2.1	
Contrasts								
B_1 vs. B_2&B_3	1	126.750	6.6	<.025	1	38.521	3.4	
B_2 vs. B_3	1	96.696	5.3	<.05	1	9.923	<1	
B C	4	34.111			4	14.310	1.3	
B S	4	170.830	9.4	<.001	4	40.821	3.6	~.05
B I	10	36.655			10	21.406	1.9	
Error b(rest)[a]	14	18.160			16	11.442		
Total (SS)	51	(2935.500)			53	1200.435		

[a]Error b for blood urea N has only 14 DF because 2 plots were missing.

Appendix Table II.16--Trial II 1971. Amounts of feed offered and consumed, and total dry matter intake.

Cow/Period	Fed all	Consumed 1	2	3	4
	kg	kg	kg	kg	kg
607 Trtm.		0	L	M	H
Hay	6.8	5.7	6.1	6.8	6.2
Cons.	11.3	10.2	10.5	10.2	7.5
DM Total		14.9	14.6	15.0	12.3
604 Trtm.		L	O	H	M
Hay	6.8	5.1	5.5	6.3	5.1
Cons.	11.3	9.3	10.5	8.4	8.5
DM Total		12.8	14.1	13.2	12.4
603 Trtm.		M	H	O	L
Hay	6.8	6.5	5.3	6.3	4.2
Cons.	9.1	6.2	6.4	8.1	6.1
DM Total		11.3	10.5	12.8	10.0
606 Trtm.		H	M	L	O
Hay	6.8	6.6	6.8	6.6	5.6
Cons.	11.3	5.8	5.2	8.2	7.7
DM Total		11.1	10.8	13.3	12.2

Appendix Table II.17--Trial II 1971. Feed composition and estimated net energy values.

Period	1	2	3	4	Average feed/refusal
Hay					
Dry matter, %	84.7	85.7	86.9	87.6	86.2 / 80
crude prot. in DM,%	18.0	13.2	17.7	17.3	16.6
Crude fiber in DM, %	28.4	35.5	28.8	30.9	30.9
Est. Net Energy, Mcal/kg[a]	1.25	1.15	1.25	1.20	1.21
Concentrate[b]					
Dry matter, %	88.8	88.3	89.0	88.5	88.7 / 82
crude prot. in DM, %	16.7	15.5	16.1	15.1	
Est.Net Energy, Mcal/kg DM[c]	the same value used throughout				2.02

[a]Estimated out from CP and CF content using NRC (1971) feed tables.

[b]For ingredients of the concentrate mixture see Table II.1, footnote b.

[c]Calculated from values assigned to the ingredients (NRC 1971) as for trial I 1971.

Appendix Table II.18--Trial II 1971. Milk yield and concentration of milk constituents.

Period	607	604	603	606	
1 Trtm.	O	L	M	H	m
Milk yield, kg.	17.99	20.61	15.27	16.83	17.68
N cons., %	.50893	.52163	.52465	.47210	
NPN cons., %	.03005	.03279	.04024	.03420	
ETP cons., %	3.055	3.119	3.091	2.794	
Fat Cons., %	2.97	3.13	2.73	2.90	
Lactose cons., %	4.92	4.75	4.52	4.61	
SNF cons. %[a]	---	---	---	---	
2 Trtm.	L	O	H	M	
Milk yield, kg.	17.27	21.15	16.60	14.45	17.37
N cons., %	.55125	.53966	.56082	.47571	
NPN cons. %	.03196	.02674	.03948	.02859	
ETP cons., %	3.313	3.272	3.326	2.849	
Fat Cons., %	3.00	3.00	2.70	3.30	
Lactose cons. %	4.77	4.87	4.65	4.79	
SNF conc. %	8.83	8.68	8.75	7.97	
3 Trtm.	M	H	O	L	
Milk yield, kg	16.93	21.94	16.76	17.22	18.21
N cons., %	.57476	.59357	.51411	.48871	
NPN cons., %	.03478	.03993	.03030	.02867	
ETP cons., %	3.445	3.523	3.087	2.935	
Fat cons., %	3.00	2.94	2.57	3.15	
Lactose cons., %	4.95	4.79	4.55	4.83	
SNF cons., %	9.36	8.83	8.80	8.59	
4 Trtm.	H	M	L	O	
Milk yield, kg	16.57	21.62	16.33	16.33	17.68
N cons., %	.58244	.54216	.53135	.47226	
NPN cons., %	.03952	.03515	.02954	.02019	
ETP cons., %	3.464	3.235	3.202	2.884	
Fat cons., %	3.03	3.13	2.10	3.20	
Lactose cons., %	4.96	4.79	4.66	4.77	
SNF cons., %	9.40	9.09	9.30	8.53	

[a]Observation missing period 1.

Appendix Table II.19--Trial II 1971. Milk production parameters: period observations for each cow and treatment, and time period means.

Trtm./Cow	603	604	606	607	Treatment means	Period (No.) means
Milk yield				kg/day		
O	16.8	21.2	16.3	18.0	18.1	(1) 17.7
L	16.3	20.6	17.2	17.3	17.9	(2) 17.4
M	15.3	21.6	14.5	16.9	17.1	(3) 18.2
H	16.6	21.9	16.8	16.5	18.0	(4) 17.7
Protein conc.				%		
O	3.28	3.44	3.01	3.25	3.25	(1) 3.23
L	3.39	3.33	3.12	3.52	3.34	(2) 3.39
M	3.34	3.46	3.03	3.67	3.38	(3) 3.47
H	3.58	3.79	3.01	3.72	3.53	(4) 3.40
Protein prod.				g/day		
O	549	728	492	584	588	(1) 572
L	553	685	529	608	594	(2) 592
M	510	748	438	621	579	(3) 632
H	594	830	507	616	637	(4) 602
NPN conc.				mg%		
O	30.3	26.7	20.2	30.1	26.8	(1) 34.3
L	29.5	32.8	28.7	32.0	30.7	(2) 31.7
M	40.2	35.2	28.6	34.8	34.7	(3) 33.4
H	39.5	39.9	34.2	39.5	38.3	(4) 31.1
ETP conc.				%		
O	3.09	3.27	2.88	3.06	3.07	(1) 3.01
L	3.20	3.12	2.94	3.31	3.14	(2) 3.19
M	3.09	3.24	2.85	3.45	3.16	(3) 3.25
H	3.33	3.53	2.79	3.46	3.28	(4) 3.20
ETP prod.				g/day		
O	517	692	471	550	558	(1) 534
L	523	643	505	572	561	(2) 557
M	472	699	412	583	541	(3) 595
H	552	775	470	574	592	(4) 567
Fat Conc.				%		
O	2.57	3.00	3.20	2.97	2.94	(1) 2.93
L	2.10	3.13	3.15	3.00	2.85	(2) 3.00
M	2.73	3.13	3.30	3.00	3.04	(3) 2.92
H	2.70	2.94	2.90	3.03	2.89	(4) 2.87
Lactose conc.				%		
O	4.95	4.87	4.66	4.62	4.78	(1) 4.70
L	4.96	4.75	4.55	4.79	4.76	(2) 4.77
M	4.92	4.79	4.65	4.83	4.80	(3) 4.78
H	4.77	4.79	4.52	4.77	4.71	(4) 4.80

Appendix Table II.20--Trial II 1971. Concentrations of blood urea nitrogen, mg/100ml.[a]

Period	Bleeding	607	604	603	606	m_b	m_p[b]
1 Trtm.		O	L	M	H		
	1	24	34	26	32	29.0	
	2	30	39	41	33	35.8	32.4
	3	--[c]	35	59	20	38.0	
2 Trtm.		L	O	H	M		
	1	21	44	55	36	39.0	
	2	43	18	44	25	32.5	35.7
	3	--	--	--	--		
3 Trtm.		M	H	O	L		
	1	53	44	19	31	36.8	
	2	38	40	38	34	37.5	37.2
	3	46	49	36	43	43.5	
4 Trtm.		H	M	L	O		
	1	59	37	37	25	39.5	
	2	55	45	41	22	40.8	40.2
	3	59	38	--	27	41.3	
Cow m		40.4	37.6	37.6	29.8		

[a]Because so many plots are missing of bleeding 3 the statistical analyses employed bleeding 1 and 2 only.

[b]B_1 & B_2 only behind the period and cow means.

[c]Plot observation missing.

Appendix Table II.21--Trial II 1971. Concentrations of blood plasma glucose, mg/100ml.

Period	Bleeding	Cow 607	604	603	606	m
1 Trtm.		O	L	M	H	
	1	69.6	72.5	68.0	68.8	69.7
	2	67.1	70.4	63.5	65.3	66.6
	3	68.4[a]	69.3	69.3	68.8	69.0
	m	68.3	70.7	66.9	67.3	68.4
2 Trtm.		L	O	H	M	
	1	66.5	72.2	70.0	65.6	68.6
	2	65.1	71.7	66.7	63.5	66.8
	3	67.5	70.3	70.5	67.0	68.8
	m	66.4	71.4	69.1	65.4	68.1
3 Trtm.		M	H	O	L	
	1	75.2	75.2	74.0	78.0	75.6
	2	63.5	73.0	70.0	69.0	68.9
	3	66.5	71.5	71.0	70.0	69.8
	m	68.4	73.2	71.7	72.3	71.4
4 Trtm.		H	M	L	O	
	1	66.0	66.0	64.0	65.0	65.3
	2	68.2	67.2	65.0	65.2	66.4
	3	59.0	65.0	66.3	65.8	64.3
	m	64.4	66.1	65.1	65.3	65.2
Cow total m		66.7	70.4	68.2	67.7	

[a]Missing observation; the value estimated by formulas of Cochran and Cox (1956), p. 125, employing data for bleeding 3 only. The value not included in AOV.

Appendix Table II.22--Trial II 1971. Layout of ANOVA II.

Source of Variance	Degrees of freedom symbol	No.
a) The Latin Square	r^2-1	15
Cows	c-1	3
Periods	p-1	3
Treatments	t-1	3
orth. contrasts		
K vs. O		1
L vs. MH		1
M vs. H		1
Error a (rest)	(r-1)(r-2)	6
b) Within LSQ plots	$(br^2-1)-(r^2-1)$	32
Bleedings	b-1	2
orth. contrasts		
B_1 vs. B_2B_3		1
B_2 vs. B_3		1
BxT	(b-1)(t-1)	6
BxP	(b-1)(p-1)	6
BxC	(b-1)(c-1)	6
Error b (rest)		12
Total	(br^2-1)	47

Appendix Table II.23--Trial II 1971. Statistical analysis for feed intake parameters.

Source of Variance	DF	MS	F	F sign.	MS	F	F sign.	MS	F	F sign.
A. Trial II		DM Intake (kg/day)			CP Intake (kg/day)			ENE Intake (Mcal/day)		
Cows	3	7.4270	27.4		.18686	26.0		30.1233	25.4	
Pers.	3	2.3558	8.7		.19310	26.8		8.1303	6.9	
Inf.Trtm.	3	2.0839	7.7	<.025	.05430	7.6	<.025	9.6224	8.1	<.025
O vs. K	1	4.6128	17.0	<.025	.12710	17.7	<.01	20.5408	17.3	<.01
L vs. MH	1	.9720	3.6		.02220	3.1		6.3860	5.4	<.10
M vs. H	1	.6730	2.5		.01361	1.9		1.9405	1.6	
Error	6	.2707			.00719			1.1859		
Total (SS)	15	(37.2246)			(1.34595)			(150.7432)		

Appendix Table II.24--Trial II 1971. Statistical analyses for milk production parameters.

Source of Variance	DF	MS	F	F sign.	MS	F	F sign.	MS	F	F sign.
Parameter:		Milk Yield (kg/day)			Fat Cons (%)			Prot. Cons. (%)		
Cows	3	23.7716			.30149			.206842		
Pers.	3	.4933			.01246			.038742		
Inf.Trtm.	3	.8148	1.2		.00277	<1		.054092	10.2	
O vs. K	1							.085008	15.8	
L vs. MH	1							.032267	6.0	
M vs. H	1							.045000	8.3	
Error(rest)	6	.7061			.04184			.005358		
Total (SS)	15	(79.4740)			1.27684			(.931175)		
Parameter:		Prot. Prod. (g/day)			NPN Cons. (mg/100ml)			NPN/Tot. N(%)		
Cows	3	47940.7			40.671			.4863		
Pers.	3	2561.1			8.933			.7576		
Inf. Trtm.	3	2632.1	3.6	<.10	98.030	29.9	<.001	2.2281	15.6	<.005
O vs. K	1	678.0	<1		180.226	54.9	<.001	4.3440	30.5	<.005
L vs. MH	1	427.7	<1		113.864	34.7	<.005	2.0242	14.2	<.01
M vs. H	1	6675.9	9.0	<.025	88.052	26.8	<.005	.3160	2.2	
Error(rest)	6	739.6			3.282			.1426		
Total (SS)	15	(163839.4)			(462.591)			(10.7852)		
Parameter:		Est. True Prot. (%)			Est. True Prot. (g/day)			Lactose Cons. (%)		
Cows	3	.172139			4173.61			.064691		
Pers.	3	.041783			2600.51			.007088		
Inf.Trtm.	3	.029043	5.1	<.05	1842.57	2.7		.005088	<1	
O vs. K	1	.041478	7.3	<.05						
L vs. MH	1	.014900	2.6							
M vs. H	1	.030752	5.4	<.10						
Error(rest)	6	.005697			671.90			.006225		
Total	15	(.763.082)			(142680.83)			(.267975)		

Appendix Table II.25--Trial II 1971. Statistical analysis for blood parameters.

	Blood urea N. conc. (mg/100ml)				Plasma glucose conc. (mg/100ml)			
	DF	MS	F	F sign.	DF	MS	F	F sign.
a) Whole plot	15				15			
Cows	3	168.031			3	26.615		
Pers.	3	82.698			3	76.763		
Inf. Trtms.	3	429.281	8.2	<.025	3	14.282	6.7	<.025
O vs. K	1	834.261	15.9	<.01	1	13.444	6.3	<.05
L vs. MH	1	221.021	4.2		1	7.933	3.7	
M vs. H	1	232.562	4.4		1	21.470	10.0	<.025
Error (a)	6	52.447			6	2.147		
b) Split plot	16				31			
Bleedgs.[a]		2.531	<1		2	29.627	7.8	<.025
B_1 vs. B_2&B_3					1	54.904	14.4	<.01
B_2 vs. B_3					1	4.350	1.1	
BxC	3	49.115	<1		6	6.242	1.6	
BxP	3	59.115	<1		6	15.133	4.0	<.05
BxT	3	61.865	<1		6	2.478	<1	
Error (b)	6	114.947			11[b]	3.821		
	31	(3557.219)			46	(610.270)		

[a]The third blood sampling time (B_3) dropped because several samples spoiled in assay.

[b]Reduction of DF error by one because one plot estimated.

Appendix Table II.26--Trial III 1971. Ingredients in concentrate mixtures.

	Mixture A (D-122)	Mixture B (D-123)
	---------%----------	[a]
Ground shelled corn	50.2	63.5
Oats	24.0	24.0
Urea	0	2.4
Soybean meal (50%)	16.9	1.2
Molasses (cane)	7.2	7.2
Trace min. salt	0.5	0.5
Dicalcium phosphate	1.0	1.0
Sodium sulfate	0.2	0.2
Added per kg		
Vitamin A	4400 IU	4400 IU
Vitamin D	2200 IU	2200 IU

[a]On wet weight basis as mixed. Urea was 2.74% of dry matter (87.5%) in mixture B.

Appendix Table II.27--Trial III 1971. Feed composition and estimated net energy values by periods.

Feed	Seqs.	Per.	DM	CP in dry matter	CF	ENE_L[a]
			%	%	%	Mcal/kg
Hay	1	1	85.0	16.7	30.8	1.30
		2	85.6	17.7	29.5	1.30
		3	83.8	15.6	34.0	1.25
	2	4	84.7	15.8	33.1	1.25
		5	84.0	18.0	34.4	1.25
		6	84.7	18.9	28.3	1.35
Corn Silage	1[b]	1	34.2	14.2	22.6	1.7
		2	33.1	14.2	20.4	1.7
		3	34.9	14.2	21.7	1.7
	2[c]	4	34.6	14.2	18.6	1.7
		5	33.2	14.2	19.8	1.7
		6	32.7	14.2	20.9	1.7
Concentrate mixture A; without urea (D-122)	1	1	88.0	17.6		2.05
		2	87.9	17.6		2.05
		3	88.3	17.6		2.05
	2	4	87.7	17.7		2.05
		5	88.1	17.7		2.05
		6	87.9	17.7		2.05
Concentrate mixture B; with 2.5% urea (D-123)	1	1	87.4	17.5		2.04
		2	87.7	17.5		2.04
		3	87.6	17.5		2.04
	2	4	87.1	17.6		2.04
		5	87.6	17.6		2.04
		6	87.3	17.6		2.04

[a]Estimated net energy for lactation (ENE_L) derived from NRC (1971) feed tables based on crude protein (CP) and crude fiber (CF) in dry matter (DM) of hay and corn silage, and on assigned values for the ingredients of concentrates (formulations in Appendix Table II.26).

[b]Urea added at ensiling.

[c]ProSil added at ensiling.

Appendix Table II.28.--Trial III 1971. Amounts of feed offered and consumed as dry matter, and total intakes of feed constituents per day.

Cow	S	F	P	T	Feeds Hay	Corn sil.	Cons. mix.A	Cons. mix.B	Sum intake DM	CP	ENE	Urea in Cons.B	NPN in Tot.N
603	1	H	all[a]		2.3	9.1	6.3 / 6.3						
			1	O	1.9	3.1		3.2	8.2	1.32	14.3	88	80	38
			2	K	1.9	3.0		5.4	10.3	1.71	18.6	148	106	39
			3	O	1.9	3.2		5.6	10.6	1.73	19.1	153	111	40
	2	L	4	O	1.8	3.1	4.7		9.6	1.56	17.2		32	13
			5	K	1.6	2.4	3.9		7.6	1.32	14.1		25	12
			6	O	1.9	2.9	5.4		10.3	1.74	18.6		30	11
607			all[a]		2.3	13.6	7.3 / 7.3						
	1	H	1	O	1.9	4.7		5.6	12.1	1.96	21.8	153	130	41
			2	K	1.9	4.5		5.6	12.0	1.96	21.6	153	127	40
			3	O	1.9	4.8		5.6	12.0	1.95	21.8	153	131	42
	2	L	4	O	1.9	4.7	5.6		12.2	1.95	21.8		49	16
			5	K	1.8	4.5	5.6		11.9	1.96	21.4		47	15
			6	O	1.9	4.5	5.6		11.9	1.98	21.4		47	15
604			all[a]		2.3	13.6	8.2 / 8.2						
	1	L	1	O	1.9	4.4	7.2		13.5	2.21	24.6		57	16
			2	K	1.9	4.4	7.2		13.6	2.23	24.8		57	16
			3	O	1.9	4.8	7.2		13.9	2.25	25.2		62	17
	2	H	4	O	1.9	4.7		7.1	13.6	2.21	24.7	194	136	38
			5	K	1.8	4.5		7.2	13.5	2.22	24.5	197	136	38
			6	O	1.9	4.4		7.1	13.5	2.24	24.7	194	133	37
606			all[a]		2.3	9.1	7.2 / 7.2						
	1	L	1	O	1.9	2.8	6.4		11.1	1.85	20.3		36	12
			2	K	1.9	2.7	6.4		11.1	1.85	20.2		35	12
			3	O	1.9	2.9	6.4		11.2	1.84	20.4		38	13
	2	H	4	O	1.9	2.7		5.1	9.7	1.58	17.3	140	91	36
			5	K	1.9	2.5		5.4	9.8	1.64	17.6	148	93	35
			6	O	1.9	2.6		5.6	10.1	1.71	18.4	153	96	35

[a]Amount of feed offered, wet basis. Figures for feeds in each period are DM consumed.

Appendix Table II.29--Trial III 1971. Intakes of crude protein (CP) and estimated net energy for lactation (ENE_L) relative to NRC (1971) standards (%).

		CP intake				ENE_L intakes			
Cow:		603	607	604	606	603	607	604	606
		---------%---------				--------%--------			
	Feed:	H	H	L	L	H	H	L	L
Per.	Inf. Trtm.								
1	O_1	100	121	110	115	92	115	113	111
2	K^1	121	121	115	120	113	117	117	112
3	O_2	123	120	114	113	126	115	118	111
	Feed:	L	L	H	H	L	L	H	H
4	O_1	128	131	130	100	119	124	131	97
5	K^1	109	130	125	113	98	121	125	105
6	O_2	146	136	133	119	131	125	134	111

Means	CP	ENE_L		CP	ENE_L
Inf. Trtm.			Feeding		
O_1	117	113	H	119	115
K^1	119	114	L	122	117
O_2	126	121			

Appendix Table II.30--Trial III 1971. Observations in milk production parameters.

(Item): Seqs.	Per.	Sub-per.	Trtm.	(1) Milk yield, kg/day Cow No. 603	607	604	606	m	d (d%)	(2) Nitrogen Conc., %[a] Cow No. 603	607	604	606	m	d (d%)
				H[d]	H	L	L			H	H	L	L		
1		1	O_1	9.53	11.63	20.12	14.02			.53900	.55125	.49525	.53200		
		2		8.80	12.02	20.46	14.11			.56000	.55475	.52063	.53288		
	1	m		9.16	11.83	20.29	14.07	13.84		(3.504)	(3.544)	(3.261)	(3.394)	3.42	
		1	K	10.50	12.07	20.57	14.56			.57750	.58625	.56175	.57575		
		2		10.95	11.05	19.80	14.31		0.45	.57925	.58800	.56000	.56525		0.28
	2	m		10.73	11.57	20.19	14.44	14.23	(3.3)	(3.688)	(3.742)	(3.575)	(3.639)	3.66	(8.3)
		1	O_2	10.39	10.98	18.57	13.88			.54355	.53760	.50400	.52255		
		2		10.12	11.34	20.21	14.22			.51870	.52920	.50750	.52745		
	3	m		10.26	11.16	19.39	14.05	13.71		(3.390)	(3.405)	(3.231)	(3.350)	3.35	
				L[d]	L	H	H			L	L	H	H		
2		1	O_1	9.16	10.59	17.51	13.13			.57750	.54600	.52325	.53550		
		2		8.55	10.57	17.12	13.27			.58800	.53550	.52325	.53900		
	4	m		8.85	10.58	17.32	13.20	12.49		(3.716)	(3.450)	(3.340)	(3.430)	3.48	
		1	K	8.39	10.50	17.96	13.02			.60550	.57730	.57050	.56700		
		2		8.39	10.86	18.35	12.88		0.50	.63700	.57750	.54950	.58450		0.28
	5	m		8.39	10.68	18.16	12.95	12.50	(4.2)	(3.960)	(3.680)	(3.573)	(3.675)	3.73	(8.1)
		1	O_2	8.35	10.41	16.78	12.36			.55125	.53783	.48650	.53463		
		2		7.77	8.75	15.60	11.95			.55125	.54425	.51538	.55563		
	6	m		8.06	9.58	16.16	12.15	11.50		(3.520)	(3.437)	(3.197)	(3.356)	3.41	

Appendix Table II.30--Continued.[c]

NPN Cons., mg/100ml[a] Cow No. 603	607	604	606	(3) m	d (d%)	(4) SNF Concentration, %[a] Cow No. 603	607	604	606	m	d (d%)	(5) Fat concentration, %[a,b] Cow No. 603	607	604	606	m	d (d%)
H	H	L	L			H	H	L	L			H	H	L	L		
25.43	29.74	27.07	29.33			9.00	9.04	8.66	8.49			3.7	3.6	3.3	3.1		
26.96	28.18	28.03	30.51			8.98	8.88	8.71	8.45			3.7	3.7	3.4	3.4		
26.18	28.93	27.55	29.91	28.2		9.00	8.96	8.69	8.47	8.78		3.70	3.65	3.35	3.25	3.49	
34.40	31.01	35.50	32.94			9.06	9.07	8.92	8.60			3.6	3.4	3.1	3.0		
29.94	30.64	33.80	32.59		4.9	9.19	9.00	9.06	8.59		0.31	3.5	3.6	3.0	2.9		-0.32
32.11	30.81	34.66	32.76	32.6	(17.7)	9.12	9.03	8.99	8.59	8.93	(3.6)	3.54	3.49	3.05	2.95	3.26	(-9.0)
26.65	25.90	27.58	27.42			8.80	8.71	8.52	8.37			3.8	4.1	3.5	3.6		
30.36	26.17	26.59	27.02			8.80	8.70	8.62	8.27			3.6	4.0	3.4	3.2		
28.47	26.04	27.06	27.22	27.2		8.80	8.71	8.57	8.32	8.60		3.70	4.05	3.45	3.40	3.66	
L	L	H	H			L	L	H	H			L	L	H	H		
29.93	26.65	28.08	25.97			9.12	9.39	9.40	7.86			2.8	3.2	2.7	3.5		
28.96	28.35	29.15	29.24			8.84	9.03	9.16	8.28			3.0	3.3	3.0	3.4		
29.48	27.50	28.62	27.61	28.3		8.99	9.21	9.27	8.07	8.89		2.90	3.25	2.85	3.45	3.10	
29.77	35.03	34.80	31.13			(8.79)	9.18	9.25	8.93			2.7	3.0	2.8	2.6		
32.40	34.66	35.98	31.31		4.0	9.45	8.91	9.01	8.84		0.16	3.6	3.7	3.1	2.9		-0.06
31.09	34.66	35.39	31.22	33.1	(13.7)	9.45	9.04	9.13	8.89	9.13	(1.8)	3.15	3.36	2.95	2.75	3.07	(-1.9)
29.92	30.19	30.82	29.50			9.17	8.68	9.57	8.98			3.2	3.5	3.0	2.9		
29.81	29.70	28.70	30.19			8.95	9.02	9.10	8.74			3.1	3.6	2.9	3.0		
29.87	29.97	29.85	29.85	29.9		9.07	8.84	9.36	8.87	9.04		3.15	3.55	2.96	2.95	3.16	

[a] Means for each cow and period are weighted by the milk volume for each subperiod.

[b] Fat test values for subperiod 1 in the last period (6) are lacking although samples were submitted for testing; the assigned values are average of the first day of infusion and the second subperiod.

[c] The arrangement is identical with that for the first part of table (Seqs., Per., Subper., Trtm.).

[d] The feed level of NPN; H = high, L = low.

Appendix Table II.30--Continued.[e]

Seq.	Per.	Trtm.	Cow No. 603	607	604	606	m	d (d%)
			Crude Prot. prod., g/day					
			H	H	L	L		
	1	O_1	321	419	662	478	470	
1	2	K[1]	396	433	722	526	519	56
	3	O_2	348	380	626	471	456	(12.1)
			L	L	H	H		
	4	O_1	329	365	578	453	431	
2	5	K[1]	332	393	649	476	460	51
	6	O_2	284	329	517	420	387	(12.5)
			Est. True Prot. conc., %					
			H	H	L	L		
	1	O_1	3.34	3.34	3.07	3.21	3.238	
1	2	K[1]	3.48	3.55	3.35	3.43	3.454	0.25
	3	O_2	3.21	3.24	3.05	3.18	3.169	(7.8)
			L	L	H	H		
	4	O_1	3.53	3.27	3.16	3.25	3.303	
2	5	K[1]	3.77	3.46	3.35	3.47	3.512	0.25
	6	O_2	3.33	3.26	3.01	3.29	3.221	(7.7)
			Est. True Prot. prod., g/day					
			H	H	L	L		
	1	O_1	306	395	623	452	444	
1	2	K[1]	373	411	676	495	489	51
	3	O_2	329	362	591	447	432	(11.6)
			L	L	H	H		
	4	O_1	312	346	547	429	409	
2	5	K[1]	316	370	608	449	436	48
	6	O_2	269	312	486	400	367	(12.4)
			FCM prod., kg/day					
			H	H	L	L		
	1	O_1	8.75	11.21	18.31	12.49	12.69	-0.26
1	2	K[1]	10.00	10.69	17.31	12.16	12.54	(-2.1)
	3	O_2	9.77	11.24	17.79	12.78	12.90	
			L	L	H	H		
	4	O_1	7.39	9.39	14.32	12.11	10.80	0.29
2	5	K[1]	7.32	9.65	15.30	10.52	10.70	(2.7)
	6	O_2	7.03	8.39	13.63	10.24	9.82	

[e]Derived values; period means (m) only.

Appendix Table II.31--Trial III 1971. Blood urea N concentration, mg/100ml.

Seq.	Per.	Inf.	Bldg.	Cow No. 603	607	604	606	m_B	m_P	m_S
				H	H	L	L			
1	1	O_1	1	30	25	35	18	27.0		
			2	35	36	35	39	36.3		
			3	42	34	34	30	35.0	32.8	
	2	K	1	25	43	36	36	35.0		
			2	25	46	35	32	34.5		
			3	48	43	40	46	44.3	37.9	
	3	O_2	1	29	24	26	27	26.5		
			2	38	42	32	37	37.3		
			3	37	44	29	30	35.0	32.9	34.5
				L	L	H	H			
2	4	O_1	1	30	34	19	28	27.8		
			2	38	41	43	39	40.3		
			3	34	37	42	34	36.8	34.9	
	5	K	1	26	26	40	37	32.3		
			2	36	33	38	41	37.0		
			3	30	34	44	55	40.8	36.7	
	6	O_2	1	38	34	25	37	33.5		
			2	30	29	28	34	30.3		
			3	13	31	37	31	28.0	30.6	34.1

Means

Feeds:	H	36.1	Infusions:	O_1	33.8	Bleedings:	B_1 30.3
	L	32.5		K	37.3		B_2 35.9
				O_2	31.8		B_3 36.6
				O_{1+2}	32.8		B_{2+3} 36.3

Appendix Table II.32--Trial III 1971. Blood plasma glucose concentration, mg/100ml.

Seq.	Per.	Inf.	Bldg.	Cow No. 603	607	604	606	m_B	m_P	m_S
				H	H	L	L			
1	1	O_1	1	66	65	55	66	63.0		
			2	67	56	61	57	60.3		
			3	61	59	60	61	60.3	61.2	
	2	K	1	67	67	70	64	67.0		
			2	63	60	60	59	60.5		
			3	59	55	65	56	58.8	62.1	
	3	O_2	1	61	67	60	65	63.3		
			2	59	56	56	51	55.5		
			3	59	64	61	55	59.8	59.5	60.9
				L	L	H	H			
2	4	O_1	1	66	66	65	63	65.0		
			2	63	48	60	61	58.0		
			3	63	56	64	62	61.3	61.4	
	5	K	1	69	72	72	69	70.5		
			2	71	55	74	66	66.5		
			3	72	63	70	66	67.8	68.3	
	6	O_2	1	68	69	69	73	69.8		
			2	68	55	64	60	61.8		
			3	61	59	68	66	63.5	65.0	64.9

Means

Feeds:	H	64.0	Infusions:	O_1	61.3	Bleedings:	B_1	66.4
	L	61.8		K	65.2		B_2	60.4
				O_2	62.3		B_3	61.9
				O_{1+2}	61.8		B_{2+3}	61.1

Appendix Table II.33--Trial III 1971. Layout of ANOVA III.

Source of Variance	Degrees of freedom symbol	No.	Df for milk paramt. O_1 & O_2 averaged
a) Between main plots; comprizing C,S,&F	(cs-1)	7	
Cows	(c-1)	3	
Sequences	(s-1)	1	
Feedings	(f-1)	1	
Error a (rest)		2	
b) Infusion treatments w/in CSF (split plot)	(tcs-1)-(cs-1)	16	8
Treatments	(t-1)	2	1
orth.contrasts			
K vs.O's		1	
O_1 vs. O_2		1	
TxF	(t-1)(t-1)	2	1
TxS	(t-1)(s-1)	2	1
TxC	(t-1)(c-1)	6	3
Error b (rest)		4	2
c)Bleedings w/in T (split-split plot)	(btcs-1) -(tcs-1)	24	
Bleedings	(b-1)	2	
orth contrasts			
B_1 vs. B_2B_3		1	
B_2 vs. B_3		1	
BxT		4	
BxF		2	
BxS		2	
BxC		6	
Error c (rest)		16	
Total	(btcs-1)	71	

Appendix Table II.34.--Trial III 1971. AOV for feed intake.

B. Trial III.		DM Intake (kg/day)	
Var. source	df	MS	F
a) Whole plot	7		
Cows	3	59.641	36.0
Seqs.	1	1.550	2.8
Feeds	1	.220	<1
Error a	2	1.104	
b) Split plot	16		
Inf. Trtm.	2	1.206	1.2
K vs. O	1		
O_1 vs. O_2	1		
T x C	6	2.038	<1
T x S	2	1.211	<1
T x F	2	1.106	<1
Error b	4	2.054	
Total (SS)	23	(70.130)	

Appendix Table II.35--Trial III 1971. Statistical analysis for milk production parameters.

	DF	MS	F	F sign.	MS	F	F sign.	MS	F	F sign.
		Milk Yield (kg/day)			Prot. Conc. (%)			NPN Conc. (mg/100ml)		
a)Whole plot	7									
Cows	3	67.6862			.04782			1.684		
Segs.	1	12.0930			.01488			3.151		
Feeds	1	.2782	<1		.00319	<1		2.176	1.3	
Error a	2	.3144			.01874			1.666		
b)Split plot	8									
Inf.Trtm.	1	1.0215	2.9		.30636	301.8	<.005	82.356	34.4	<.05
IxC	3	.1016	<1		.00108	1.1		3.268	1.2	
IxS	1	.0109	<1		--			.600	<1	
IxF	1	.1464	<1		.00099	<1		.176	<1	
Error b	2	.3547			.00102			2.395		
Total (SS)	15	(218.1536)			(.51156)			(111.425)		
		FCM Yield (kg/day)			Prot. Prod. (g/day)			NPN/Tot. N (%)		
a)Whole plot	7									
Cows	3	43.655			68433.3			.4936		
Seqs.	1	18.104			12210.2			.0203		
Feeds	1	.112	<1		324.0	<1		.0637		
Error a	2	1.440			410.4			.2547		
b)Split plot	8									
Inf.Trtm.	1	.003	1		11990.2	52.0	.025	.9653	2.6	
IxC	3	.195	<1		544.3	2.4		.0088	1	
IxS	1	.326	<1		9.0	<1		.0203	1	
IxF	1	.136	<1		72.3	<1		--		
Error b	2	.426			226.4			.3669		
Total (SS)	15	(152.525)			(232812.0)			(3.820)		

Appendix Table II.35--Continued.

	DF	MS	F	F sign.	MS	F	F sign.	MS	F	F sign.
		Est. True Prot. (%)			SNF Conc. (%)			Fat Conc. (%)		
a)Whole plot	7	.05335			.67572			.20212		
Cows	3	.01363			.22801			.41281		
Seqs.	1	.00243	2.0		.02176	1.1		.03516	1.7	
Feeds	1	.00121			.01948			.02088		
Error a	2									
b) Split Plot	8									
Inf. Trtm.	1	.25075	113.2	<.01	.16201	3.3		.14631	5.4	<.25
IxC	3	.00094	<1		.01777	<1		.08462	1.0	
IxS	1	--			.00764	<1		.13637	5.0	
IxF	1	.00098	<1		.00949	<1		.00530	<1	
Error b	2	.00222			.00959			.02723		
Total (SS)	15	(.47355)			(1.29650)			(1.41425)		
		Est. True Prot. (g/day)			SNF Prod. (g/day)			Fat Prod.(g/day)		
a)Whole plot	7									
Cows	3	59106.3			537055.2			49452.3		
Seqs.	1	10816.0			59902.6			36576.6		
Feeds	1	256.0	<1		105.1	<1		67.2	<1	
Error a	2	401.0			1447.1			3088.4		
b)Split plot	8									
Inf.Trtm.	1	10000.0	84.7	.025	22788.9	14.7		519.8	<1	
IxC	3	454.8	3.9		583.2	<1		1977.6	<1	
IxS	1	6.3	<1		2141.8	1.4		1242.6	<1	
IxF	1	72.3	<1		1897.1	1.2		204.5	<1	
Error b	2	118.1			1553.8			1149.8		
Total (SS)	15	(200871.8)			(1698370.9)			(194333.0)		

Appendix Table II.36--Trial III 1971. Statistical analysis for blood parameters.

		Blood urea N (mg/100 ml)			Plasma glucose (mg/100ml)		
	DF	MS	F	F sign.	MS	F	F sign.
a) Whole plot	7						
Cows	3	30.4953	2.3		59.0509	2.1	
Seqs.	1	4.0138	<1		284.0139	9.9	<.10
Feeds	1	224.0138	21.7	<.05	82.3479	2.9	
Error a	2	10.3472			28.6250		
b) Split plot	16						
Inf. Trtm.	2	188.0416	5.6	<.10	97.7639	11.9	<.025
K vs. O	1	323.9999	9.7	<.05	184.5069	22.5	<.01
O_1 vs. O_2	1	52.0833	1.6		11.0208	1.3	
IxC	6	54.0231	1.6		17.5231	2.1	
IxS	2	35.1042	1.0		63.0139	7.7	≈.05
IxF	2	57.3473	1.7		7.0972	<1	
Error b		33.5522			8.2153		
c) Split-split plot	48						
Bleedgs.	2	285.0416	8.0	<.01	235.0139	28.1	<.001
B_1vs.B_2&B_3	1	546.0624	15.9	<.001	444.5069	53.2	<.001
B_2 vs. B_3	1	6.0208	<1		25.5208	3.1	<.10
BxC	6	5.5231	<1		47.3842	5.7	<.025
BxS	2	31.9306	<1		2.3472	1	
BxF	2	125.6806	3.5		8.8472	1.1	
BxI	4	88.3959	2.5		8.9098	1.1	
Error c	32	35.5408			8.3637		
	71						
Total (SS)		(3768.8750)			(2140.3195)		