THE REQUIREMENT OF METHIONINE AND TOTAL SULFUR AMINO ACIDS IN THE PRE-RUMINANT CALF

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ABSTRACT

THE REQUIREMENT OF METHIONINE AND TOTAL SULFUR AMINO ACIDS IN THE PRE-RUMINANT CALF

By

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The qualitative and quantitative requirement of amino acids in calves is essentially unknown. The amino acids essential for growth in calves have not been determined but was assumed to be the same as those essential for growth in rats, because they are required at the tissue level in both ovine and bovine animals, and because calves fed gelatin have inferior performance when compared with those fed milk protein. The requirements of methionine and total sulfur amino acids have been studied, but the estimates vary from .23 to more than .58 g per day per kg metabolic weight. The requirement of lysine has been reported to be 1.75 to 1.95% of dry matter.

The requirement of methionine and total sulfur amino acids was studied in 20 male Holstein calves employed in a two-period changeover design with five dietary levels of methionine (1.86, 2.48, 3.10, 3.72, and 4.34 g/16 g N). This was done in ten two by two latin squares where two

calves represent the rows and two periods (9 to 15 and 21 to 27 days of age) represent the columns. The calves were fed milk replacer containing 25% of the total protein (18.08%) as crystalline L-amino acids as the only feed. Prepared milk (13%) solids were fed at the daily rate of 10% of body weight in two equal meals 12 hours apart.

The response criteria were average daily gain, digestibility of dry matter and crude protein, nitrogen balance, and plasma methionine and urea nitrogen levels before and two hours after feeding on the first and the last day of each period. Using these methods plus the difference between fasting and post feeding plasma methionine levels, we estimated methionine requirements ranging from 2.75 to 2.95 g per 16 g N, except when digestibility of dry matter and plasma urea nitrogen were used as response criteria. All diets contained 1.05 g cystine per 16 g N. If the assumption is made that the requirement of sulfur amino acids is that of methionine only or 45% methionine plus 55% cysteine, then the requirement of total sulfur amino acids is 3.80 to 4.00 g per 16 g N, or .25 to .26 g per day per kg metabolic weight. The requirement of the remaining essential amino acids was estimated from the above value and amino acid composition of the 40 week old calf fetus.

When poor health due to factors other than treatments was encountered, then average daily gain,

digestibility, nitrogen balance, and plasma urea nitrogen were less sensitive to diet than was plasma methionine. These data suggest that three days on the experimental feed are sufficient to estimate the amino acid requirement in calves by plasma amino acid levels. Poor health due to bacterial infections of the gastrointestinal tract could not be related to dietary methionine but the severity tended to increase at the highest methionine intake. At that dietary level, plasma methionine tended toward a plateau instead of increasing linearly with intake as expected. The cause of the plateau is unknown but it may have been caused by decreased methionine absorption, or increased deamination of amino acids due to gluconeogenesis, as indicated by increased plasma urea nitrogen levels. Whether gluconeogenesis from amino acids is stimulated because energy becomes limiting for vital functions in scouring calves, or is brought about by a direct stimulation of glucocorticoid secretion by high level of free methionine in the diet is not known.

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TABLE OF CONTENTS

	Page
LIST OF TABLES	vi
LIST OF FIGURES	xi
LIST OF ABBREVIATIONS	xiii
INTRODUCTION	1
REVIEW OF LITERATURE	3
Methods for Assessment of Protein and Amino Acid Requirements	3
Growth and Nitrogen Retention	4
Growth Assay	5
Nitrogen Retention Methods	6
Plasma Amino Acids	11
Feeding Methods	13
Effect of Short Term Fast	16
Breaking Point	17
Carcass Analysis	18
Urinary Urea	18
Plasma Urea Nitrogen	19
Amino Acid Oxidation	20
Energy and Protein Requirements of Non Ruminating Calves	22
Energy and Milk Requirements of Calves Fed Milk	22
Protein Requirement	24
Endogenous Losses	29
Digestibility and Biological Value of Milk Proteins	32

Page

Protein Sources in Milk Replacers 34
Amino Acid Requirements 38

Calves 38
Factors Affecting Plasma Amino Acid Levels in Calves 39
Amino Acid Infusion in Ruminating Calves and Lambs 40
Amino Acid Requirements of Other Species 41

Blood Urea Nitrogen in Calves and Lambs . . . 43

MATERIALS AND METHODS 44

Animals 44
Diets 46
Feeding Schedule 51
Body Weight 53
Nitrogen Balance 53
Blood Collection and Processing. 54
Experimental Design and Statistics 56

RESULTS 59

Body Weight, Daily Gain, and Health. 59
Digestibility 66
Nitrogen Balance 70
Plasma Amino Acids 78
Plasma Urea Nitrogen 90
Hematocrit 92

DISCUSSION 97

Body Weight, Daily Gain, and Health 97
Digestibility. 100
Nitrogen Balance 102
Plasma Amino Acids 104
Plasma Urea Nitrogen 110
Hematocrit 111
Sulfur Amino Acid Requirement in Baby Calves . . . 112

CONCLUSIONS 117

APPENDIX 119

BIBLIOGRAPHY 139

LIST OF TABLES

Table		Page
1.	Energy requirement of calves fed milk; digestible energy per day	23
2.	Milk requirement of calves fed only milk; liters per day	24
3.	Digestible energy requirements of calves fed milk (kcal/day)	25
4.	Relationships between N retention and the daily supply and requirement of apparently digestible protein in calves	27
5.	Relationships between N retention and daily gain in calves	28
6.	Loss of endogenous urinary N (UE) in calves	30
7.	Loss of metabolic fecal N (MF); g/100 g dry matter ingested	31
8.	Apparent digestibilities of milk dry matter (DM) and milk protein in calves at different ages; %	33
9.	The amino acid requirements of rats, chicks, turkeys, and pigs	42
10.	Calves omitted from the experiment before completion due to poor health or mortality	45
11.	Assignment of calves to blocks, date of birth, dam's identification number and treatments	47
12.	Composition of the milk replacer	48

Table Page

13. The amino acid content in whole milk, commercial milk replacer and prepared amino acid mixtures 49

14. Plan of feeding, weighing, collection, and blood sampling 52

15. The effect of dietary methionine on body weight (initial and final), average daily gain, and fecal score of calves fed graded levels of methionine 60

16. Estimated relationship between dietary methionine levels (X,%) and average daily gain (ADG, g) in calves. Adjusted observations 63

17. The effect of dietary methionine levels on the digestibility of dry matter (DM) and crude protein (CP) and related factors in calves 67

18. The effect of dietary methionine (%) on nitrogen balance and related parameters of calves 71

19. The effect of dietary methionine (%) on the daily N-content and N-balance per 100 kg initial body weight (g N/day/100 kg BW) . . . 76

20. Estimated relationships between dietary methionine levels (X, %) and N-balances expressed as total for experimental period (NB_1, g), g N daily per 100 kg initial BW (NB_2), and g N daily per kg $BW^{.73}$ (NB_3), respectively. Adjusted observations. 77

21. The effect of dietary methionine on plasma concentrations of methionine, valine, leucine, and isoleucine (µM/100 ml) in calves 80

22. Estimated relationships between dietary methionine levels (X, %) and plasma methionine concentrations (Y, µM/100 ml). Adjusted observations 83

Table | Page

23. The effect of dietary methionine levels on plasma urea nitrogen levels in calves on the first and the last day of the experimental period 91

24. The effect of dietary methionine levels on blood hematocrits in calves 94

25. The relationship between average daily gain (ADG, g) and fecal (F, kg), and urinary (U, liters) excretion in calves . . . 99

26. Methionine needs of the baby calf determined by various methods 113

27. The requirement of total sulfur amino acids in the young calf as estimated by several workers 115

28. Calculated requirement of essential amino acids in the young calf 116

A1. Analysis of variance for initial and final body weight, average daily gain, and fecal score of calves fed graded levels of methionine 119

A2. Analysis of variance for the relationship between dietary methionine levels (X, %) and final body weight (BW, kg), average daily gain (ADG, g), and fecal score (FS), respectively. Adjusted observations 120

A3. Analysis of variance for the relationship between fecal excretion (F, kg) and average daily gain (ADG, g), and the estimated regression. Unadjusted observations 121

A4. Analysis of variance for the relationship between fecal excretion (F, kg) and urine excretion (U, liters), and the estimated regression. Unadjusted observations 121

A5. Analysis of variance for the relationship between urine excretion (U, liters) and average daily gain (ADG, g), and the estimated regression. Unadjusted observations 122

Table Page

A6. Analysis of variance for dry matter (DM) content (%) in milk replacer, milk weigh back, feces, and digestibilities of DM and crude protein (CP) in calves 123

A7. Analyses of variation for the relationship between dietary methionine levels and the digestibility of dry matter (DM) and crude protein (CP) in calves. Adjusted observations 124

A8. Analyses of variance for the relationship between fecal excretion (X, kg) and the digestibility of dry matter (DM) and crude protein (CP) in calves 125

A9. Analysis of variance for N-balance data 126

A10. Analysis of variance for the relationship between urine excretion (U, liters) and urine N-concentration (NU, %), and the estimated regression. Unadjusted observations 127

A11. Analysis of variance for the relationship between fecal excretion (F, kg) and the fecal N-concentration (NF, %) for all observations, and for period 1 (NF_1) and for period 2 (NF_2), respectively, and the estimated regressions. Unadjusted observations 128

A12. Analysis of variance for N-balance data expressed as g N/day per 100 kg initial body weight 129

A13. Analysis of variance for the relationship between dietary methionine levels (X, %) and N-balances expressed as total for the experimental period (NB_1, g), g N daily per 100 kg initial BW (NB_2), and g N daily per kg $BW^{.73}$ (NB_3), respectively. Adjusted observations 130

A14. Analysis of variance for plasma concentrations of methionine, valine, leucine, and isoleucine 131

Table Page

A15. Analysis of variance for the relationship between dietary methionine (X, %) and the plasma methionine concentration (M, µM/100 ml). Adjusted observations 133

A16. Analysis of variance for the relationship between dietary methionine (X, %) and the plasma methionine concentration (M, µM/100 ml). Group E deleted. Adjusted observations 135

A17. The relationship between dietary methionine levels (X, %) and plasma urea nitrogen (PUN) in calves on the first (1) and the last (2) day of the experimental period 137

A18. The relationship between dietary methionine levels (X, %) and hematocrits (HEM) in calves on the first (1) and the last (2) day of the experimental period 138

LIST OF FIGURES

Figure		Page
1.	The relationship between graded levels of methionine (X) and average daily gain (ADG, Y) in calves	64
2.	The relationship between dietary methionine levels and the digestibility of dry matter (DM) and crude protein (CP) in milk replacers for calves	68
3.	The relationship between dietary methionine (X) levels and N-balance (Y) in calves	79
4.	The relationship between dietary methionine levels and plasma methionine concentrations before feeding on the first day of sampling in calves	86
5.	The relationship between dietary methionine (X) levels and plasma methionine (Y) concentrations two hours after feeding on the first day of sampling in calves	87
6.	The relationship between dietary methionine (X) levels and plasma methionine (Y) concentrations before feeding on the last day of sampling in calves	88
7.	The relationship between dietary methionine (X) levels and plasma methionine (Y) concentrations two hours after feeding on the last day of sampling in calves	89
8.	The relationship between dietary methionine levels and plasma urea nitrogen (PUN) on the first (1) and the last (2) day of the experimental period	93

Figure Page

9. The relationship between dietary methionine levels and packed blood cell volume (HEM) in calves on the first (1) and the last (2) day of the experimental period 96

10. The relationship between dietary methionine levels and the difference (DIF) between the linear model for before feeding and the cubic model for two hours after feeding on the first (1) and the last (2) day of sampling in calves 109

LIST OF ABBREVIATIONS

ADG = average daily gain

AP = available protein

BV = biological value

BW = body weight

CP = crude protein

DCE = 1,2-dichloroethane

DCP = apparently digestible protein

DDS = distillers dried solubles

DM = dry matter

DSM = dried skim milk

EAA = essential amino acid(s)

FS = fecal score

G = nitrogen retained in gain

MF = metabolic fecal nitrogen

MR = milk replacer(s)

N-balance = nitrogen balance

PAA = plasma amino acid(s)

PUN = plasma urea nitrogen

S = dermal loss

TP = digestible true protein

UE = endogenous urinary nitrogen

INTRODUCTION

The function of dietary protein is to serve as a source of amino acids for anabolic processes in the body. The most economical protein will be that which provides a mixture of amino acids, both essential and nonessential, in the proportions needed by the body (Munro, 1964b). The importance of this concept was very clearly demonstrated by Dean and Scott (1965) in their development of an amino acid reference diet for early growth in chicks. A final mixture of amino acids containing the equivalent of 17.69% protein gave as good a growth rate as a practical ration of the corn-soybean meal type containing the equivalent of 26.20% protein.

With the increasing use of milk replacers in calf nutrition and the desire to replace dried skim milk in the replacers by other protein sources, it becomes increasingly important to obtain knowledge of the qualitative and quantitative requirement of amino acids in calves to maintain good nutrition.

The amino acids essential for growth in calves have not been determined, but may be assumed to be the same as those essential for growth in rats. The amino

acids essential for rats are required at the tissue level in both ovine and bovine animals (Downes, 1961; Black et al., 1952), and calves fed gelatin (tryptophane deficient) have inferior performance when compared with calves fed milk protein (Blaxter, 1950; Blaxter and Wood, 1952a).

Only limited information is available concerning the quantitative requirement of amino acids in non-ruminating calves. At the outset of this study the requirement of methionine had been studied only by Patureau-Mirand et al. (1973). Since then, an experiment on the requirements of total sulfur amino acids and lysine was reported by Tzeng (1974) and Williams and Smith (1975) investigated the requirement of total sulfur amino acids.

REVIEW OF LITERATURE

Studies of protein metabolism have traditionally been divided into the intermediary metabolism of protein and amino acids, and protein nutrition (Munro, 1964). Within the studies of protein nutrition two main lines of research have been the evaluation of protein quality and assessment of protein and amino acid requirements (Munro, 1964).

The objective of this literature review have been limited to: (a) comparisons of when and how various methods have been used in assessment of dietary need of protein and amino acids and factors influencing interpretation of the results, and (b) the energy and protein requirements of the non-ruminating calf.

Methods for Assessment of Protein and Amino Acid Requirements

Knowledge of the protein and amino acid requirements of various species is important in the preparation of highly nutritious feeds which can sustain good health at least cost. The dietary requirement of domestic animals may also be defined as the level which will produce optimal production. However, this can only be done

when it is beyond the aspect of "maximum health." The most widely used methods in assessment of dietary needs of protein and amino acids have been the growth assay and the nitrogen retention methods. In the case of amino acids, plasma amino acid (PAA) concentrations, carcass analysis, urine urea, plasma urea nitrogen (PUN) concentrations, amino acid oxidation, and others also have been suggested.

The objective of this section is to compare the various methods with respect to when and how they can be used, and features influencing interpretation of the results.

Growth and Nitrogen Retention

Balance studies were first conceived by Boussingault (1839; Munro, 1964) in studies with milk cows in which the total intake of C, H, O, and N was compared with the total output of these in urine, feces, and milk. The concept of balance of income and outgo was rapidly adopted and Voit (1831-1908; Munro, 1964) developed the nitrogen balance (N-balance) as a precise tool for the study of protein metabolism.

Another important concept in the use of these methods was the principle of diminishing returns. This principle was probably first formulated by Liebig (1855; Brody, 1945) under the name of the "law of the minimum" which may be shown to be a special case of, if not

identical with, the principle of diminishing returns (Brody, 1945). This principle has since been widely used in animal experiments by feeding various experimental groups graded levels of the nutrient in question.

The rapid response and sensitivity of both growth and N retention methods to changes in the dietary protein and amino acid supply are mainly because: (a) all of the acids needed by higher organisms are obtained together from the proteins of foodstuffs, (b) except for minor quantities required for special purposes, all amino acids are used simultaneously for the synthesis of tissue proteins, and (c) there is essentially no storage of free amino acids in the body (Harper, 1964; Munro, 1970).

Growth Assay.--The law of diminishing returns applies closely to the amino acid requirement except when physiological limits to response to nutrient variations are attained, i.e., maturity, or at low levels of a specific nutrient intake. Other factors complicating interpretation of growth data are reserve stores, synthesis in the animal, or small residual quantities in the diet may be an important fraction of the supply the animal receives (Almquist, 1947, 1953).

Interpretation of the growth assay is made most conveniently by the use of logarithms for the abscissa (Almquist, 1953; Brookes et al., 1972). Advantages of this method of interpretation are: (a) bad data are made

more conspicuous, (b) requirements can be more readily estimated although no specific data happen to coincide with the full requirements, provided there are sufficient data to establish the limits of requirements, and (c) full use may be made of the submaximal data to establish the response lines (Almquist, 1953).

In order to obtain a reasonable degree of confidence in the measurements obtained the animals have to be fed the experimental diet for a considerable length of time. With increasing size of experimental animals the daily amount of feed per animal increases. Therefore, the biggest drawback to the applicability of this method in amino acid requirement studies is probably the time factor because of the high cost of mixtures of purified amino acids.

Nitrogen Retention Methods.--Nitrogen retention can be measured by either the factorial method or the N-balance method.

By the factorial method requirements are estimated by summation of N lost and amounts of protein synthesized, and the assumption that N consumed can be used with 100% efficiency (e.g., Williams et al., 1974). The formula for the total dietary requirement of digestible true protein (TP) in grams per day is (ARC, 1965):

$$(1)\quad TP = (6.25)(100/BV)(UE + S_1 + S_2 + MF + G + P + L)$$

where UE = urinary endogenous loss

S_1 = loss of N in hair and scurf

S_2 = N retention in wool by adult sheep

MF = metabolic fecal N

G = N retention in live-weight gain

P = N retention during pregnancy in fetal tissues

L = output of N in milk

BV = biological value of protein.

The corresponding requirement of apparent digestible protein (DCP) is obtained from (1) by subtracting (6.25)(MF) and is therefore:

$$(2)\quad DCP = (6.25)(100/BV)(UE + S_1 + S_2 + G + P + L) + (6.25)(BV)(100/BV - 1)$$

Both TP and DCP are dependent on MF and hence on the dry matter (DM) intake, which make them inconvenient for purposes of tabulation (ARC, 1965). Therefore, the term MF was excluded and the calculated value was termed available protein (AP; ARC, 1965):

$$(3)\quad AP = (6.25)(100/BV)(UE + S_1 + S_2 + G + P + L)$$

Assessment of the minimum endogenous urinary excretion (UE) is difficult, but a plateau is normally obtained after 5 to 10 days of N depletion (Williams et al., 1974). In the general case the sum of S_1 and S_2 is termed the dermal loss (S) and is equal to the total loss of N in

sweat, desquamated epithelium, hair and nails, menstrual, seminal, nasal and oral secretions, and excretions from wounds and N retention in hair (Williams et al., 1974). The coefficient of variation in (UE + S + G) is 15 to 20% (Williams et al., 1974). The metabolic fecal N (MF) often shows variations from the average of more than 80% (Williams et al., 1974), and the variation is due to: (a) the difficulty in assuring proper assignment of collections to respective metabolic periods and complete fecal collection, (b) fecal N excretion is influenced by the quantity and composition of the food consumed, and (c) values for infants and children are lower in g per day but represent a higher percentage of the total N loss. In case of growing animals, N retained in gain (G) is calculated as the difference of values obtained from animals sacrificed at the beginning and animals sacrificed at the end of the growth period, or by the use of standard values (Williams et al., 1974). The latter add error to the method as does the difference method, although to a lesser degree (Williams et al., 1974). Furthermore, the sacrifice technique cannot be used for human research, and often not for large domesticated animals (cattle, horses) due to the cost of the experimental unit.

The N-balance method is a measurement of the least amount of N that will maintain N equilibrium in adults or satisfactory growth and N retention in the young (Williams

et al., 1974). The N-balance technique has been discussed by Albanese (1959), Wallace (1959), Allison and Bird (1964), and Williams et al. (1974), and the method can be formulated as:

$$(4)\quad NB = I - (U + F + S)$$

where NB = N-balance

I = dietary N intake

U = N excretion in urine

F = N excretion in feces

S = dermal N loss

Improvement in the N-balance obtained by stepwise additions of the limiting nutrient give a measure of the need in a specifically depleted organism (Albanese, 1959; Allison and Bird, 1964), and may be used for essential amino acid (EAA) requirement determinations only under rigidly controlled conditions (Nasset, 1956). The N-balance is the sum of gains and losses of all the tissues of the body and equilibrium may not be identical with good nutrition (Allison and Bird, 1964).

The possible errors in conversion of N retention to expected body composition are: (a) the factor used to convert N to proteins, (b) the ash content of the body, (c) the water content of the body, (d) the fat content of the body, and (e) the possibility of cumulative errors (Wallace, 1959). The most pertinent of these factors is

the cumulative error (Wallace, 1959), because, even with the most refined and meticulous technique, a finite quantity of the measured intake is lost in feeding and, similarly, a finite portion of the excreta is not recovered. When output is subtracted from intake, these losses increase the error. Wallace (1959) concluded that a 2% loss of intake and a similar loss of excreta will result in a 30 to 40% increase in the N-balance when the two, ordinarily very large numbers, are subtracted to obtain what is usually the very small balance value. Further, the common practice of not including the dermal losses may further increase the error by at least 20%. Finally Wallace (1959) pointed out, that the higher the concentration of nitrogen in the food, the greater will be the losses and the error in the balance.

Changes in body water content in excess of 4 to 5% occur only in abnormal clinical conditions (Wallace, 1959). However, changes in body water have been invoked to explain N retention in excess of correlative body growth. In the 4 month old child 33% of the retained N is being stored without equivalent gain of body weight (BW).

Both the factorial approach and the N-balance method for measuring N retention have disadvantages, but in work with large domesticated animals the N-balance is usually preferable to the sacrifice method because of cost.

Plasma Amino Acids

Utilization of PAA data in studies of amino acid requirements was suggested by Almquist (1954), and McLaughlan and Morrison (1968), and a direct relationship between plasma and dietary lysine was demonstrated by McLaughlan et al. (1961). Since then PAA have been compared with growth and N retention methods in growing rats (Morrison et al., 1961b; McLaughlan and Illman, 1967; Stockland et al., 1970), in growing chicks (Zimmerman and Scott, 1965), in growing pigs (Mitchell et al., 1968b), and in young adult men (Young et al., 1968, 1971, 1972).

Morrison et al. (1961b) fed growing rats graded levels of lysine. After an initial lag, plasma lysine rose rapidly in response to added dietary lysine and reached a maximum at a dietary lysine concentration about .2% units greater than that found necessary for maximum growth. When Zimmerman and Scott (1965) fed young growing chicks suboptimal and superoptimal dietary concentrations of lysine, arginine, and valine in a basal diet of crystalline amino acids previously determined to have an optimal combination of amino acids (Dean and Scott, 1965), the first limiting amino acid remained at a very low level in the blood irrespective of the severity of the amino acid deficiency until the dietary level exceeded that needed to maximize growth. When the dietary amino acid level was in excess of requirement for maximum weight gain,

that amino acid accumulated rapidly, and in a linear manner in the plasma, even though the greatest dietary concentration used was more than twice that required. Zimmerman and Scott (1965) did not obtain a maximum plateau in the PAA response curve, and concluded that the shape of the PAA curve (broken-line response curve) can be used to determine the amino acid requirement of the chick. The broken-line response curve and its validity as described by Zimmerman and Scott (1965) has since been confirmed by Mitchell et al. (1968b) in studies with growing pigs fed graded levels of lysine, isoleucine, leucine, and histidine when compared with maximum N-balance; and by Stockland et al. (1970) in growing rats fed graded levels of lysine when compared with maximum average daily gain and gain per unit feed consumed; and by Young et al. (1971, 1972) in young adult men given graded levels of tryptophane, valine, and lysine when compared with N-balances.

McLaughlan and Illman (1967) found an almost linear relationship between plasma and dietary concentrations of lysine, isoleucine, leucine, threonine, tryptophane, and histidine. The requirement was considered to be the dietary level at which the plasma level after feeding was equal to the normal fasting level, and was in close agreement with average values obtained by other methods. The

lack of a broken-line response curve was believed to reflect the duration of the test period.

Feeding Methods.--Even though the same typical broken-line response curve has been obtained in most experiments where PAA were used as response criteria the feeding methods used and the length of the fasting period before bleeding have varied.

The cost of purified amino acids is a limiting factor in experiments for assessment of the amino acid requirements, and becomes greater with increasing size of the experimental animal. Therefore, if the feeding period necessary in the PAA assay is shorter than that needed for assessment by growth and N retention the PAA method may become increasingly useful for determining amino acid status (Mitchell et al., 1968b).

Chicks fed suboptimal levels of dietary lysine for up to 33 days showed no accumulation of lysine in the plasma (Zimmerman and Scott, 1965). However, Morrison et al. (1961b) did not obtain the typical plasma response curve when rats were fed a test diet for three weeks and blood was collected from non-fasted animals.

Test periods of 14 to 17 days in length and blood collection 6 hours after the last food presentation, combined with different feeding methods was studied in growing rats by Stockland et al. (1970). Feeding for one hour every 12 hours gave the typical response curve. When the

diet was fed ad libitum, plasma response was more variable because the procedure provided no way of controlling the time that the rat last consumed its food. They also combined the ad libitum feeding for 17 days with an additional two day period where feed was offered ad libitum for one hour every 12 hours before bleeding. In this case the plasma levels started to accumulate prior to dietary levels associated with maximum daily gain and growth per unit feed. It was concluded that continued uptake of the assigned diets combined with slow weight gains during the short period of controlled feeding lead to an accumulation of lysine in the plasma. They suggested a period of "metabolic adaptation" to different feeding methods is needed.

Zimmerman and Scott (1965) obtained a typical plasma response curve when chicks were fed a pre-test diet for 7 days and then fed the test diet for 7 days or more and blood was collected after a 24 hours fast. Mitchell et al. (1968b) also obtained the typical plasma response curve when pigs were used in a 5 day test experiment (7 days pre-test), where blood was collected 6 hours after feeding on the last day on the test diet. The pigs were offered 3 equal meals at one hour intervals and then the remaining ad libitum until bleeding. Young et al. (1971) found no change in the plasma tryptophane curve in young adult men between days 3 and 6 of feeding the experimental

diet. Morrison et al. (1961c) fed diets containing 10 or 20% protein to rats and obtained the same response in test periods of 3 and 7 days duration. They also found that rats fasted for 19 hours and then fed diets containing 10% protein of bread or fish flour origin had higher plasma lysine levels when fed fish flour. The response within protein was the same one and 3 days after introduction of the diet. When the diets were fed for two hours only there was no difference between proteins.

A broken-line response curve was not obtained and the amino acid requirement could not be determined by the ordinary procedure in the following cases: when McLaughlan and Illman (1967) fed rats pre-test and test diets for 3 days; when McLaughlan et al. (1961) fed rats lysine-deficient test diets for one or two days; and when Mitchell et al. (1968b) offered pigs the test diet for the first time on the evening before bleeding. Further, Zimmerman and Scott (1967a) did not obtain a broken-line response curve when chicks were fed an isolated soy-protein glucose diet for 4 days, fasted one day, fed a complete amino acid mixture for 3 days, and then fed the test diet ad libitum for 6 hours, or 1/12 of the feed every 30 minutes for 6 hours. The chicks were bled 4 hours after the 6 hour test period.

The evidence presented suggests that the critical length of the feeding period in the PAA assay is 3 to 5

days after the diet is introduced or the feeding method has been changed. Zimmerman and Scott (1967a), Mitchell et al. (1968b), and Stockland et al. (1970) suggested that this length of time is necessary for changes of the metabolic system responsible for protein synthesis to utilize all of the first limiting amino acid available in the blood and for depletion of labile endogenous sources of amino acids.

Effect of Short Term Fast.--Chicks fasted for 3, 6, 12, or 24 hours showed progressive accumulation of several amino acids in the blood (Zimmerman and Scott, 1967b). When a non-protein diet was fed before fasting, the concentration of EAA was below the levels noted when fasted. The plasma tryptophane concentration was unchanged in young adult men during 12 through 17 hours of an overnight fast (Young et al., 1971). After feeding, the tryptophane concentration dropped within 2 hours and was lowest at 3 hours, suggesting accelerated amino acid utilization for hepatic protein synthesis during absorption.

Growing rats fed superoptimal levels of lysine for one hour every 12 hours for 14 days and then bled 1, 2, 4, and 6 hours post feeding showed linear increases in lysine with increasing dietary lysine (Stockland et al., 1970), but plasma lysine levels after a 6 hour fast tended to be less than the responses obtained after 1, 2, and 4 hours of fasting, and the latter three were equal.

When animals are "metabolically adapted," uptake and incorporation of amino acids into tissue proteins is very rapid. This was clearly demonstrated by Neale and Waterlow (1974) when they gave ^{14}C-isotopes of lysine and leucine by stomach tube to rats fed a low casein diet. The highest specific radioactivity of CO_2 was found one hour after administration. If the fall-off was considered exponential, then the half-life was approximately 1.5 hours. Three hours after leucine isotope administration, protein-bound radioactivity in liver and muscle was 97.7 and 93.7%, respectively. For lysine, the respective values were 92.4 and 75.9%. At all other time intervals (3 hours to 15 days, 15 to 20 days, and 20 to 30 days) all radioactivity was protein-bound and not detectable in the free amino acid fraction.

Breaking Point.--Plasma lysine in chicks started to accumulate at a dietary level approximately 10% in excess of that required to maximize weight gain (Zimmerman and Scott, 1965). However, Mitchell et al. (1968b) concluded that deviations in calculated requirements determined by N-balance and PAA response were not larger than what could be explained by the experimental error, and that plasma data seemed to be the more sensitive of the two methods. Part of the discrepancies between the two methods may be related to an underestimation by the

N-balance method when dermal losses are not included (Young et al., 1971, 1972).

Carcass Analysis

Williams et al. (1954) stated that "an effective method of determining the requirements of a growing animal for these (essential) amino acids may be to determine, first, the requirement in grams per day of one amino acid, such as lysine, and then to estimate the requirements of the others from the proportion existing between the essential amino acids and lysine in the body of the animal, these proportions to be determined by amino acid assays of the entire carcass, or by amino acid assay of a dominant tissue such as muscle." The validity of the method was confirmed by Williams et al. (1954). They showed that the amino acid content of the whole carcass of rats, chicks, and pigs had comparable patterns of amino acids within each species at different stages of growth and also a remarkable similarity among species. The similarities in the amino acid pattern is also reflected in estimated requirements.

Urinary Urea

Brown and Cline (1974) suggested that total urinary urea excretion may indicate protein quality and assess amino acid requirements of swine and other non ruminants. This conclusion was reached because swine fed a corn diet

deficient in lysine showed a significant decrease in urinary urea when supplemented with graded levels of lysine. The decrease was linear, but the quadratic effect approached significance on day three.

Plasma Urea Nitrogen

Lambs fed 12 and 35% protein in a milk replacer showed higher levels of PUN than lambs fed a 24% protein diet (Bergen and Potter, 1975). Increased PUN in lambs fed 12% dietary protein was believed related to the catabolic state of the animals, whereas immediate degradation of excessive amino acids appeared to cause elevated PUN levels in lambs fed the 35% protein diet. The low PUN levels in lambs fed 24% dietary protein was explained by better utilization of the amino acids for anabolic purposes.

Brown and Cline (1974) and Williams and Smith (1975) showed that PUN levels decreased until the amino acid requirement was met and then remained constant when pigs and calves were fed increasing levels of dietary lysine and methionine, respectively. Williams and Smith (1975) showed good agreement between methionine requirements determined by PUN and PAA, but Brown and Cline (1974) concluded that urinary urea was more precise.

Amino Acid Oxidation

The validity of amino acid oxidation measurements in assessing amino acid requirements for growth (Brookes et al., 1972), and for maintenance (Neale and Waterlow, 1974) have been studied.

Brookes et al. (1972) injected growing rats with .4 μCi L-lys-U-^{14}C-hydrochloride (240 Ci/mole) in .5 ml of .9% saline by heart puncture and collected the expired air with respiration chambers. Release of $^{14}CO_2$ in the first 6 hours after injection was equal to at least 95% of the total expected for 24 hours. The amount of lysine oxidized remained at a low and relative constant level at suboptimal levels of dietary lysine and accumulated linearly at superoptimal intakes. The response seems analogous to that of PAA, but should be subject to less transient change since amino acid flux is large compared to pool size. The technique appears specific for the amino acid under study. Adaptation of the oxidative mechanisms was complete at day 3 of test diet introduction. The advantage of the oxidation technique over the growth assay is the time factor, although more expensive equipment is required; compared to the PAA method, oxidation is less complex mechanically.

In rapidly growing animals, size changes complicate the interpretation of amino acid requirement determined by growth. Of necessity growth data is interpreted more

broadly than estimates from amino acid oxidation or PAA (Brookes et al., 1972). Requirements determined by amino acid oxidation are usually somewhat lower than by growth assay.

Based on the assumption that all carbon chains of amino acids are completely oxidized and not converted to fat, and that the loss of amino acids in urine is negligible, Neale and Waterlow (1974) studied the amino acid requirement for maintenance of rats. They assumed that the maintenance requirement was the net rate of loss from the body which had to be replaced, i.e., the endogenous loss; and that such a loss could be measured by the output of $^{14}CO_2$. The endogenous rate of amino acid loss as isotope can be measured only if all the amino acids oxidized have the same specific activity. Uniform labelling was reached at day 20 after ^{14}C-labelled lysine or leucine had been given by stomach tube. Disadvantages to the method are that in order to determine the change in the amount of radioactivity retained in the body, it is necessary to make measurements on different groups of animals at different time intervals. This introduces uncertainties. The loss is the difference between two large values, and therefore cannot be measured very precisely. Due to day to day fluctuations in the endogenous loss of amino acids, caused by variations in physical activity and food intake, it was necessary to calculate

the rate of loss from the average excretion of $^{14}CO_2$ expressed as a fraction of the dose remaining in the body over the dose remaining in the body over the period of observation. The maintenance requirements determined by this method were so different from others that they could not be explained by experimental error (Neale and Waterlow, 1974), and prevention of coprophagy was suggested to increase the maintenance requirement.

Energy and Protein Requirements of Non Ruminating Calves

Nutrient requirements of growing domesticated animals must be considered in terms of age and weight, maintenance, maximum growth, highest retention of ingested nutrients, and the minimum cost per unit of product (Jacobson, 1969).

Energy and protein requirements and digestion in the milk-fed calf have been reviewed by Jacobson (1969), Roy (1970), Porter (1969), Huber (1969), ARC (1965), and Radostits and Bell (1970).

Energy and Milk Requirements of Calves Fed Milk

Requirements of energy for maintenance and growth, and of milk for young calves have been estimated by Roy *et al.* (1958, 1963, 1964), Blaxter and Wood (1951b, 1952a), Brisson *et al.* (1957), Bryant *et al.* (1967), and McGilliard *et al.* (1969; Jacobson, 1969) (Tables 1 and 2). There is

Table 1.--Energy requirement of calves fed milk; digestible energy per day.[a]

Source	Maintenance (per kg BW)	Growth (per g gain)
Blaxter and Wood, 1952a	53.8	--
Blaxter and Wood, 1952a	(79.5)[b]	--
Brisson et al., 1957	44.7	2.68[c]
McGilliard et al., 1969	41	3.82
Roy et al., 1964	--	3.02
Bryant et al., 1967	48.2[d]	3.70[d]
Mean	46.9	3.31

[a]DE = 46.9 BW + 3.31 G; where DE = digestible energy per day (kcal), BW = body weight (kg), G = gain per day (g).

[b]Per m^2 body surface at 2.5 times maintenance.

[c]Kcal per g gain.

[d]BWG = .269 DE - 12.96; at 3 to 7 weeks of age
BWG = .237 DE - 11.37; at 3 weeks of age
BWG = .265 DE - 13.44; at 7 weeks of age
where BWG = body weight gain (g/day), DE = digestible energy (kcal) per day.

Table 2.--Milk requirement of calves fed only milk; liters per day.

BW[a] kg	Gain; g/day			10% of BW[a]
	0	227	454	
27.2	2.15[b]	2.92	3.68	2.72
36.3	2.75	3.51	4.27	3.63
45.4	3.34	4.11	4.87	4.54
Equation[c]	2.64	3.66	4.68	--

[a]BW = body weight, kg

[b]Roy et al., 1958.

[c]Blaxter and Wood (1952a); G = 222.7 M - 588.75 where G = gain (g/day), M = milk per day (liters).

some variation in average energy requirement and the simple mean is slightly below that determined by Roy et al. (1958) by covariance analysis of data from 232 shorthorn and 92 ayrshire calves, and since revised by Roy et al. (1963) (Table 3). If the simple mean values in Table 1 are used in the formulas estimated by Bryant et al. (1967; Table 1), then the values in Table 3 by Roy et al. (1963) overestimate the daily gain and the calculated values underestimate it.

Protein Requirement

The protein requirement of calves has been determined in N-balance and growth response experiments. The

Table 3.--Digestible energy requirements of calves fed milk (kcal/day).

BW[c] kg	Gain; g/day							
	0		500		1000		1500	
50	2427[a]	(2345)[b]	4489	(4000)	6551	(5655)	8613	(7310)
75	4130	(3581)	6191	(5172)	8253	(6828)	10314	(8483)
100	5662	(4690)	7724	(6345)	9786	(8000)	11848	(9655)

[a]Roy et al., 1963.

[b]DE = 46.9 + 3.31 G (see Table 1).

[c]BW = body weight, kg.

N-balance studies have shown that the N retention increases with increasing amounts of dietary protein (Brisson et al., 1957); Blaxter and Wood, 1951c, 1952a; Lassiter et al., 1963; Bryant et al., 1967). Above 24% protein, increases in N retention are not significant. In growth studies, 20% dietary protein has generally been sufficient (Lassiter et al., 1963; Brisson et al., 1957; Cunningham et al., 1958; Huber et al., 1964; Bowman et al., 1965) (Table 4). However, when fed only 20% protein, calves slaughtered at 91 kg live weight had less edible carcass and more edible fat than those fed 25% protein (Bowman et al., 1965). Crane and Hansen (1965) found no effect of 10 or 20% fat in diets containing 24% protein when milk was fed ad libitum.

By the use of the following equation (ARC, 1965):

$$(5)\quad \log N = .966 \log BW - 1.518;\ (S.D. \pm .28)$$

where N = nitrogen, kg

BW = body weight, kg

it has been calculated that one kg carcass gain contains 2.5% N. If it is considered that a calf is gaining weight at the rate of .5 kg per day the N gain will be 12.5 g. This is an absolute minimum and slightly lower than estimated by the relationships in Table 5 (13.4 g/day). The positive N retention at zero gain (Table 5) suggests a pronounced ability to conserve protein in the young

Table 4.--Relationships between N retention and the daily supply and requirement of apparently digestible protein in calves.

Equation[a]	Source	Calculated requirement	
		ADN[a,b]	AP[a,b]
NB_H = .81 ADN[b] - 5.64	Blaxter and Wood, 1951c	23.5	147
NB_H = .81 ADN[b] - 4.22	Blaxter and Wood, 1951c	21.8	136
NB_L = .81 ADN[b] - 3.13	Blaxter and Wood, 1951c	20.4	128
NB = .972 ADN[b] - 11.0	Blaxter and Wood, 1952a	25.1	157
ADN[c] = 65.3 + 32.2 G	Brisson et al., 1957	19.4	121
BWG = .269 DE - 12.96	Bryant et al., 1967	--	---
NB = 6.87 DE - 292.86	Bryant et al., 1967	12.8	80
	McGilliard et al., 1969[d]	--	139

[a]Abbreviations: NB = N retention (g/day); ADN = apparently digestible N; BWG = gain (g/day); DE = digestible energy (kcal/kg BWG; G = gain (g/day); AP = available protein (ADN x 6.25); H = 22, M = 18, L = 14% protein.

[b]g/day

[c]mg/kg body weight

[d]In Jacobson, 1969.

Table 5.--Relationships between N retention and daily gain in calves.

Equation[a]	Source	NR[a,b,c]
$NR^c = .0262\ G^c + .703$	Blaxter and Wood, 1951c	13.8
$NR^d = 18.58\ G^d + 74.79$	Bryant et al., 1967	13.0
Mean		13.4

[a]Abbreviations: NR = N retention; G = body weight gain; BW = body weight.

[b]Calf: BW = 50 kg, G = .5 kg/day.

[c]g/day.

[d]mg/kg BW.

(Bryant et al., 1967). Other possible explanations are deposition of lean muscle tissue occurring concomitantly with depletion of lipid and glycogen (or water), as well as biological variation and experimental error (Bryant et al., 1967). The water balance becomes a very important factor, especially if diarrhea occurs.

The mean retention of 13.4 g N per day in a 50 kg calf gaining weight at the rate of .5 kg per day was applied to the inverse relationships between N retention and the daily requirement of (apparently) digestible protein (Table 4). Results are shown in Table 4 and are in good agreement with recommendations by Roy et al. (1970); but slightly below those when protein is supplied

through milk (13% DM, 25% of DM as protein) fed at the rate of 10% of BW. If the same feeding procedure were used with milk containing only 20% protein (DM basis) the diet would supply a little less than the calculated digestible N requirement (130 g/day). However, the utilization of calculated available protein is inversely related to the protein content in the diet (Blaxter and Wood, 1951c). Therefore, calculated values are in good agreement with the conclusion that 20% protein is sufficient for the milk-fed calf.

Endogenous Losses.--In order to estimate the N retention by the factorial method and the biological value (BV) of milk protein, it is necessary to know the loss of endogenous urinary N (UE), the loss of metabolic fecal N (MF), and the loss of N in hair and scurf (S).

The loss of UE was determined by Blaxter and Wood (1951a, b, c), Cunningham and Brisson (1957), and Roy et al. (1963, 1964); their results are very much alike (Table 6). When Blaxter and Wood (1951c) fed five ayrshire calves semisynthetic milk diets containing 22, 18, and 14% protein on a DM basis, rates of UE losses (slopes) were constant for the three percentages of protein, but the average UE loss increased with amount of protein in the diet. When calves were starved the loss of urinary N was much higher (250 mg/kg BW/day) than in mature

Table 6.--Loss of endogenous urinary N (UE) in calves.

Equation[a]	UE, mg N/kg[a] BW	UE, mg N/kg[a] $BW^{.73}$	Source
	81.9	---	Blaxter and Wood, 1951a
$UE_H = .21\ ADN_H + 5.30$	--	---	Blaxter and Wood, 1951c
$UE_M = .21\ ADN_M + 3.71$	--	---	Blaxter and Wood, 1951c
$UE_L = .21\ ADN_L + 2.13$	--	---	Blaxter and Wood, 1951c
	65.3	186	Cunningham and Brisson, 1957
	--	224	Roy et al., 1963
$UE = .262\ ADN + 3.38$	62.9	184	Roy et al., 1964

[a]Abbreviations: UE = endogenous urinary N, mg; ADN = apparently digestible N, g; BW = body weight; H = 22, M = 18, L = 14% protein in dry matter.

ruminants of the same body size (goat, sheep) or the same species (Blaxter and Wood, 1951b).

The excretion of metabolic fecal N (MF) in calves was determined by Harris and Loosli (1944), Lofgreen and Kleiber (1953), Blaxter and Wood (1951a, b, c), Cunningham and Brisson (1957), and Roy et al. (1963, 1964) (Table 7). The values obtained by Blaxter and Wood (1951a, c) and Cunningham and Brisson (1957) are as high as those determined for ruminating calves. These studies may have overestimated MF because N-free diets were fed as milk replacers, whereas Roy et al. (1963, 1964) used calves fed whole milk through 10 weeks of age. Lofgreen and Kleiber (1953) used ^{32}P-labelled casein. Blaxter and Wood (1951a)

Table 7.--Loss of metabolic fecal N (MF); g/100 g dry matter ingested.

MF	Source
.27	Lofgreen and Kleiber, 1953
.427	Blaxter and Wood, 1951a
N = 1.2 + .3 P[a]	Blaxter and Wood, 1951c
.334	Cunningham and Brisson, 1957
.19	Roy et al., 1963, 1964
.372[b]	Harris and Loosli, 1944

[a]Abbreviations: N = g N in dry feces; P = pct. of total cal. as protein.

[b]Ruminating calves.

suggested that the quantity of fecal DM rather than DM intake determines the excretion of MF.

The loss of N in hair and scurf have been estimated to be $.02BW^{.73}$ (g/day) (ARC, 1965). There is no information on the loss of N in sweat, but it seems unlikely to be of importance (ARC, 1965).

Digestibility and Biological Value of Milk Proteins

Digestible protein is more meaningful, if accurately determined, than total crude protein in rations for milk fed calves (Jacobson, 1969). Digestibilities of DM and protein in whole milk are high (Table 8), and not affected by age and amount of milk fed (Roy et al., 1964; Blaxter and Wood, 1952a; Bryant et al., 1967). The true digestibility of casein is high (Lofgreen and Kleiber, 1953), whereas the apparent digestibility of dried skim milk is lower than whole milk and shows a slight tendency to increase with increasing protein content of the diet (Lassiter et al., 1963; Blaxter and Wood, 1951a; Bryant et al., 1967; Bowman et al., 1965).

The estimated biological value of milk proteins varies from 32 to 79 for casein and from 60 to 92 for whole milk and dried skim milk (Blaxter and Wood, 1951c, 1952a, c; Brisson et al., 1957; Roy et al., 1963, 1964).

Table 8.--Apparent digestibilities of milk dry matter (DM) and milk protein in calves at different ages; %.

Protein Source		Digestibility DM	Digestibility Protein	Source
Whole milk	4 wk[a]	97.5	95.1	Roy et al., 1964
	7 wk	96.1	93.4	Roy et al., 1964
	10 wk	97.2	96.4	Roy et al., 1964
Dried skim milk	15.2[b]	87.0	78.6	Lassiter et al., 1963
	18.7	88.6	80.7	Lassiter et al., 1963
	24.1	88.5	86.2	Lassiter et al., 1963
	30.9	91.6	90.6	Lassiter et al., 1963
		94.0	--	Blaxter and Wood, 1951a
	3 wk[a]	--	86.1	Bryant et al., 1967
	7 wk	--	90.1	Bryant et al., 1967
Casein		--	93.5[c]	Lofgreen and Kleiber, 1953
N-free		77.0	--	Blaxter and Wood, 1951a

[a]Age in weeks.

[b]Percent protein in milk replacer.

[c]True digestibility.

Protein Sources in Milk Replacers

An increasing demand and price for fluid milk for human consumption has caused development of milk replacers (MR). The major protein source in MR is dried skim milk (DSM), but others have been investigated.

Mixing one part soy flour with nine parts of warm water was an early attempt to replace whole milk (Shoptow, 1936). When used in an otherwise nutritionally adequate diet replacement of up to 30% of the protein from DSM with soy flour normally resulted in inadequate gains and poor N retention, but results were variable (Noller et al., 1956; Porter and Hill, 1963; Neiman-Sorensen et al., 1965; Colvin and Ramsey, 1968, 1969; Gorrill and Thomas, 1967; Klausen et al., 1969). Soy flour is low in lysine and methionine compared to milk but supplements of these amino acids showed only slight improvement in MR containing 26 to 28% protein (Neiman-Sorensen et al., 1965; Klausen et al., 1969). Spray drying of 7 parts of soy flour with one part whey solubles was used with moderate success up to a maximum of 43% of the dry non-fat solids in MR, but supplemented methionine (.25%) had no effect on growth of calves (Stein and Knodt, 1954; Stein et al., 1954). When soy was subjected to acid (pH 4.0) or alkali for 5 hours at 37 C the nutritional value was markedly improved; but the mechanism of improvement was not identified (Colvin and Ramsey, 1968, 1969). When isolated soy

protein (71% protein) supplied 50% of the MR protein without supplemental methionine or 70% with methionine, weight gains were equal to those of calves fed a MR, where DSM or whole milk were the only protein sources (Gorrill and Thomas, 1967; Gorrill and Nicholson, 1969; Schmutz et al., 1967). Dry matter and N digestibilities of isolated soy protein were lower than for all the milk protein sources and were not affected by supplemental methionine (Gorrill and Nicholson, 1969). Calves fed isolated soy protein as the only protein source showed N retentions and digestibilities equivalent to casein (Porter and Hill, 1963). The difference between untreated soy flour and isolated soy protein may in part be attributed to a high content of soybean trypsin inhibitor (Gorrill and Thomas, 1967). Soy flour containing 50% protein and supplying 60% of the protein in MR caused weight loss, decreased activities of trypsin and chymotrypsin in the pancreas and intestinal contents, and decreased in vitro protein digestibilities. Isolated soy protein gave results equal to whole milk or replacers containing only milk protein.

Weight gains and apparent digestibilities of N, ether extract, and energy showed significant linear declines when increasing levels of distillers dried solubles (DDS) were substituted for DSM and lactose (Bryant et al., 1967). However, it was concluded that DDS

can replace 35% of the digestible protein in herd-replacement diets without severe impairment of growth.

Defatted fish flour substituted for up to 40% of the protein in a MR had no consistent effect on daily gains, incidence of diarrhea, or protein digestion or retention (Slade and Huber, 1965; Harshbarger and Gelwicks, 1965; Huber and Slade, 1967). However, at 60 to 67% replacement, marked decreases in growth and N utilization, and increases in diarrhea were observed; and at 100% death occurred (Huber and Slade, 1967). These data are in contrast to the equal gains in calves fed a commercial MR where defatted fish flour and whey powder were the only protein sources (Sorensen and Lykkeaa, 1968). These differences may be due to species differences in fishes used, heat damage which led to unavailability of lysine and sulfur amino acids, alkylation of sulfhydryl groups by 1,2-dichloroethane (DCE) to produce thioether linkages with a resultant decrease in cystine, methionine, and histidine, or a marked deficiency of vitamin E, which may be compounded by the presence of about 3% highly unsaturated fat (Morrison and Sabry, 1963; Morrison and Munro, 1965; Genskow et al., 1969; Makdani et al., 1971a, b, c). Supplements of histidine (.075%) and methionine (.20%) resulted in additive and parallel increments in growth of rats for all fish flours used, and extraction with isopropanol resulted in a more nutritious fish flour than

when DCE was used (Makdani et al., 1971a, b). Genskow et al. (1969) demonstrated that plasma levels of histidine and tyrosine decline, and that methionine and arginine increase when increasing levels of fish flour (zero to 100% of protein) are added to MR fed to calves.

Single cell protein from oil products supported the same rate of gain when substituted for 67% of the DSM protein in a MR containing 20% protein (Lykkeaa et al., 1973). When single cell protein replaced more than 67% of the protein, daily gain was significantly lower, probably due to a low methionine content (1.5 versus 2.4% in DSM protein).

From the evidence presented it may be concluded that lower digestibility, toxic residues, or inadequacy of essential amino acids or vitamins (due to genetic or preparational factors) make protein sources other than DSM inferior for milk replacers. Supplementation with the limiting amino acids, vitamins, and/or alkali treatment to increase digestibility or removal of toxic factors render alternate sources more suitable. However, most of these sources can be successfully used for partial replacement of milk protein.

Amino Acid Requirements

Calves.--The young non-ruminating calf is as dependent on its diet for EAA as is man, dog, or rat as clearly demonstrated by Blaxter (1950) and by Blaxter and Wood (1952c) when they fed calves dried skim milk, casein, or gelatin. The requirement of the individual EAA is virtually unknown. Patureau-Mirand et al. (1973) determined the methionine requirement of calves by combining results from two experiments where 12 calves were fed 2.6, 3.0 and 3.5, and 3.5 and 4.5 g methionine daily, as a supplement to commercial MR (24.5 and 26.4% protein). The methionine content in the blood (mg/100 g blood; Y) showed a curvilinear relationship with dietary methionine (X, mg met/kg $BW^{\cdot 73}$; $Y = .0883e^{6.12X}$; $r = .94$). From these results they concluded that calves gaining 1.0 kg or more BW per day require .58 g methionine per kg $BW^{\cdot 73}$. Expressed on a MR basis, that equals 9 g methionine per kg MR solids, or 3.5 g methionine per 16 g N when the protein content is 26.4%. They found no effect of graded levels of supplementary cysteine. Moreover, amino acid supplements had no significant effect on growth or feed intake.

Tzeng (1974) studied the requirement of methionine and lysine in non-ruminating calves. They fed MR where the protein was replaced by a mixture of crystalline amino acids. The requirement of methionine was determined to be

1.65% of DM by weight gain and N retention and 1.15% of DM by PAA. The requirement of lysine was 1.8, 1.75, and 1.95% of DM when determined by weight gain, N-retention, and PAA, respectively.

Williams and Smith (1975) estimated the total sulfur amino acid requirement in 2 non-ruminating calves fed successively increasing amounts of methionine (.8 to 4.8 g) as supplement to a diet containing .25 kg whole milk, .53 kg synthetic milk (Smith, 1959). Casein was omitted, and 270 ml aqueous solution of amino acids was fed per kg milk (.05 kg per kg BW). They obtained the typical broken-line response for plasma methionine, and the inverse curve for PUN. From the results obtained the total sulfur amino acid requirement was determined to be 4.2 to 4.8 g per day, or .23 to .26 g per kg $BW^{.73}$. It was concluded that cow's milk fed to promote .25 kg daily gain provides no surplus of sulfur amino acids (3.2 g met + 1.0 g cys).

The three experiments report widely differing requirements and suggest a need for additional information.

Factors Affecting Plasma Amino Acid Levels in Calves.--Leibholz (1966) fed calves a commercial MR from birth to 4 weeks of age and showed significant negative correlations between age and plasma levels of serine, proline, glutamine, methionine, leucine, lysine, and histidine. Proline declined very rapidly, and the remaining

amino acids showed no change with age. Williams and Smith (1973, 1975) found little variation with age.

Calves bled before the morning feeding at 10 am and at one hour intervals until 4 pm and thereafter every two hours until 10 am the following morning showed decreased concentrations between 10 am and one pm of total PAA and most individual amino acids (Williams and Smith, 1973, 1975). No marked change in total PAA was observed after the evening feeding at 5 pm.

The variation in plasma levels of methionine isoleucine, leucine, phenylalanine, tyrosine, and total PAA is significantly greater between animals than within animals in both non-ruminating and ruminating calves (Williams and Smith, 1973, 1974a, b, c, 1975).

Amino Acid Infusion in Ruminating Calves and Lambs.--Amino acids administered orally do not survive rumen degradation in lambs (Papas et al., 1974). However, intraperitoneal infusion into calves of a mixture of methionine, lysine, tryptophane, histidine, and arginine improved N-balance compared to a mixture of the remaining EAA or single constituents of the mixture (Hall et al., 1974). Williams and Smith (1974a, b, c) infused graded levels of methionine into calves (110 to 160 kg BW) fed 20 g N as decorticated, extracted groundnut meal. Plasma methionine was markedly increased in excess of 4.4 g L-methionine. Estimated flow of methionine and cysteine

from the rumen was 9.8 and 4.9 g per day, respectively. This led to an estimated total requirement of sulfur amino acids of 19.1 g per day, and is in good agreement with the estimated requirement of 22.4 g per day for 274 kg steers (Fenderson and Bergen, 1975). Infusion of lysine, threonine, or tryptophane did not show the broken-line response curves, suggesting that they were supplied in adequate amounts from rumen digesta under those circumstances (Williams and Smith, 1974c; Fenderson and Bergen, 1975).

Amino Acid Requirements of Other Species.--Recommended dietary levels of the EAA have been given for growth in rats, chicks, turkeys, and pigs (NRC 1971a, 1972a, 1973a), but are essentially unknown for mink, fox, cat, guinea pig, hamster, monkey, mouse, rabbit, horse, sheep, and cattle (NRC, 1968a, b, 1966, 1971b, 1972a, b, 1973b). Variations in requirements were discussed by Hegsted (1963). Table 9 shows recommendations as a percent of the diet and as a percent of the dietary protein. The variation in recommended needs of the various species, when expressed as percent of the dry feed, is almost completely eliminated when expressed as a percent of the dietary protein. Boomgaardt and Baker (1973) found that in chicks fed 14 to 23% dietary crude protein, the lysine requirement was constant when expressed as percent of the crude protein.

Table 9.--The amino acid requirements of rats, chicks, turkeys, and pigs.[a]

Age, wk	Rat	Chick (replacement)			Turkey		Pig	
		0-6	6-14	14-20	8-11	11-14	5-10[b]	10-20[b]
				Percent of dry diet				
Arg	.67	1.2	.95	.72	1.3	1.1	.28	.23
His	.33	.4	.32	.24	.45	.35	.25	.20
Ile	.61	.75	.6	.45	.85	.75	.69	.56
Leu	.83	1.4	1.1	.84	1.5	1.3	.83	.68
Lys	1.	1.1	.9	.66	1.2	1.0	.96	.79
Met[c]	.67	.75	.6	.45	.7	.58	.69	.56
Phe[d]	.89	1.3	1.05	.78	1.4	1.20	.69	.56
Thr	.56	.7	.55	.42	.8	.70	.62	.51
Trp	.17	.2	.16	.12	.2	.17	.18	.15
Val	.67	.85	.7	.5	.95	.80	.69	.56
				Percent of dietary protein				
Arg	5.2	6.0	5.9	6.0	5.9	5.8	1.3	1.3
His	2.5	2.0	2.0	2.0	2.0	1.8	1.1	1.1
Ile	4.7	3.8	3.8	3.8	3.9	3.9	3.1	3.1
Leu	6.4	7.0	6.9	7.0	6.8	6.8	3.8	3.8
Lys	7.7	5.5	5.6	5.5	5.5	5.3	4.4	4.4
Met[c]	5.2	3.8	3.8	3.8	3.2	3.1	3.1	3.1
Phe[d]	6.8	6.5	6.6	6.5	6.4	6.3	3.1	3.1
Thr	4.3	3.5	3.4	3.5	3.6	3.7	2.8	2.8
Trp	1.3	1.0	1.0	1.0	.9	.9	.8	.8
Val	5.2	4.3	4.4	4.2	4.3	4.2	3.1	3.1

[a]From NRC, 1971a, 1972a, 1973a.

[b]Weight, kg.

[c]Or 45% met and 55% cys.

[d]Or 55% phe and 45% tyr.

Blood Urea Nitrogen in Calves and Lambs

Increasing PUN with increasing levels of dietary protein (15.2 to 30.9%) have been reported for calves (Lassiter et al., 1963), and lambs (Potter and Bergen, 1974). The PUN also increases with increasing age in calves fed whole milk (Roy et al., 1964). Milk-fed calves had significantly higher PUN levels than ruminating calves fed zero to 55.6% of the dietary N as urea (Leibholz and Naylor, 1971). In calves fed a commercial MR, Leibholz (1966) found a sharp increase in PUN from birth to one week of age and then an almost linear decline until dry feed was introduced. These results are in contrast to the absence of age effects in grazing lambs, yearling- and adult-sheep (Torell et al., 1974), and calves fed whole milk at 35 and 84 days of age (Williams and Smith, 1973, 1975). Torell et al. (1974) and Williams and Smith (1973, 1975) found only slight changes with time of sampling after a meal and larger variations between animals than within animals.

A semisynthetic diet supplemented with graded levels of methionine led to marked and linear decreases in plasma urea when the supplement was suboptimal and to only low and almost constant responses at superoptimal levels of methionine (Williams and Smith, 1973, 1975).

MATERIALS AND METHODS

The objective of this study was to determine the effect of feeding graded levels of methionine to the young milk-fed calf in an attempt to learn more concerning its methionine requirement. Weight gain, digestibility, N-balance, plasma methionine, and plasma urea nitrogen were used in assessing methionine effects.

Animals.--Twenty male Holstein calves were employed in a four-week, two-period changeover experiment with five dietary levels of methionine. Thirteen of the calves were born in the M.S.U. dairy herd and 7 were bought from nearby Michigan dairy farms. Due to calf disease problems in the herd, compounded with stress of the metabolism stalls, 12 calves succumbed before completion of the experimental period. Results for these calves were omitted and the first calf born after the death of the original calf was used as a replacement. Scours from bacterial infections unrelated to treatments were the major reason for deaths among the calves (Table 10).

At the average age of 3.7 days (range, one to 9 days) the calves were confined to metal metabolism stalls at the M.S.U. Dairy Research Center. The calves were

Table 10.--Calves omitted from the experiment before completion due to poor health or mortality.

Block	Calf	Date of birth	Dam	Omission	
				Age, days	Remarks
1	285	Dec. 11, 1974	1251	23	Died; diarrhea.
2	126	Jul. 12, 1974	1302	3	Died; diarrhea.
4	269	Nov. 8, 1974	1254		Crippled front legs
5	284	Dec. 10, 1974	1321	5	Died; diarrhea.
6	275	Nov. 9, 1974	Bought	17	Died; diarrhea.
6	283	Dec. 4, 1974	1319	12	Died; diarrhea.
6	288	Dec. 18, 1974	1213	20	Died; diarrhea.
6	294	Dec. 31, 1974	1331	11	Died; diarrhea.
8	127	Jul. 12, 1974	1176	2	Died; diarrhea.
8	194	Sep. 21, 1974		8	Died; diarrhea.
9	290	Dec. 27, 1974	1154	13	Died; diarrhea.
9	295	Jan. 10, 1975	1195	18	Died; diarrhea.

blocked in groups of two according to date of birth, and each calf received two dietary levels of methionine; one during period 1 (9 to 15 days of age) and one during period 2 (21 to 27 days of age). The blocks were randomized as follows: block 3, 7, 8, 4, 10, 2, 6, 5, 1, and 9, before assignment of calves. The assignment of calves to blocks and treatments is given in Table 11 along with the calf's date of birth and the dam's identification number.

Diets.--The five dietary levels of methionine representing 75, 100, 125, 150, and 175% of the methionine in milk protein (treatments A, B, C, D, and E, respectively) were obtained by preparing experimental milk replacers containing 20% crude protein from a commercial, non-medicated milk replacer[1] (20% crude protein), crude lactose,[1] and crystalline amino acids.[2] The commercial milk replacer was prepared from dried skim milk, dried whole whey, animal fat, casein, premix of vitamins and minerals, and calcium carbonate (Table 12). Its assayed amino acid content showed good agreement with literature values for whole milk (Table 13) when determined by the method previously described by Makdam et al. (1971b).

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[1]Supplied by Milk Specialties Co., P. O. Box 278, Dundee, Illinois 60118.

[2]General Biochemicals, Chagrin Falls, Ohio.

Table 11.--Assignment of calves to blocks, date of birth, dam's identification number and treatments.

Block	Calf	Date of Birth	Dam	Period 1	Period 2	Remarks
1	292	Dec. 31, 1974	1196	A[a]	B	285[c]
	286	Dec. 13, 1974	1260	B	A	
2	273	Nov. 8, 1974	Bought	A	C	
	274	Nov. 9, 1974	Bought	C	A	126
3	125	Jul. 10, 1974	1227	A	D	
	128	Jul. 16, 1974	1147	D	A	
4	262	Oct. 29, 1974	1252	A	E	
	270	Nov. 11, 1974	1168	E	A	269
5	282	Dec. 1, 1974	1116	B	C	
	287	Dec. 14, 1974	1314	C	B	284
6	296	Jan. 22, 1975	1231	B	D	275, 283, 288, 294
	276	Nov. 10, 1974	Bought	D	B	
7	129	Jul. 20, 1974	1306	B	E	
	130	Jul. 31, 1974	1205	E	B	
8	259	Oct. 17, 1974	Bought	C	D	127, 194
	260	Oct. 17, 1974	Bought	D	C	
9	289	Dec. 21, 1974	1311	C	E	
	300[b]	Jan. 27, 1975	1328	E	C	290, 295
10	271	Nov. 3, 1974	Bought	D	E	
	272	Nov. 7, 1974	Bought	E	D	

[a]Treatment.

[b]Fed whole milk in period 1 due to lack of feed and amino acids. Data for calf 295 used in period 1.

[c]Number of calf replaced.

Table 12.--Composition of the milk replacer.[a]

Ingredient	% of DM
Dried skimmed milk[b]	32.0
Dried whole whey	43.8
Animal fat[c]	20.0
Casein[d]	3.0
Premix[e]	.6
Calcium carbonate	.6
Guaranteed analysis:	
Crude protein, not less than	20.0
Crude fat, not less than	20.0
Crude fiber, not more than	.15
Vitamin A, not less than	15,000[f]
Vitamin D_3, not less than	3,000[f]

[a]Supplied by Milk Specialties Co., P. O. Box 278, Dundee, Illinois 60118.

[b]37% protein.

[c]Preserved with BHA.

[d]Processed into a high fat ingredient before mixing.

[e]Soy lecithin, Polyoxyethylene glycol mono and dioleates, Vitamin A palmitate (stability improved), Vitamin D_3 source: D-activated animal sterol, Vitamin E supplement, Vitamin B_{12} supplement, Folic acid, Choline chloride, Riboflavin, Niacin, Calcium Pantothenate, Thiamine mononitrate, Calcium carbonate, Copper sulfate, Cobolt sulfate, Zinc sulfate, Ethylene diamine dihydroiodide, Sodium silico aluminate, Manganese sulfate, Magnesium oxide.

[f]USP units per lb.

Table 13.--The amino acid content in whole milk, commercial milk replacer[c] and prepared amino acid mixtures.[a]

Amino acid	Whole milk[b]		Milkreplacer[c,i]		Supplemental amino acid mixture[d]				
	Uncorr.	Corr.	Uncorr.	Corr.	A	B	C	D	E
EEA									
Arg	3.40	3.25	3.67	3.44	———		3.44	———	
His	2.78	2.66	2.47	2.31	———		2.31	———	
Ile	5.78	5.52	7.27	6.81	———		6.81	———	
Leu	9.49	9.07	9.21	8.62	———		8.62	———	
Lys	8.25	8.14	9.10	8.52	———		10.66[e]	———	
Met	2.66	2.54	2.65	2.48	0	2.48[g]	4.96	7.44	9.92
Phe	4.29	4.10	4.99	4.67	———		4.67	———	
Thr	3.92	3.75	5.19[f]	4.86	———		4.86[h]	———	
Trp	1.31	1.25	--	1.23	———		1.23	———	
Val	6.48	6.19	6.85	6.41	———		6.41	———	
Total EAA	48.36	46.47	51.40	49.35					
NEAA									
Ala	3.39	3.24	3.89	3.64	———		none	———	
Asp	6.98	6.67	8.86	8.30	———		none	———	
Cys	.77	.67	1.12	1.05	———		1.05	———	
Glu	22.34	21.35	19.38	18.14	37.02	35.80	34.58	33.35	32.13
Gly	1.91	1.83	2.23	2.09	18.89	18.23	17.64	17.02	16.39
Pro	9.82	9.39	9.07	8.49	———		none	———	
Ser	5.43	5.19	5.10	4.77	———		none	———	
Tyr	5.63	5.38	4.45	4.17	———		4.17	———	

Table 13.--Continued.

Amino acid	Whole milk[b]		Milkreplacer[c,i]		Supplemental amino acid mixture[d]				
	Uncorr.	Corr.	Uncorr.	Corr.	A	B	C	D	E
Total NEAA	56.27	53.72	55.41	50.65					
Total AA	104.63	100.19	106.81	100.00					

[a]g per 16 g N.

[b]Source: NRC, 1969; NDC, 1965; Smidth, 1971.

[c]Supplied by Milk Specialties Co., P. O. Box 278, Water and Illinois Sts., Dundee, Illinois 60118.

[d]General Biochemicals, Chagrin Falls, Ohio. Grade L-amino acids assayed in accordance with the methods outlined in National Research Council Publication No. 719.

[e]L-lysine, HCl. [f]Not determined.

[g]Methionine free. [h]Allo free.

[i]Average of three determinations. Dr. W. G. Bergen, Ruminant Nutrition Laboratory, Animal Husbandry, Michigan State University.

A mixture containing 15% crude protein was prepared from 75% commercial milk replacer and 25% crude lactose. The 5% additional protein was added as a mixture of L-crystalline amino acids. The amino acid mixture contained the EAA's, cysteine, and tyrosine in the same ratios as determined for the commercial milk replacer. The remaining non-essential N was formulated from 50% glycine-N and 50% glutamic acid-N (Table 13). In order to make the experimental milk replacers isonitrogenous, the content of glycine and glutamic acid decreased with increasing levels of methionine. Amino acids were first weighed[1] and mixed; then they were added to the appropriate amount of crude lactose. This mixture was then blended with the commercial milk replacer in a batch mixer, and stored at room temperature.

Feeding Schedule.--The calves were fed colostrum for 3 days and whole milk until placed on experimental diets which were the only feed from 6.5 days (range 4 to 9 days) to 28 days of age. One to two days were used for a gradual change from whole milk to the experimental replacer and from the treatment of period 1 to that of period 2. Each dietary change was followed by a 3-day adjustment period. Periods 1 and 2 were from 9 to 15 and 21 to 27 days of age, respectively (Table 14). Milk

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[1]Sartorious scale.

Table 14.--Plan of feeding, weighing, collection, and blood sampling.

Age days	Treatment	Weighing	Total collection urine + feces	Blood samples	Remarks
	Birth				
1	Colostrum				
2	Colostrum				
3	Colostrum	+			
4	3M + 2A[a,c]	+			Adjustment
5	2M + 3A	+			Adjustment
6	5A				Adjustment
7	A				Adjustment
8	A	+			Adjustment
9	A	+	+	+	Period 1
10	A	+	+		Period 1
11	A		+	(+)[b]	Period 1
12	A		+		Period 1
13	A		+	(+)	Period 1
14	A	+	+		Period 1
15	A	+	+	+	Period 1
16	3A + 2B[c]	+			Adjustment
17	2A + 3B				Adjustment
18	5B				Adjustment
19	B				Adjustment
20	B	+			Adjustment
21	B	+	+	+	Period 2
22	B	+	+		Period 2
23	B		+	(+)	Period 2
24	B		+		Period 2
25	B		+	(+)	Period 2
26	B	+	+		Period 2
27	B	+	+	+	Period 2
28	B	+			

[a]Parts of total feed.

[b]Were collected in the first six calves only.

[c]Abbreviations: M = whole milk, A = treatment A, B = treatment B.

replacer was diluted with water to 13% solids and was fed by nipple pail at 10% of body weight daily in two equal meals (5% each) at 6 am and 6 pm. At each feeding the replacer powder was mixed with warm water (27 C) by use of a hand beater. Initially, the calves had free access to water (calves 125, 128, 129, 130, 259, and 260), but later additional water was restricted to .5 to 1.5 liters after each meal, because some calves became nibblers and would not drink their allotted feed at the following feeding. The first .5 liters was used to wash down milk residue in the nipple pails.

Body Weight.--Body weight of the calves was determined on three consecutive days at the beginning and at the end of each experimental period (Table 14).

Nitrogen Balance.--The calves were confined to metal metabolism stalls from 4 to 28 days of age during which all urine and feces voided were collected daily (Table 14). Urine was collected in plastic buckets containing about 30 ml 33% sulfuric acid. Feces was collected in open trays placed underneath the calves. All milk weigh back, urine, and feces were refrigerated until the last sampling in each period, whereupon composited samples were frozen at -20 C until analysis. The nitrogen content in feed, milk weigh back, urine, and feces was determined by the Kjeldahl method.

Blood Collection and Processing.--In the first 6 calves blood was collected at 9, 11, 13, and 15 days of age in period 1, and at 21, 23, 25, and 27 days of age in period 2. In the remaining calves blood was collected at 9 and 15 days of age in period 1, and 21 and 27 days of age in period 2 (Table 14). The number of collection days was reduced because initial analyses showed only slight differences between days in plasma methionine levels, the stress introduced by blood sampling would be less, and the high number of analyses decreased. On each day of sampling blood was collected before feeding and 1, 2, 4, and 6 hours after feeding. Each sample made up 20 ml.

In the first four calves (calf 125, 128, 129, and 130) a permanent jugular catheter was established. Because of difficulty in maintaining the cannula in metabolism stalls the remaining calves were collected by jugular puncture.[1] After collection, blood was transferred to 10 ml evacuated tubes[2] containing 37 mg potassium oxalate as anticoagulant and 37 mg sodium fluoride as glycolysis inhibitor. The tubes were gently inverted and placed in an ice bath until processing.

Blood processing was initiated 7 to 10 hours after collection of the first sample. From inverted tubes, whole

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[1]Single Draw Vacutainer Needle (silicone coated, 20 gauge). Becton-Dickinson, Rutherford, N.J. 07070.

[2]Vacutainer, non-sterile, 10 ml. Becton-Dickinson, Rutherford, N.J. 07070.

blood was transferred to hematocrit tubes and the remaining whole blood was centrifuged at 6000 x g for 10 minutes at 0 C.[1] Plasma was pipetted into centrifuge tubes, mixed with .1 ml 50% sulfosalicylic acid and .1 ml Nor-leucine (1 mM) standard per ml plasma, and placed in ice bath from 30 minutes to 2 hours before centrifugation at 35,000 x g for 15 minutes at 0 C. The protein-free supernatant was frozen until analyses. A sample of unprocessed plasma was also frozen for later analysis.

Plasma was analyzed for methionine, valine, leucine, and isoleucine by ion-exchange chromatography on an amino acid analyzer[2] by Dr. W. G. Bergen.[3]

Unprocessed plasma was assayed for urea nitrogen by the method described by Fawcett and Scott (1960), and since modified by Kulasek (1972), using the reagents described by Okuda et al. (1965).

Hematocrit was determined by centrifugation of whole blood in standard hematocrit tubes in an International centrifuge at 65% of maximum speed for 10 minutes by Universal timer.

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[1]Sorvall Superspeed RC2-B Automatic Refrigerated Centrifuge.

[2]TSM-1 amino acid analyzer,[R] Michigan State University.

[3]Ruminant Nutrition Laboratory, Animal Husbandry, Michigan State University.

Experimental Design and Statistics.--The experiment was conducted as a two-period, changeover design with five dietary levels of methionine and 20 calves in 10 two x two latin squares. In each square two calves represent rows and two periods represent columns. All data with equal numbers were analyzed statistically according to the model (6) described by Gill and Magee (1976).

$$(6)\quad \hat{Y}_{ijk} = \mu + D_i + \rho_j + \tau_k + E_{(ijk)}$$

where $\hat{Y}_{ijk}$ = observation in i th calf in j th period on k th treatment,

D_i = effect of i th calf,

ρ_j = effect of j th period,

τ_k = effect of k th treatment, and

$E_{(ijk)}$ = error

Treatment means (adjusted for differences in individual calf means) were calculated as described (7) by Gill and Magee (1976):

$$(7)\quad \bar{y}'_k = \bar{y}\ldots + \hat{\tau}_k$$

where $\bar{y}'_k$ = adjusted mean for the k th treatment,

$\bar{y}\ldots$ = overall mean,

$\hat{\tau}_k = [2(t-1)\ \bar{y}..._k - \sum_{i(k)}^{2(t-1)} \bar{y}_i..]/t$,

t = number of treatments

$\bar{y}..._k$ = observed (unadjusted) mean for the k th treatment, and

$\bar{y}_{i..}$ = observed mean for the i th calf among those given the k th treatment in either period.

Unadjusted treatment means are not reported. Differences between adjusted treatment means were tested by Dunnett's t-test, using treatment B as control (Kirk, 1968).

The relationship between dietary methionine levels and assay parameters was analyzed by multiple regression analysis according to a third degree polynomial (8) and its reduced forms:

$$(8)\quad \hat{Y} = \hat{\beta}_0 + \hat{\beta}_1 x + \hat{\beta}_2 x^2 + \hat{\beta}_3 x^3$$

where $\hat{Y}$ = predicted assay response, and

x = relative dietary methionine level (100 = 2.48 g met/16 g N).

The procedure was as follows: The relationship was determined for dietary methionine level and the adjusted mean for the parameter in question if the overall treatment effect was significant ($P < .10$). If the regression so determined was significant ($P < .25$), then the regression analysis was repeated on the full data set after observations had been adjusted according to equation (9):

$$(9)\quad Y_{adj.\ obs.} = Y_{obs.} - (\bar{Y}_{obs.\ trt.} - \bar{Y}_{adj.\ trt.})$$

where $Y_{adj.\ obs.}$ = adjusted observation,

$Y_{obs.}$ = observed observation,

$\bar{Y}_{obs.\ trt.}$ = observed treatment mean, and

$\bar{Y}_{adj.\ trt.}$ = adjusted treatment mean.

Regression coefficients different from zero ($P < .05$) were then selected and a final prediction equation was calculated. If the relationship was curvilinear, minimum and/or maximums were determined according to (10) for quadratic regressions and approximated according to (11) for regressions involving only linear and cubic terms:

(10) $X_{min/max} = -(\hat{\beta}_1/2\hat{\beta}_2)$, and

(11) $X_{min/max} \simeq \pm \sqrt{\hat{\beta}_1/3\hat{\beta}_3}$ (ignoring imaginary part of root, $\sqrt{-1}$).

RESULTS

Body Weight, Daily Gain, and Health.--Body weights (BW) at the beginning and at the end of the experimental period, as well as average daily gain (ADG) and fecal score (FS) are given in Table 15, and the corresponding analyses of variance is in Table A1.

The average initial BW was 43.2 kg and did not differ between treatment groups. However, the initial BW varied among calves ($P < .001$), and was lower in period 2 than in period 1 ($P < .01$). The lower BW at the beginning of period 2 can probably be explained by poor health and weight loss in period 1 as will be discussed later. The average final BW was 42.8 kg and tended to differ between treatment groups ($P < .10$). The final BW varied among calves ($P < .001$), but there was no difference between periods. Multiple comparisons among treatment means showed that the calves in group E weighed less than the calves in group A, C, and D ($P < .05$), but group B did not differ from any other group.

The ADG tended to differ between treatment groups ($.25 > P > .10$), and was less in period 1 than in period 2 ($P < .10$). There were no significant differences among

Table 15.--The effect of dietary[c] methionine[d] on body weight (initial and final), average daily gain, and fecal score of calves fed graded levels of methionine.

	Treatment					s.e.	Overall Mean	Period	
	A	B	C	D	E			1	2
Body weight, kg									
Initial	43.7[a]	42.8[a]	42.7[a]	43.1[a]	43.8[a]	± .5	43.2	43.7	42.7
Final	43.6[a]	42.3[a,b]	43.1[a]	43.1[a]	41.6[b]	± .4	42.8	42.6	42.9
Avg. daily gain, g	-12[a]	-72[a]	59[a]	12[a]	-306[b]	± 94	-64	-168	41
Fecal score	2.22[a]	2.31[a]	1.89[a]	1.98[a]	2.78[a]	±.33	2.24	2.94	1.54

[a,b]Means sharing a common superscript are not significantly different ($P < .05$).

[c]Relative Met conc: 100 = 2.48 g Met/16 g N.

[d]Fecal score = $[(1) D_1 + (2) D_2 + (3) D_3 + (4) D_4 + (5) D_5] / 7$,

where D_1 = no. of days with < 250 g feces,
D_2 = no. of days with 251 to 500 g feces,
D_3 = no. of days with 501 to 1000 g feces,
D_4 = no. of days with 1001 to 2000 g feces, and
D_5 = no. of days with > 2000 g feces.

calves. The weight loss in period 1 explains the lower initial weight in period 2 than in period 1, and the low ADG in period 2 explains why the final BW was the same in period 1 and 2. Multiple comparisons among treatment means for ADG showed that group E gained less (or lost more) than any other group ($P < .05$), and that differences between groups A, B, C, and D were not significant.

A FS was calculated for each calf according to the formula in (12):

$$(12)\quad FS = [(1)D_1 + (2)D_2 + (3)D_3 + (4)D_4 + (5)D_5]/7,$$

where FS = fecal score

D_1 = number of days with < 250 g feces,

D_2 = number of days with 251 to 500 g feces,

D_3 = number of days with 501 to 1000 g feces,

D_4 = number of days with 1001 to 2000 g feces, and

D_5 = number of days with > 2000 g feces.

The analysis of variance showed no significant differences between treatment groups or between calves, but the FS was higher in period 1 than in period 2 ($P < .001$), and may in part explain the low ADG and final BW for period 1.

The relationship between dietary methionine and final BW, ADG, and FS, respectively, was determined by the described procedure for multiple regression analyses of the full model and its reduced forms. The determined models for adjusted means and the calculated regressions

for adjusted observations are given in Table 16, and the analysis of variance is in Table A2. The calculated regressions showed no significant correlation between dietary methionine levels and final BW or FS. For ADG the analysis showed relatively equal significance levels for the regression coefficients of the full model. The prediction equation for this relationship showed that 26% of the variation in ADG was due to dietary methionine levels. However, only the regression coefficient of the quadratic term was significant, and accordingly, only 10% of the variation in ADG was due to dietary methionine.

As may be seen from Table 15 the trends for ADG and FS tend to indicate decreasing ADG with increasing frequency of scouring (increasing FS = increasing fecal excretion). Therefore, the relationship between ADG and fecal excretion was determined for uncorrected observations by multiple regression analyses of a third degree polynomial and its reduced forms. The results are given in Table A3, and show that 56% of the variation in ADG was due to variation in fecal excretion (scouring). Further, fecal excretion was found to account for 14% of the variation in urine excretion (Table A4), and urine excretion accounted for 46% of the variation in ADG (Table A5). Therefore, a combined relationship between ADG and fecal and urine excretion is suggested from the multiple regression analyses. A prediction equation was:

Table 16.--Estimated relationship between dietary methionine levels (X,[a] %) and average daily gain (ADG, g) in calves. Adjusted observations.[b]

Model determined from adjusted means		Calculated models from adjusted observations				
		Regression coefficients				
Model	Sign. of β_i	β_0	β_1	β_2	β_3	R
(3)	P < .25	3208.	-92.52[c]	.8452*	$-.2465(10^{-2})$[c]	.51*
(2)	P < .25	-951.2	17.80[c]	$-.7924(10^{-1})$*	--	.44*
(1)	P < .25	88.75	--	$-.9035(10^{-2})$*	--	.32*

[a]Relative Met conc: 100 = 2.48 g Met/16 g N.

[b]Adjusted observation = Observation - (Observed mean - Adjusted mean).

[c]P < .10.

*P < .05.

Model (3): $Y = \beta_0 + \beta_1 x + \beta_2 x^2 + \beta_3 x^3$

Model (2): $Y = \beta_0 + \beta_1 x + \beta_2 x^2$

Model (1): $Y = \beta_0 + \beta_2 x^2$

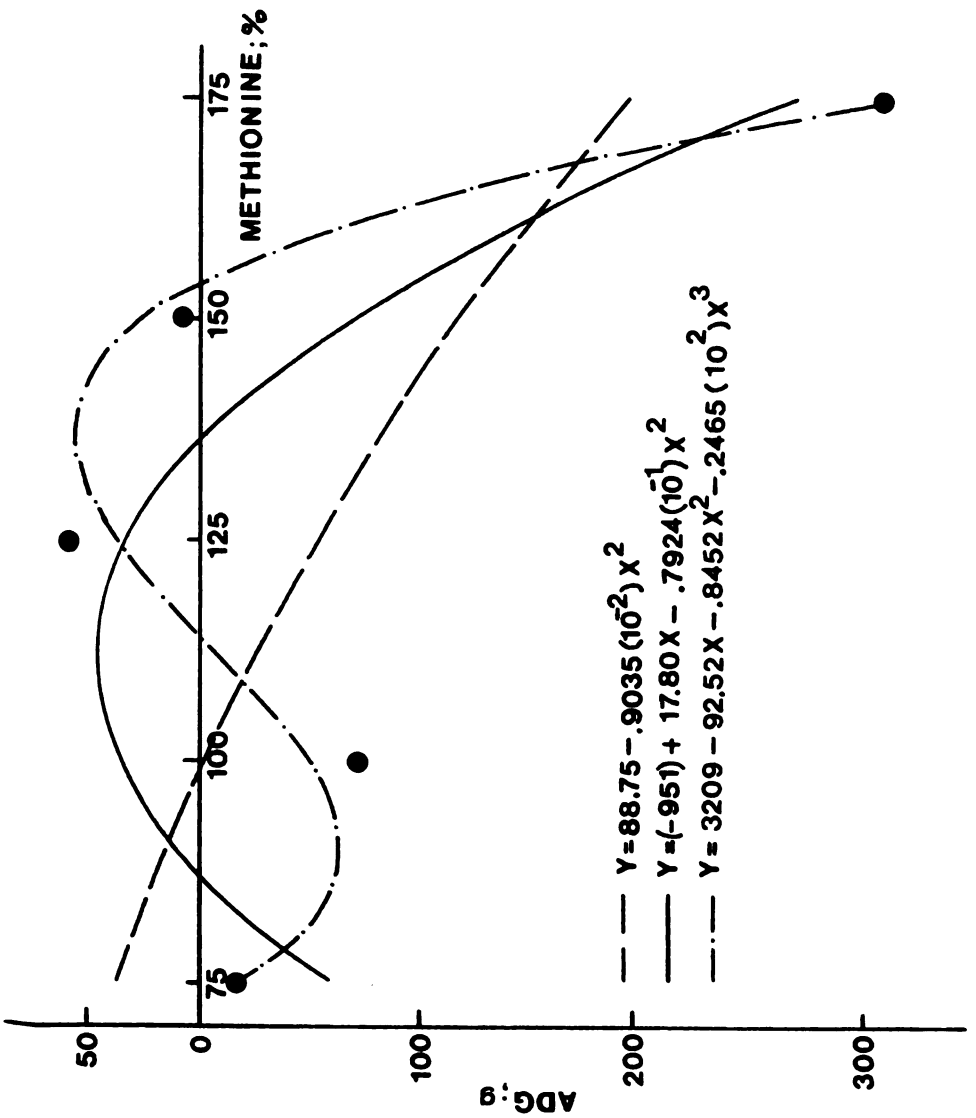

Fig. 1.--The relationship between graded levels of methionine (X) and average daily gain (ADG, Y) in calves.

$$\hat{ADG} = -274.8 - 110.8\ F + 16.34\ F^2 - .7450\ F^3 + 24.76\ U - .2753\ U^2;\ R = .88,$$

where $\hat{ADG}$ = average daily gain (g),

F = fecal excretion in period (kg), and

U = urine excretion in period (liters).

The equation showed that 77% of the variation in ADG can be explained by variation in fecal and urine excretion. It also shows that the ADG decreases with increasing fecal excretion (scouring), and that a compensatory decrease in urine excretion in milder cases of scouring prevents a dramatic decrease in ADG.

It was often observed that scouring calves did not respond to common oral and injected antibiotics (CIBA scour powder, Entefur tablets, Lingomycin,Combiotic, and Penecillin). A fecal swab from one calf showed infection with Kleibsiella bacteria which is resistant to all common antibiotics except Gentamycin and Polymyxin B. Therefore, it may be concluded that weight losses, poor health (scouring), and high death losses in calves before two weeks of age were largely due to bacterial infections of the gastrointestinal tract, which tended to mask dietary methionine effects. When the full model for the relationship between dietary methionine levels and ADG was reduced to the quadratic model, dietary methionine levels still accounted for 20% of the variation in ADG (Table 16). This

model shows maximum daily gain at the 115% level of methionine; equivalent to 2.85 g per 16 g N.

Digestibility.--The average dry matter (DM) content in milk replacer, milk weigh back, and feces are given in Table 17 along with the digestibilities for DM and crude protein (CP). The corresponding analyses of variance are given in Table A6.

The DM content in milk replacer and milk weigh back varied among calves ($P < .05$), and the DM content in milk replacer was higher in period 1 than in period 2 ($P < .01$). The differences among calves and between periods for milk replacer may be explained by a variable number of feed batches per treatment, whereby a different number of calves per group received the various batches. For DM in milk weigh back, differences among calves may be due to variable amounts of milk weigh back (Table A9). The DM content in feces was higher in period 2 than in period 1 ($P < .001$) and may be explained by more severe scouring in period 1 than in period 2 (Table A1). The DM content in milk replacer, milk weigh back, and feces did not differ among treatment groups.

The digestibility of DM did not vary among calves, but tended to be higher in period 2 than in period 1, and to differ among treatments ($P < .10$). Multiple comparisons among treatment means showed that the digestibility for

Table 17.--The effect of dietary methionine levels[a] on the digestibility of dry matter (DM) and crude protein (CP) and related factors in calves.

	Treatment					s.e.	Overall Mean	Period	
	A	B	C	D	E			1	2
DM content, %:									
Milk replacer	97.80	97.76	97.80	97.77	97.62	± .07	97.75	97.85	97.65
Milk weigh back	75.59	50.70	67.00	67.60	29.29	±15.25	58.04	58.01	58.06
Feces	19.04	16.46	17.96	18.21	13.97	± 2.83	17.13	11.26	23.00
Digestibility, %:									
Dry matter[b]	87.3[a,b]	79.4[a,b]	88.2[a]	86.8[a,b]	75.3[b]	± 3.6	83.4	80.7	86.1
Crude protein	72.8[a,b]	59.6[a,b]	69.0[a,b]	73.4[a]	52.9[b]	± 5.3	65.5	59.4	71.7

[a]Relative methionine concentration: 100 = 2.48 g per 16 g N.

[b]Means not sharing a common superscript are significantly different ($P < .05$).

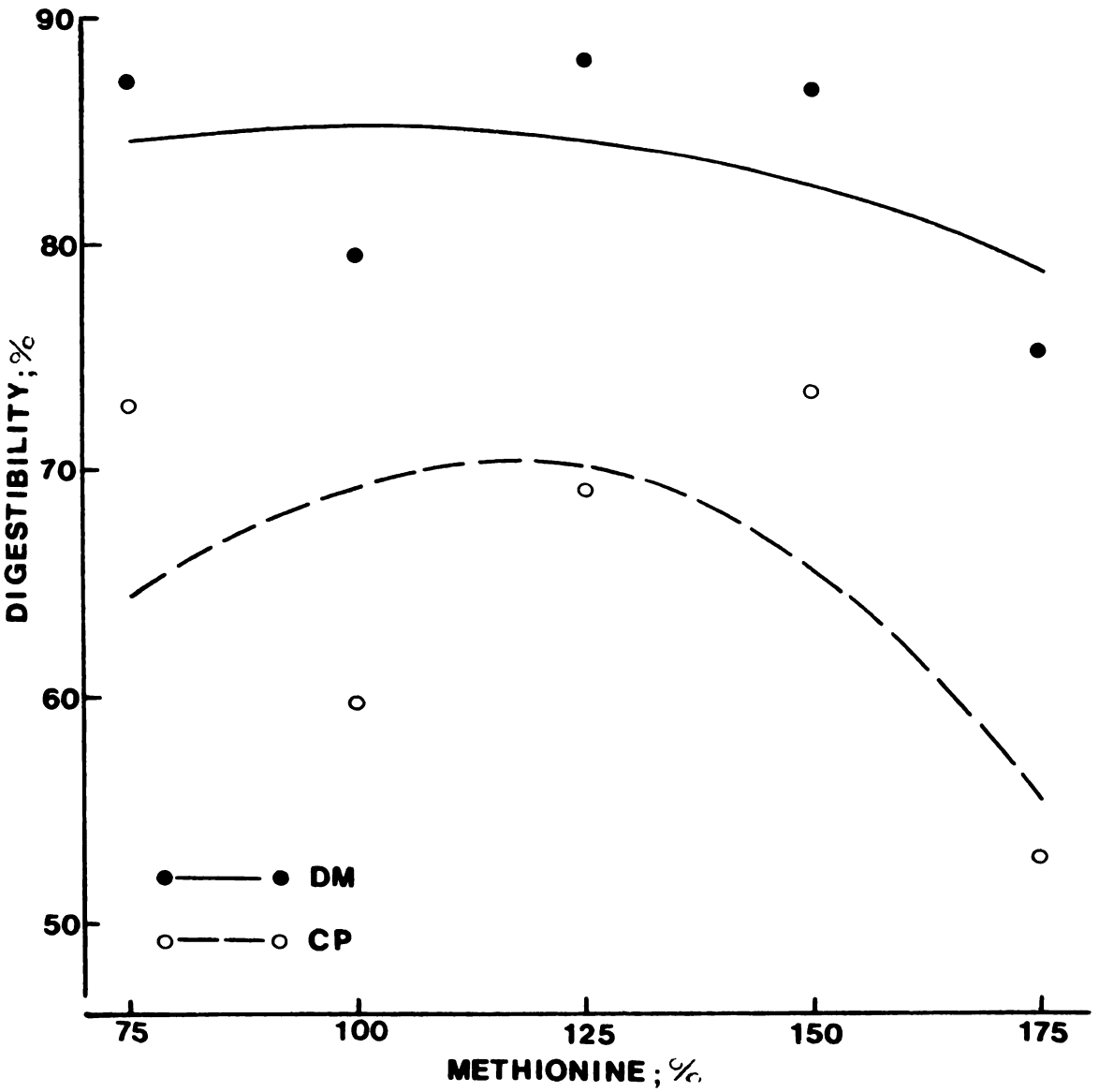

Fig. 2.--The relationship between dietary methionine levels and the digestibility of dry matter (DM) and crude protein (CP) in milk replacers for calves.

group C was higher than for group E ($P < .05$) but none of the differences among other groups were significant.

The digestibility of CP did not differ among calves but was higher in period 2 than in period 1 ($P < .01$), and tended to differ among treatments ($P < .10$). Multiple comparisons among treatments showed that the digestibility was higher for group D than for group E ($P < .05$). None of the differences between other groups were significant.

The relationship between dietary methionine levels and the digestibilities was analyzed by multiple regression, and the results are given in Table A7. For the digestibility of DM only the linear and cubic terms are significant, and only 6% of the variation is due to dietary methionine. The prediction equation showed a maximum dry matter digestibility when methionine was 101% of that in milk. This is equivalent to 2.5 g per 16 g N. For the digestibility of CP dietary methionine levels accounted for 20% of the variation when the full model was used. However, only the cubic term was significant, and if the model was reduced to the linear and the cubic terms, dietary methionine accounted for only 11% of the variation in CP digestibilities. This prediction equation showed a maximum at the 117% level; equivalent to 2.90 g per 16 g N.

Since scouring was a problem and digestibilities may be expected to decrease with increased defecation, the

relationship was determined by multiple regression of a third degree polynomial and its reduced forms (Table A8). For DM and CP 34 and 56% of the variation in digestibility, respectively, was due to variation in fecal excretion. In healthy calves 89 and 77% of the DM and CP, respectively, were digested. The respective values declined linearly at the rate of 1.3 and 2.7% per kg increase in fecal excretion.

Nitrogen Balance.--Data on nitrogen balance (N-balance) and related parameters are given in Table 18, and the corresponding analyses of variance in Table A9. The amount of milk replacer powder varied among calves ($P < .001$), but did not differ between periods or treatments. The variation in feed intake among calves may be explained by the difference in BW of calves, since prepared milk replacer containing 13% solids was fed at 10% of BW throughout the trial. The volume of milk weigh back tended to vary among calves ($P < .10$), but no significant differences were found between periods or among treatments. The volume of urine varied among calves ($P < .01$) and was larger in period 2 than in period 1 ($P < .05$). Correspondingly the amount of feces was greater in period 1 than in period 2 ($P < .001$). The amount of feces did not vary significantly among calves, and neither the volume of urine nor the amount of feces differed significantly among treatment groups. The higher urine

Table 18.--The effect of dietary methionine[c] (%) on nitrogen balance and related parameters of calves.

	Treatment					s.e.	Overall Mean	Period	
	A	B	C	D	E			1	2
Fresh volume									
Milkreplacer, g[d]	3955[a,b]	3899[a,b]	3876[a]	3874[a]	3995[b]	± 33	3920	3913	3927
Milk weigh back, ml[e]	953	1731	1183	1226	4049	±1044	1828	1661	1996
Urine, ml	28774[a,b]	29286[a,b]	31388[a]	30425[a,b]	18594[b]	±3523	27693	24654	30732
Feces, g	3754	4618	2669	3795	7609	±1453	4489	7014	1964
N concentration, %									
Milkreplacer[d]	2.919[a]	2.917[a]	2.887[a,b]	2.900[a,b]	2,842[b]	±.018	2.893	2.906	2.880
Milk weigh back[e]	.028	.197	.066	.070	.113	±.049	.095	.093	.097
Urine	.239	.328	.204	.232	.462	±.081	.293	.381	.254
Feces	1.306	1.289	1.402	1.316	1.060	±.235	1.275	.817	1.733
N content, g									
Milkreplacer[d]	115.5	113.9	112.0	112.4	113.6	± 1.3	113.5	113.7	113.2
Milk weigh back[e]	.5	6.5	2.1	2.4	7.6	± 2.5	3.8	2.8	4.8

Table 18.--Continued.

	Treatment					s.e.	Overall Mean	Period	
	A	B	C	D	E			1	2
Urine	59.2	67.6	61.2	48.0	72.2	± 8.6	61.7	61.9	61.4
Feces	30.4[a,b]	38.6[a,b]	31.7[a,b]	27.4[a]	46.4[b]	± 5.0	34.9	43.4	26.4
N balance, g	25.3[a,b]	1.2[a,b]	16.9[a,b]	34.6[a]	-12.6[b]	±11.7	13.1	5.6	20.6

[a,b]Means sharing a common superscript are not different ($P < .05$).

[c]Relative Met conc: 100 = 2.48 g Met/16 g N.

[d]Before dilution to 13% solids.

[e]After dilution to 13% solids.

volume in period 2 than in period 1, and the lower amount of feces in period 2 than in period 1, may be explained in part by the apparent inverse relationship between the volume of urine and feces, and a decreased incidence of scouring in period 2 compared to period 1 (Tables 15 and A4).

The N concentration in milk replacer varied among calves ($P < .01$), and tended to be higher in period 1 than in period 2 ($P < .10$), and to differ among treatment groups ($P < .10$). Multiple comparisons among treatment means showed that milk replacer E contained less N than milk replacer A and B ($P < .05$). The differences in the N concentrations in milk replacers may be due to a variable number of batches per treatment group (Group A, B, C, D, and E: 3, 3, 2, 3, and 2 batches, respectively), and a different N concentration in commercial milk replacer used for batches one and two, compared to three (20.1 versus 18.1% crude protein in dry matter). The different numbers of batches in different treatment groups was caused by the high loss of calves for certain treatments and the resulting necessity of preparing new feed for the replacement calves. The crude protein content in the milk replacers was equivalent to 18.24, 18.23, 18.04, 18.13, and 17.76% of the dry matter in ration A, B, C, D, and E, respectively. This was lower than the planned 20%. The N concentrations in milk weigh back and urine did not differ between calves, periods, or treatments. The fecal N concentration was lower in period 1

than in period 2 ($P < .001$), but no significant differences were found among calves or treatments. The N concentration in urine and feces appears to be inversely related to the excretion of feces and urine (Table 18). The relationships were analyzed by a third degree polynomial and its reduced forms. The analyses showed that 72% of the variation in the N concentration in urine was due to variation in urine excretion (Table A10), and that 79% of the variation in fecal N concentration was related to variation in the fecal excretion (Table A11).

The total amount of N in feed varied among calves ($P < .001$), probably because of BW differences among calves, and different concentrations of N in the feed. No significant differences were found between periods or among treatments for milk replacer, milk weigh back, and urine, nor among calves for the latter two and fecal excretion. The fecal N excretion was greater in period 1 than in period 2, suggesting that the daily N excretion is not constant with a simple inverse relationship between the amount of feces and the fecal N concentration.

Nitrogen balances did not differ among calves or between periods, but tended to differ among treatment groups ($P < .10$). Multiple comparisons among treatment means showed that N retention for group E was less than for group D ($P < .05$), but was equal for groups A through D. The average N deposition for groups A through D was

19.5 g for the total period and equivalent to 17.4 g body protein per day. This is in disagreement with an average weight loss of 13 g per day for the four groups.

To eliminate the difference in initial BW, the data for N content and N-balance were recalculated on an equal BW basis (Table 19). The analyses of variance are given in Table A12. The recalculated data show the same differences as the original values, but differences in N consumption among calves were less ($P < .05$), and N intake even greater for period 2 ($P < .05$). The differences among calves may be explained by different N concentrations in milk replacers, and the difference between periods by the feeding practice. If a calf lost weight in period 1, the feed was not adjusted downward to correspond with the lower initial BW of period 2.

The relationship between dietary methionine levels and the N-balance was also analyzed by multiple regression. The results are given in Tables 20 and A13. When the full model was used differences in dietary methionine levels accounted for 29% of the variation in the N-balance. However, only the cubic term is significant, and accordingly, only 5% of the variation in N-balance can be attributed to dietary methionine levels. If the N-balance is recalculated as g N retained per day per kg $BW^{.73}$, then the linear term approaches significance (Table 20), and the amount of variation explained by dietary methionine

Table 19.--The effect of dietary methionine[c] (%) on the daily N-content and N-balance per 100 kg initial body weight (g N/day/100 kg BW).

	Treatment					s.e.	Overall Mean	Period	
	A	B	C	D	E			1	2
N content, g									
Milkreplacer	37.79	38.05	37.50	37.25	37.10	± .50	37.54	37.13	37.95
Milk weigh back	.25	2.17	.64	.80	2.46	± .83	1.26	.93	1.59
Urine	19.19	22.48	20.72	16.42	23.58	±2.65	20.48	20.32	20.64
Feces	10.18	13.09	10.96	8.98	15.14	±1.67	11.67	14.17	9.17
N-balance	8.16[a,b]	.32[a,b]	5.18[a,b]	11.06[a]	-4.08[b]	±3.88	4.13	1.71	6.55

[a,b]Means sharing a common superscript are not different ($P < .05$).

[c]Relative Met conc: 100 = 2.48 g Met/16 g N.

Table 20.--Estimated relationships between dietary methionine levels (X,[a] %) and N-balances expressed as total for experimental period (NB_1, g) g N daily per 100 kg initial BW (NB_2), and g N daily per kg $BW^{.73}$ (NB_3), respectively. Adjusted observations.[b]

Variable	Model determined from adjusted means: Model	Sign. of β_i	Calculated models from adjusted observations: Regression coefficients β_0	β_1	β_2	β_3	R
NB_1	(3)	P < .25	903.2	-23.87	.2041	$-.5578(10^{-3})$**	.54**
NB_1	(2)	P < .25	-24.63	.6100	--	$-.1592(10^{-4})$**	.28
NB_1	(1)	P < .10	22.95	--	--	$-.0408(10^{-4})$**	.23
NB_2	(3)	P < .25	292.2	-7.716	$.6590(10^{-1})$	$-.1799(10^{-3})$**	.52**
NB_2	(1)	P < .10	7.300	--	--	$-.0131(10^{-4})$**	.22
NB_3	(3)	P < .25	9.445	-.2442[c]	$.2050(10^{-2})$	$-.0552(10^{-4})$**	.57**
NB_3	(2)	P < .25	.1290	$.1605(10^{-3})$[c]	--	$-.0008(10^{-4})$**	.30
NB_3	(1)	P < .10	.2542	--	--	$-.0005(10^{-4})$**	.29

[a] Relative Met conc: 100 = 2.48 g N/16 g N.

[b] Adjusted observation = Observation - (Observed mean - Adjusted mean).

[c] P < .10.

**P < .01.

Model (3): $Y = \beta_0 + \beta_1 x + \beta_2 x^2 + \beta_3 x^3$

Model (2): $Y = \beta_0 + \beta_1 x + \beta_3 x^3$

Model (1): $Y = \beta_0 + \beta_3 x^3$

is slightly increased. Inclusion of the linear term in the N-balance for the whole period increases the R^2 value slightly, and a maximum is found at 113% methionine; equivalent to 2.80 g per 16 g N (Figure 3).

Plasma Amino Acids.--Plasma concentrations of methionine, valine, leucine, and isoleucine before feeding and two hours after feeding are given in Table 21, and the corresponding analyses of variance is in Table A14.

Plasma concentrations for the four amino acids did not vary significantly among calves, except for the methionine concentration two hours after feeding on the last day of the experimental period ($P < .10$).

Plasma concentrations of valine, leucine, and isoleucine were higher in period 1 than in period 2 before feeding on the last day of the experimental period ($P < .05$, $P < .10$, and $P < .10$, respectively). These were the only significant differences between periods. However, for all amino acids at all sampling hours, plasma concentrations tended to be higher in period 1 than in period 2. Excepted from this trend are plasma methionine before feeding on the first day, and two hours after feeding on the last day of the experimental period.

Only plasma methionine concentrations were affected by treatments. The plasma concentrations tended to differ due to treatment before feeding on the last day of the experimental period ($P < .10$), and two hours after

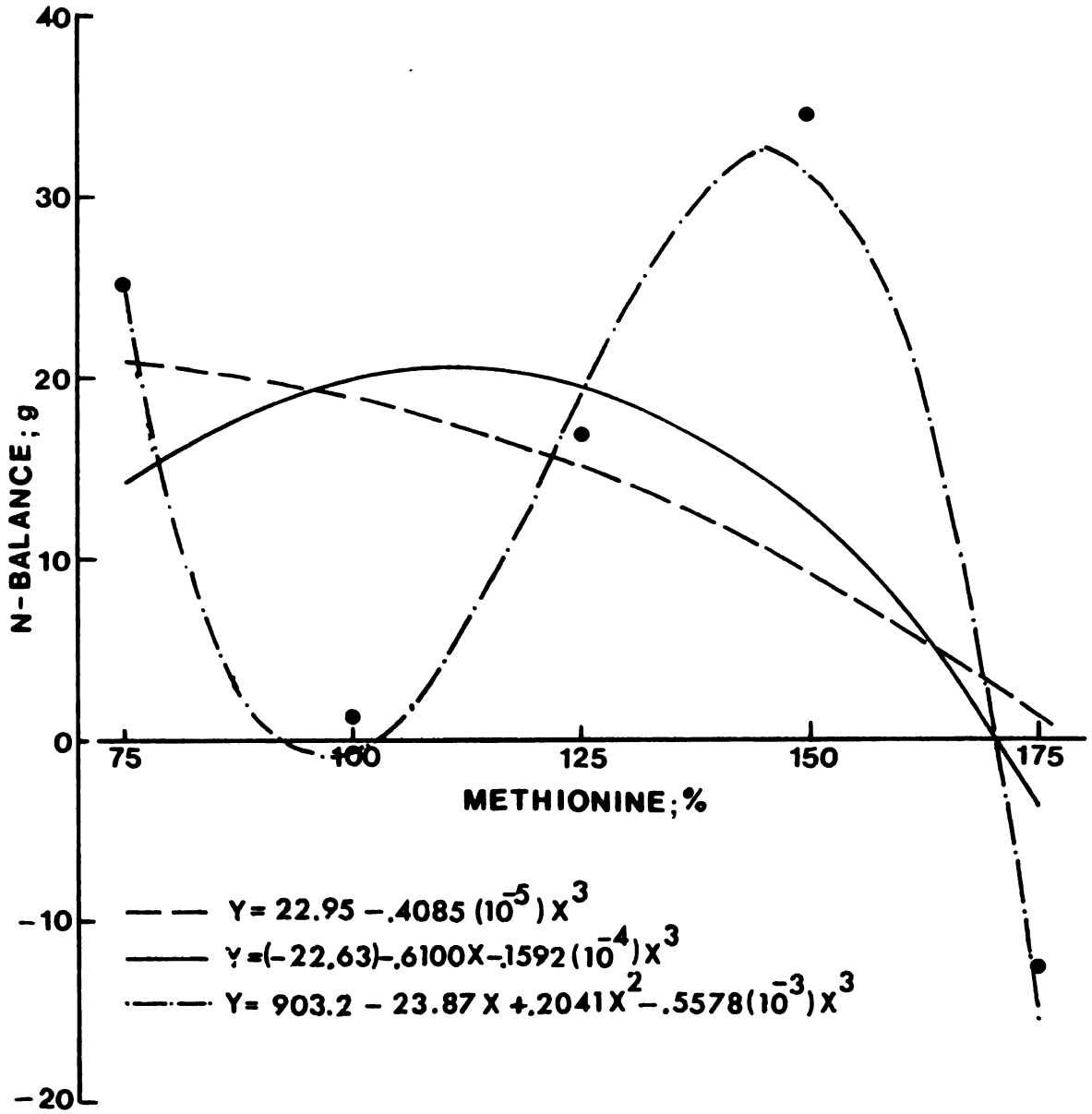

Fig. 3.--The relationship between dietary methionine (X) levels and N-balance (Y) in calves.

Table 21.--The effect of dietary methionine[e] on plasma concentrations of methionine, valine, leucine, and isoleucine (μM/100 ml) in calves.

	Treatment					s.e.	Overall Mean	Period	
	A	B	C	D	E			1	2
Methionine									
First day, T-0[f]	2.61	2.86	2.99	3.41	3.63	± .61	3.10	3.06	3.14
First day, T-2[g]	2.48[a,b]	1.53[b]	3.70[a,c]	5.69[d,c]	4.47[a,c]	± .60	3.58	3.66	3.49
Last day, T-0[h]	2.17[a]	3.05[a,b]	3.02[a,b]	3.34[a,b]	4.18[b]	± .44	3.15	3.26	3.04
Last day, T-2[i]	1.97[a]	2.19[a]	3.95[a,b]	5.27[b]	5.62[b]	± .62	3.80	3.65	3.95
Valine									
First day, T-0	17.47	25.68	17.99	9.29	21.51	±6.16	18.39	20.24	16.53
First day, T-2	19.24	16.72	19.78	21.64	19.63	±2.22	19.40	19.97	18.83
Last day, T-0	17.84	20.04	16.86	13.88	19.99	±2.02	17.72	19.66	15.78
Last day, T-2	20.96	22.00	20.39	15.37	21.85	±1.95	20.11	21.09	19.14
Leucine									
First day, T-0	7.50	10.72	8.10	4.83	8.81	±2.47	7.99	8.81	7.17
First day, T-2	8.98	7.02	9.21	10.01	8.46	±1.01	8.73	9.13	8.34

Table 21.--Continued.

	Treatment					s.e.	Overall Mean	Period	
	A	B	C	D	E			1	2
Last day, T-0	7.82	8.84	6.66	6.13	9.69	±1.02	7.83	8.55	7.10
Last day, T-2	9.31	9.83	9.47	7.28	9.49	± .90	9.08	9.28	8.87
Isoleucine									
First day, T-0	10.37	9.38	9.62	10.56	11.14	±1.17	10.21	10.58	9.85
First day, T-2	13.41	9.85	14.17	13.81	13.63	±1.66	12.98	13.16	12.79
Last day, T-0	11.94	13.21	10.35	8.60	13.28	±1.44	11.48	12.54	10.42
Last day, T-2	14.12	13.83	13.38	10.15	13.89	±1.38	13.07	13.66	12.49

[a,b,c,d]Means sharing a common superscript are not different ($P < .05$).

[e]Relative Met conc: 100 = 2.48 g Met/16 g N.

[f]First day of the experimental period. Blood collected before feeding.

[g]First day of the experimental period. Blood collected two hours after feeding.

[h]Last day of the experimental period. Blood collected before feeding.

[i]Last day of the experimental period. Blood collected two hours after feeding.

feeding on both the first and the last day of sampling ($P < .01$). Multiple comparisons among means before feeding on the last day of sampling showed that the plasma concentration in group A was lower than the concentration in group E ($P < .05$). Concentrations for neither group were different from those of groups B, C, and D. The equivalent comparisons two hours after feeding on the first day of sampling showed that plasma methionine concentrations in A were lower than in D ($P < .01$), and that B was lower than C ($P < .05$), D and E ($P < .01$). Plasma methionine was relatively stable at the lowest level of dietary methionine, but increased with increasing intake. However, at the highest methionine level plasma methionine decreased. The same trend shown two hours after feeding on the first day of sampling was also observed on the last day of sampling with the exception that plasma methionine continued to increase on treatment E even though the rate of increase was less than at lower intakes.

The relationship between plasma and dietary methionine concentrations was analyzed by multiple regression, and the results are given in Tables 22 and A15. On the first day of sampling the linear model approached conventional significance for the collection before feeding, whereas both the linear and the cubic regression coefficients were significant for the samples two hours after feeding. On the last day of the experimental

Table 22.--Estimated relationships between dietary methionine levels (X,[a] %)[b] and plasma methionine concentrations (Y, μM/100 ml). Adjusted observations.

Model determined from adjusted means		Model calculated from adjusted observations Regression coefficients				R
Model[g]	Sign. level of β_i	β_0	β_1	β_2	β_3	
Dependent variable: M-10[c]						
(3)	P <	2.989	$-.1736(10^{-1})$	$.2015(10^{-3})$	$.0046(10^{-4})$	.28[h]
(1)	P < .10	1.805	$.1035(10^{-1})$	--	--	.28[h]
Dependent variable M-12[d]						
(3)	P <	55.28	-1.457	$.1257(10^{-1})$	$.3374(10^{-4})$	.67***
(2)	P < .05	-1.858	$.5063(10^{-1})$	--	$-.0037(10^{-4})$	.53***
Dependent variable: M-20[e]						
(3)	P <	-11.38	.3495	$.2817(10^{-2})$	$.0759(10^{-4})$	.55***
(2)	P < .25	1.421	$.1161(10^{-1})$	--	$.0011(10^{-4})$	.52***
(1)	P < .10	.9975	$.1722(10^{-1})$	--	--	.52***
Dependent variable: M-22[f]						
(3)	P <	20.86	-.5513	$.4991(10^{-2})$	$.1336(10^{-4})$	.70***
(2)	P < .25	-1.820	$.4719(10^{-1})$	--	$-.0011(10^{-4})$	.68***

Table 22.--Continued.

Model determined from adjusted means		Model calculated from adjusted observations Regression coefficients				R
Model[g]	Sign. level of β_i	β_0	β_1	β_2	β_3	
(1)	P < .10	-1.396	$.4158(10^{-1})$	--	--	.68***

[a]Relative Met concentration: 100 = 2.48 g Met/16 g N.

[b]Adjusted observation = Observation - (Observed mean - Adjusted mean).

[c]Plasma met conc. on the first day of the experimental period; before feeding.

[d]Plasma met conc. on the first day of the experimental period; two hours after feeding.

[e]Plasma met conc. on the last day of the experimental period; before feeding.

[f]Plasma met conc. on the last day of the experimental period; two hours after feeding.

[g]Model (3): $Y = \beta_0 + \beta_1 X + \beta_2 X^2 + \beta_3 X^3$

Model (2): $Y = \beta_0 + \beta_1 X + \beta_3 X^3$

Model (1): $Y = \beta_0 + \beta_1 X$

[h]P < .10.

***P < .001.

period only the linear regression coefficient was significant for blood collected before feeding and two hours after feeding. For the plasma methionine concentrations two hours after feeding on both the first and the last day of sampling the estimated regressions (where only the significant regression coefficients were included) do not show good agreement with the adjusted means (Figures 5 and 7). Therefore, the estimated full models are also shown in the figures. These estimated full models show an approximately linear increase between 110 and 140% of the methionine in milk. If the point where the linear increase commences is assumed to be the requirement, then the estimated need for methionine in the baby calf is about 2.73 g per 16 g N.

Since the decrease in adjusted means between groups D and E was unexpected, the regression analyses were repeated without group E. The analyses of variance (Table A16) gave the same results as the original analyses except for the sampling two hours after feeding on the first day of the experimental period. For that sampling, the estimated regression was changed to a second degree polynomial with the optimum level at about 93% of that of milk. However, the full model follows the one calculated for all data (including group E) and displays a linear slope between the 110 and 140% level (Figure 5). For the sampling two hours after feeding on the last day of the

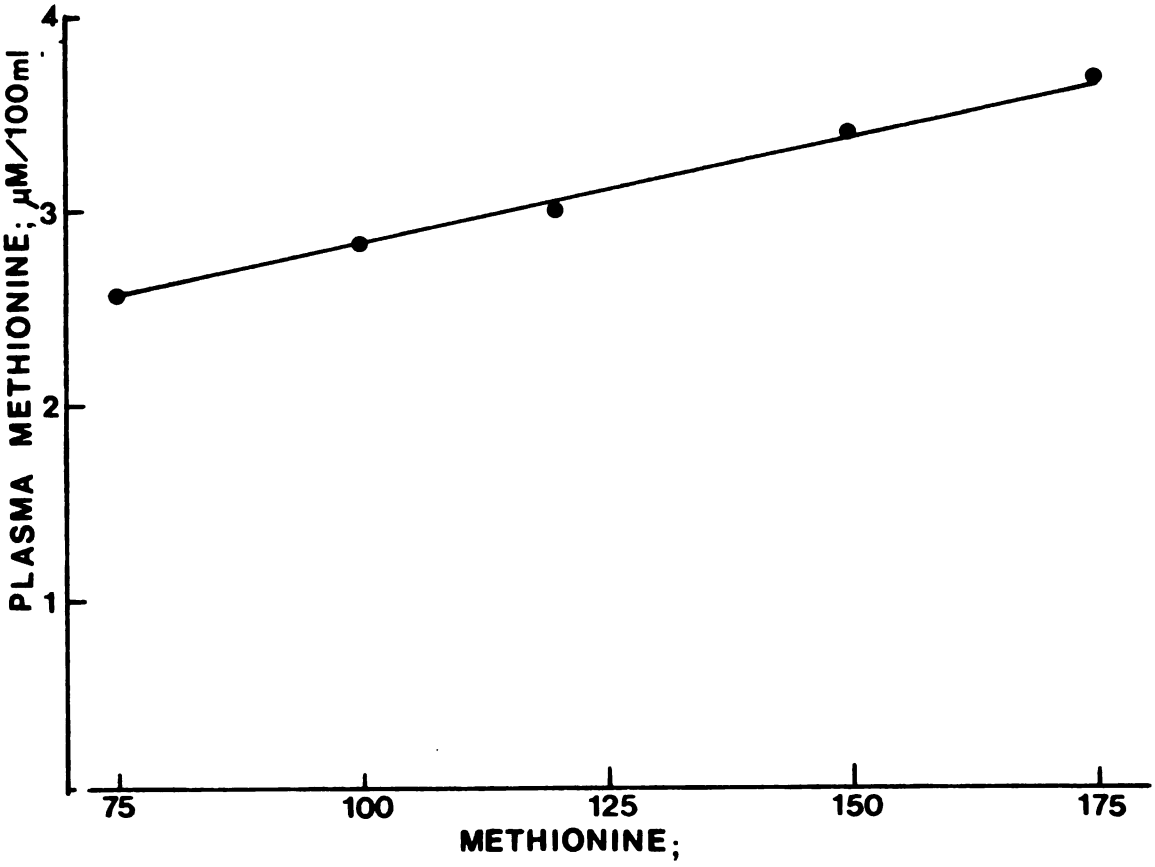

Fig. 4.--The relationship between dietary methionine levels and plasma methionine concentrations before feeding on the first day of sampling in calves.

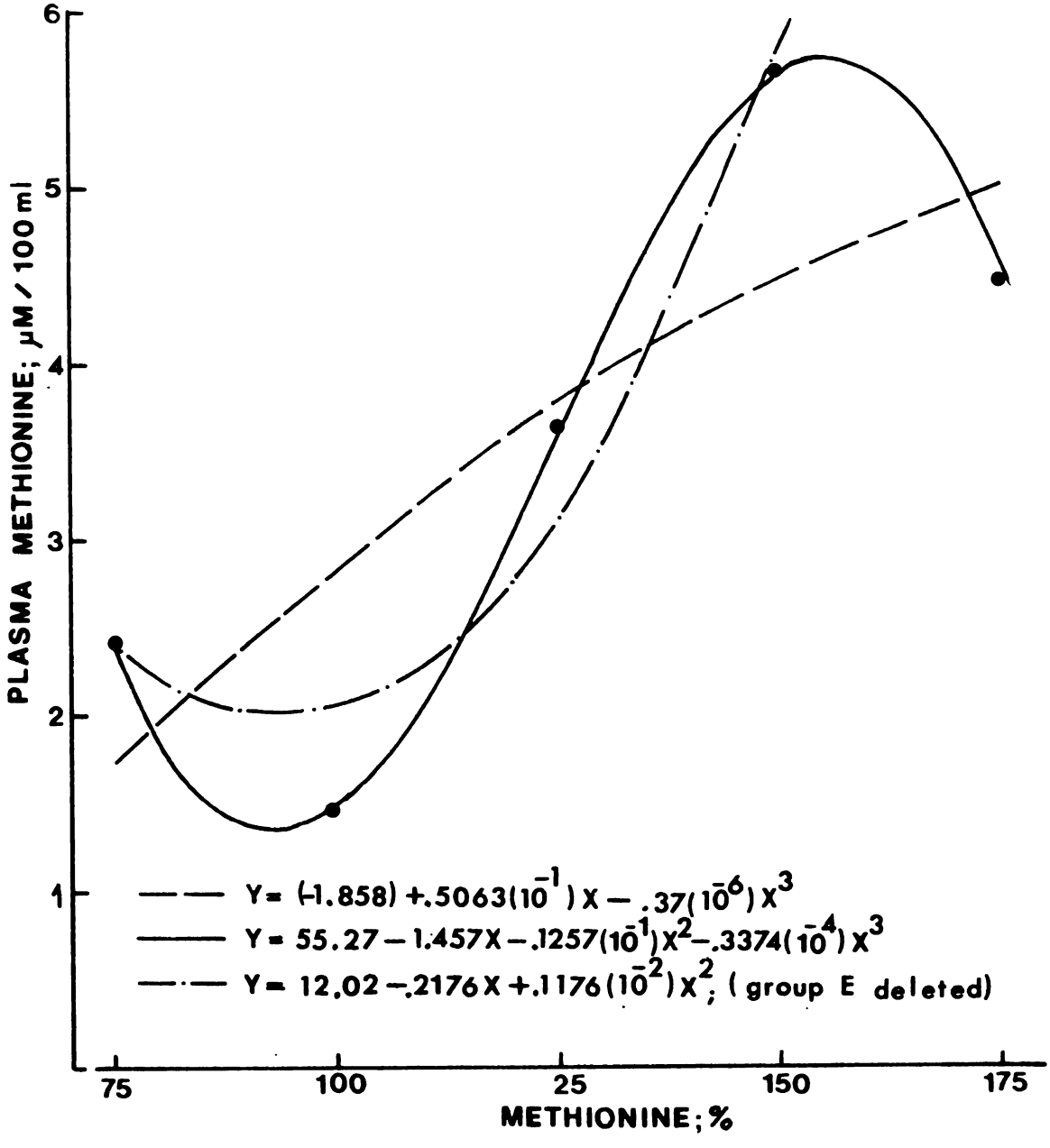

Fig. 5.--The relationship between dietary methionine (X) levels and plasma methionine (Y) concentrations two hours after feeding on the first day of sampling in calves.

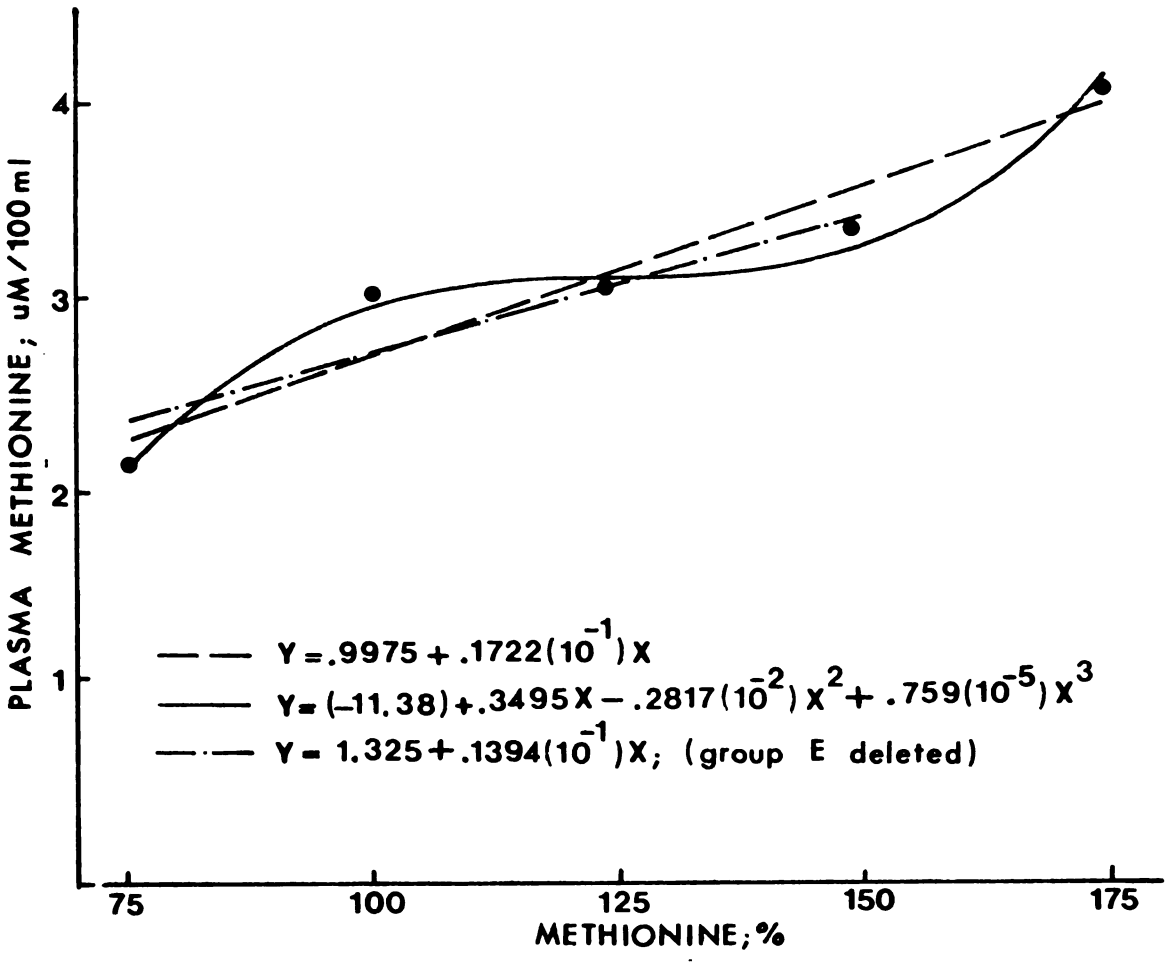

Fig. 6.--The relationship between dietary methionine (X) levels and plasma methionine (Y) concentrations before feeding on the last day of sampling in calves.

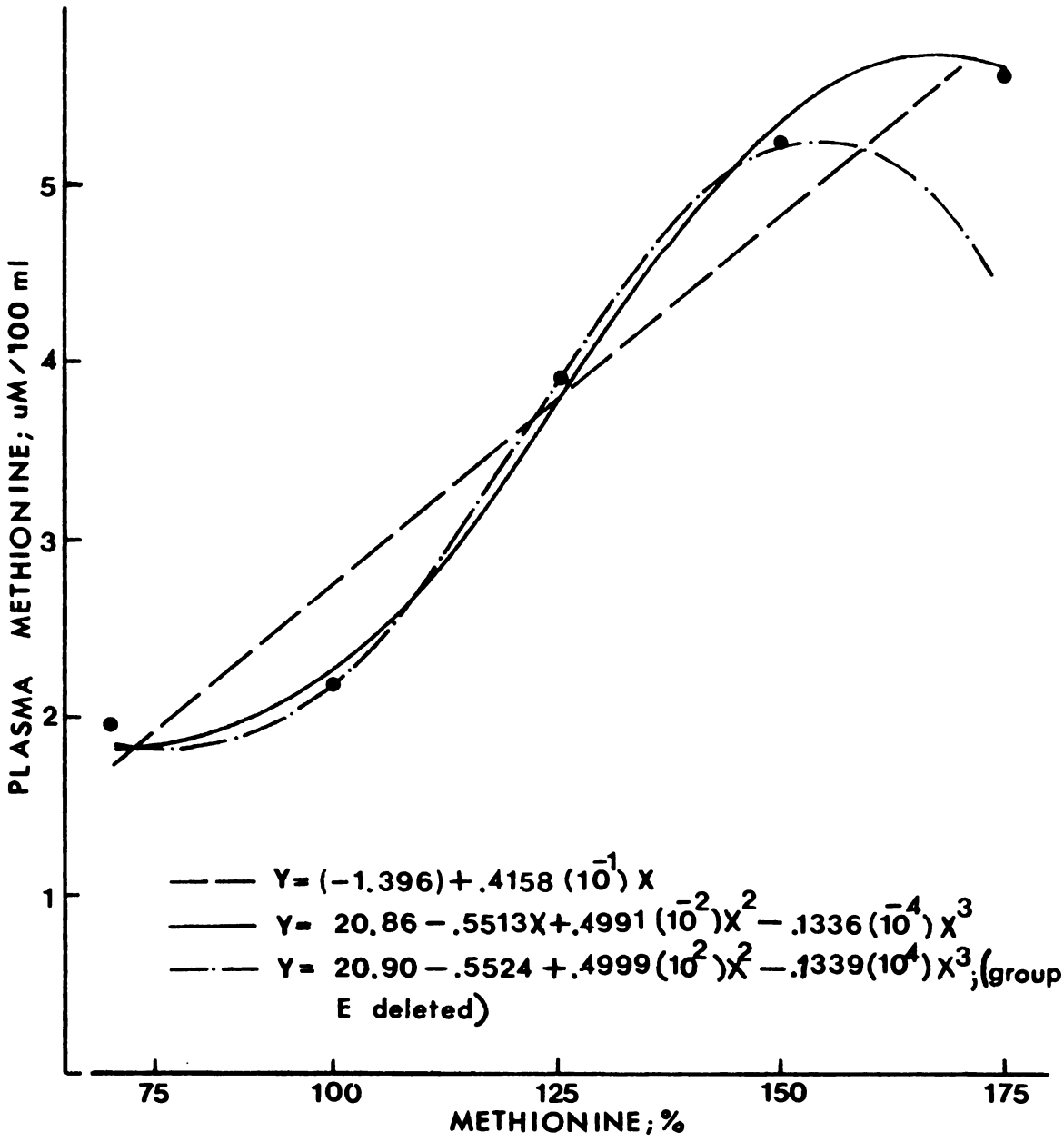

Fig. 7.--The relationship between dietary methionine (X) levels and plasma methionine (Y) concentrations two hours after feeding on the last day of sampling in calves.

experimental period both the second and the third degree polynomials parallel the original full model, and show an approximately linear increase from 110 to 140%. The recalculated regressions did not change the estimated methionine need for the baby calf of 2.73 g per 16 g N.

Plasma Urea Nitrogen.--Plasma urea nitrogen (PUN) levels on the first and the last day of the experimental period are given in Table 23. Each value represents the average of 5 sampling hours per calf. Since the data are incomplete (no observations for calves 125, 128, 129, and 130; incomplete for calves 259 and 260), and because of the experimental design used, treatment effects could not be tested by the ordinary analysis of variance. Instead a t-like test with approximate degrees of freedom (Welch, 1938) was used.

For the first day of the experimental period PUN was higher in group E than all other groups ($P < .05$). None of the differences between groups A, B, C, and D were significant. On the last day of the experimental period PUN levels in group A and E, were higher than for groups B, C, and D ($P < .05$). The difference between periods was not significant.

The relationship between dietary methionine levels and PUN was analyzed by a third degree polynomial and its reduced forms. The dependent variable used was not adjusted and was the average of 5 sampling hours for each

Table 23.--The effect of dietary methionine levels on plasma urea nitrogen levels in calves on the first and the last day of the experimental period.

Day of Experiment		Treatment[d]				
		A	B	C	D	E
First	$\bar{X}$[e]	8.30[a,c]	8.63[a]	8.55[a,c]	7.51[c]	11.00[b]
	s.e.[f]	±.37	±.28	±.37	±.47	±.77
	n[g]	30	35	40	30	30
Last	$\bar{X}$	11.18[a]	9.59[b]	9.15[b]	9.20[b]	11.25[a]
	s.e.	±.37	±.48	±.39	±.40	±.75
	n	24	35	40	30	40

[a,b,c]Means sharing a common superscript are not different ($P < .05$).

[d]Each mean includes five determinations per calf.

[e]Treatment mean.

[f]Standard error of the mean.

[g]Number of observations.

calf, because inspection of individual values revealed only small differences. The results of the regression analyses are given in Table A17. On the first day of the experimental period dietary methionine levels accounted for 15% of the variation in PUN and a complicated relationship was described by the full model (Figure 8). For the last day of the experimental period only the square term tended to be significant and dietary methionine accounted for only 8% of the variation when the quadratic model was used. The prediction equation shows good agreement with the treatment means (Figure 8) and the minimum PUN at 125% suggest a requirement of 3.1 g methionine per 16 g N. For both days of sampling the low correlation between the independent and the dependent variables probably reflects a larger than normal error because the values were not corrected for incomplete block (calf) differences according to equation (9).

Hematocrit.--The average effect of dietary methionine on the packed cell volume (hematocrit) is given in Table 24. Since the data are incomplete they were subjected to a t-like test like that used for PUN. On the first day of the experimental period hematocrits were lower for group B and higher for group E than for any of the other groups ($P < .05$). Differences between group A, C, and D were not significant. On the last day of the experimental period the same trend was observed, but

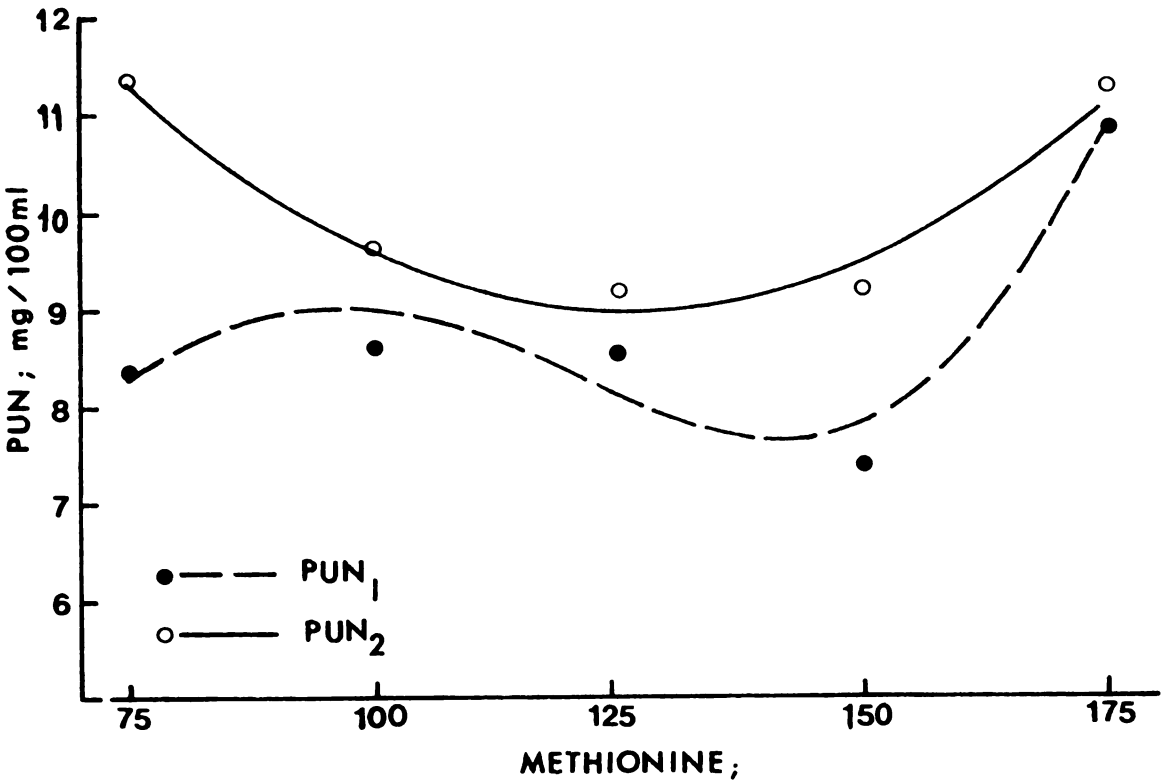

Fig. 8.--The relationship between dietary methionine levels and plasma urea nitrogen (PUN) on the first (1) and the last (2) day of the experimental period.

Table 24.--The effect of dietary methionine levels on blood hematocrits in calves.

Day of Experiment		Treatment[d]				
		A	B	C	D	E
First	$\bar{X}$[e]	37.87[a]	34.57[b]	37.46[a]	39.00[a]	44.57[c]
	s.e.[f]	±1.13	±.89	±1.08	±1.06	±1.30
	n[g]	30	30	35	25	30
Last	$\bar{X}$	37.47[a]	34.73[b]	36.71[a,b]	40.80[c]	42.33[c]
	s.e.	±.81	±.92	±1.41	±1.35	±1.77
	n	30	30	35	25	30

[a,b,c]Means sharing a common superscript are not different ($P < .05$).

[d]Each mean includes five determinations per calf.

[e]Treatment mean.

[f]Standard error of the mean.

[g]Number of observations.

differences between groups B and C, and between D and E were not significant.

The relationship between dietary methionine levels and hematocrit was analyzed by a third degree polynomial and its reduced forms. Results are given in Table A18. It was found that dietary methionine levels accounted for 25 and 16% of the variation in hematocrit on the first and the last day of the experimental period when a quadratic prediction equation was used. The equations show good agreement with the treatment means (Figure 9) and suggest the minimum need for methionine is 2.48 to 2.60 per 16 g N. The observed changes in hematocrit are in general agreement with the observed trend for fecal score and fecal excretion data (Tables 15 and 18).

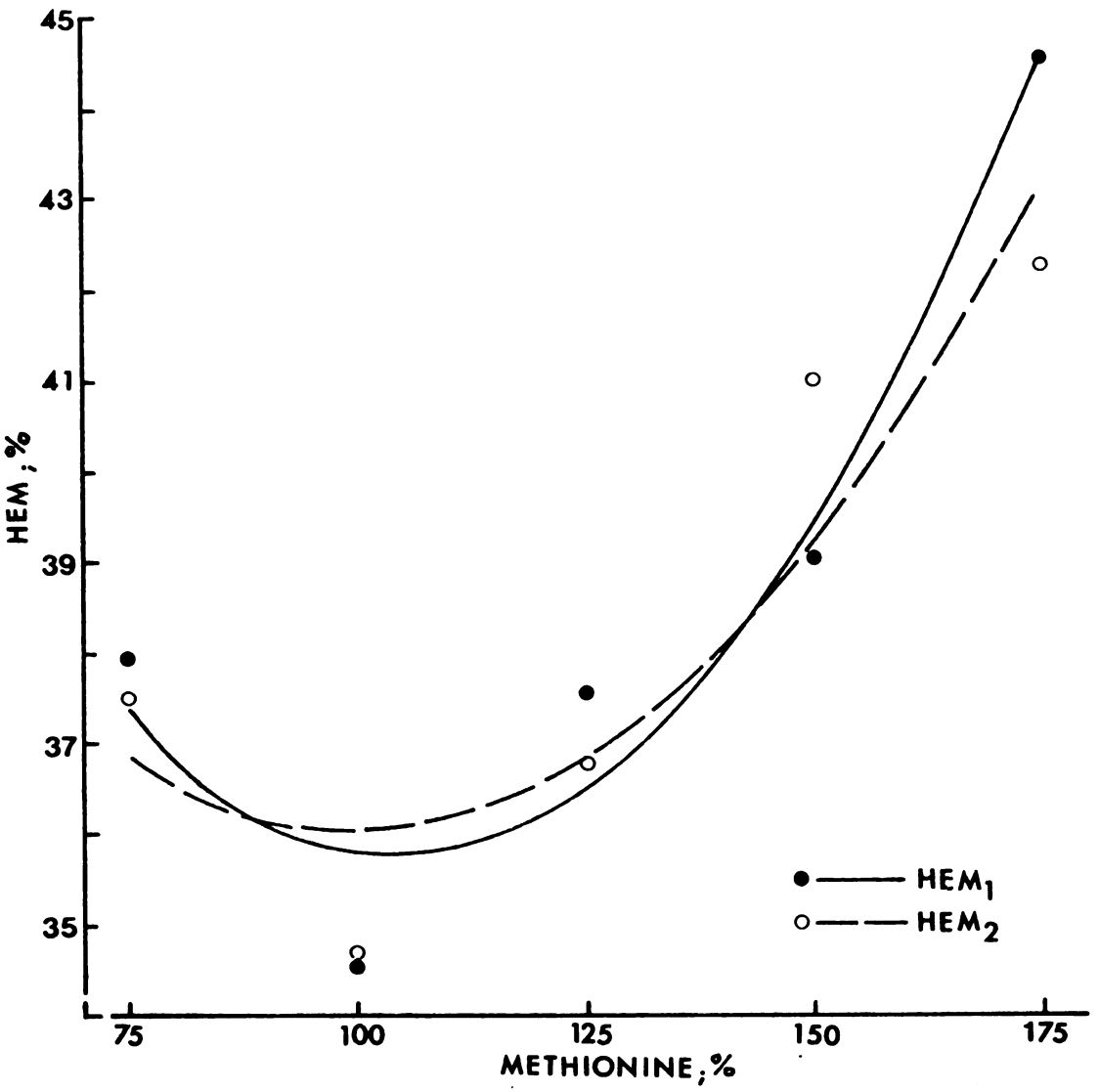

Fig. 9.--The relationship between dietary methionine levels and packed blood cell volume (HEM) in calves on the first (1) and the last (2) day of the experimental period.

DISCUSSION

Body Weight, Daily Gain, and Health.--Male Holstein calves fed graded levels of methionine from one to four weeks of age lost weight in the first two weeks after birth. In the following two weeks average daily gain (ADG) was not sufficiently high for full compensation of the weight loss (Table 15). The ADG did not differ significantly among treatment groups fed 75 to 150% of the methionine content in milk protein, but weight loss was greatly increased for the 175% diet (Table 15). The lack of ADG response at the lower levels of dietary methionine is in agreement with observations in calves by Patureau-Mirand et al. (1973). However, it is in disagreement with an expected response according to the law of diminishing returns (Almquist, 1953). This may be explained by factors such as age, health, and length of the study period.

At the age involved in the present study, the ADG in healthy calves will not normally exceed 200 g and may be expected to be even lower in the first two weeks after birth. Any health problems would be superimposed on the age effect and would decrease the ADG. In the present

study, scouring was a problem in period 1 (9 to 15 days of age) and was caused by bacterial infections of the gastrointestinal tract which seemed unrelated to treatment. It was also found that 56 and 46% of the variation in ADG was due to variation in fecal and urine excretion, respectively (Tables A3 and A5). When the two factors were combined they accounted for 77% of the variation in ADG and excluded dietary methionine as a significant predictor in an equation determined by multiple regression. Fecal scores and hematocrit values also suggest an excessive water loss in feces without a sufficient increase in water intake or decrease in urinary excretion to prevent dehydration. The prediction equation indicates that ADG would be maximized in calves when fecal excretion is less than 150 g per day (no scouring) and daily urinary excretion is 5 to 7 liters (Table 25). The optimization effect of urine excretion may be related to a minimum water excretion for removal of excretory metabolites and maintenance of water balance in calves. Because of the age and health factors the length of the study period is critical. The ADG is determined as the difference between two large values (body weight), and a short study period does not allow scouring calves to fully recover and compensate for weight losses due to scouring.

Even though the ADG response appears insensitive for determination of amino acid requirements in short

Table 25.--The relationship between average daily gain (ADG, g) and fecal (F, kg), and urinary (U, liters) excretion in calves.

Urinary excretion/ week, liters	Fecal excretion/week, kg						
	1	3	5	7	9	11	13
10	-150[a]	-260	-293	-285	-272	-288	-370
20	15	-95	-128	-120	-107	-123	-205
30	125	15	-18	-10	3	-13	-96
40	180	70	37	45	58	42	-41
50	180	70	36	45	58	42	-41
60	125	14	-19	-11	3	-14	-96

[a] $ADG = -274.8 - 110.8F + 16.3F^2 - .7450F^3 + 24.76U - .2753U^2$; $R = .88$; ($P < .001$).

term experiments, dietary methionine levels accounted for 26 and 20% of the variation in ADG when fitted to third and second degree polynomials, respectively (Table 16). The third degree polynomial describes an unexpectedly complicated relationship between dietary methionine levels and ADG, but upon reduction to the quadratic form the curve becomes parabolic (Figure 1), and shows maximum gain at the 115% level; suggesting a methionine requirement of 2.85 g per 16 g N.

Digestibility.--The overall digestibility of dry matter (DM) was 83.4% and was only slightly lower than in earlier studies with calves fed similar amounts of protein (Lassiter et al., 1963). Digestibilities were lower in period 1 than in period 2 (Table 17) with values for period 2 close to those reported by Lassiter et al. (1963). Because scouring was a problem, and the digestibility was negatively correlated with fecal excretion (Table A8), differences between the present study and literature values (Table 8) may be assumed to be caused by age and health factors rather than diet. However, fecal scores showed that increasing levels of dietary methionine caused some increase in scouring (Table 15).

Dietary methionine accounted for only 6% of the variation in the digestibility of DM when fitted to a third-degree polynomial without the quadratic term. The prediction equation so determined had a maximum

digestibility at 101%; suggesting a methionine requirement of 2.50 g per 16 g N.

The overall digestibility of crude protein (CP) was 65.5% and lower than reported previously for dried skim milk and casein (Table 8; Lassiter et al., 1963; Blaxter and Wood, 1951a; Bryant et al., 1967; Lofgreen and Kleiber, 1953). The difference may be related to age, protein level, and protein type. Bryant et al. (1967) found that digestibility increased with age. Lassiter et al. (1963) demonstrated an increased digestibility of CP with increasing amounts in the diet. In the present study the digestibility of CP was 71.7% for period 2; slightly lower than for calves fed 15.2% dietary protein in the study of Lassiter et al. (1963). These data suggest that the digestibility of CP decreases when 3 to 5% protein equivalent is replaced by crystalline amino acids; however, the decrease in this study probably was magnified by scouring since the two variables were negatively correlated (Table A8).

Dietary methionine levels accounted for 20% of the variation in the digestibility of CP when fitted to a third-degree polynomial (Table A7). However, the quadratic term was not significant and consequently only 11% of the variation was accounted for by dietary methionine. When the quadratic term was excluded the polynomial showed

a maximum in digestibility at 117%; suggesting a methionine requirement of 2.90 g per 16 g N.

The low correlations between dietary methionine and the digestibility of DM or CP, and the high negative correlation between these digestibilities and fecal excretion (scouring), indicate that digestibilities become an insensitive method for determination of the amino acid requirement in calves with diarrhea.

Nitrogen Balance.--The overall nitrogen balance (N-balance) for the experimental period was 13.1 g (Table 18). This is equal to 2.2 g N retained per day per 50 kg body weight (BW), and is much less than in older calves gaining weight at the rate of .5 kg per day (Table 5, Blaxter and Wood, 1951c; Bryant et al., 1967). However, the N-balance was positive even though calves lost weight. Bryant et al. (1967) explained such a discrepancy in the young calf by biological variation, experimental error, and a pronounced ability to conserve protein by deposition of lean muscle tissue concomitantly with depletion of lipid, glycogen, and water.

Experimental error may be the best explanation since a 2% loss of each intake and excreta would result in a 30 to 40% increase in the N-balance (Wallace, 1959). In the present study scouring was a problem. With increasing severity it becomes difficult to secure consumption of allotted feed and total collection of excreta. Error

introduced by incomplete collection of excreta is partly reduced because the N concentration in both feces and urine is decreased with increased excretion (Tables A10 and A11).

A general dehydration in the young calf can be an important factor in the discrepancy between N-balance and ADG. Wallace (1959) found that 33% of retained N is stored without an equivalent gain in BW in the 4 months old child. In the present study a long term general dehydration was probably enhanced by a more rapid dehydration due to scouring since increased water excretion often took place without a compensatory increase in intake even if offered. Further, scouring may lead to depletion of glycogen and lipid stores and enhance the inverse relation between ADG and N-balance. This is supported by the observation that digestibilities for both DM and CP decreased linearly with increased fecal excretion (Table A8). The discussed factors, along with an expected low N retention in the first weeks after birth (due to low ADG) make the N-balance method less suitable for determination of amino acid requirements in short term experiments in calves.

Even though the N retentions were low, 25% of the variation could be attributed to dietary methionine levels when fitted to a third degree polynomial (Table 20). However, the relationship was unexpectedly complicated

(Figure 3). Upon reduction to the linear and cubic terms, it only accounted for 9% of the variation in N retention. The selection of terms to be included was based on the significance of the linear and cubic terms for the relationship between dietary methionine levels and the daily N retention per kg $BW^{.73}$ (Table A13). The prediction equation so determined showed a maximum N retention at the 113% level; suggesting a methionine requirement of 2.80 g per 16 g N.

Plasma Amino Acids.--The plasma methionine concentrations reported for the present study (Table 21) are in good agreement with those reported by Patureau-Mirand et al. (1973) but a little higher than those determined by Williams and Smith (1973, 1975).

Differences among calves only approached significance for plasma methionine two hours after feeding on the last day of sampling. This is contrary to the findings by Williams and Smith (1973; 1974a, b, c; 1975) that the variation among calves is greater than the variation within calves.

No differences were found between periods (age), except for valine, leucine, and isoleucine before feeding on the last day of sampling. These results are partly in agreement with those by Williams and Smith (1973, 1975), who found little variation with age. However, valine, leucine, and isoleucine concentrations tended to

decrease with age at both sampling hours. For methionine, the age effect was variable. These results support those by Leibholz (1966), that plasma methionine and leucine are negatively correlated with age.

Plasma methionine was the only amino acid measured that was affected by dietary methionine levels (Table 21). The absence of a response to diet in plasma methionine levels before feeding (12 hours fast; Figure 4 and 6) is in disagreement with findings by Zimmerman and Scott (1965) who found a typical broken-line response for the amino acid under study when chickens were bled 24 hours after the last feeding, and all other EAA were at requirement levels. The reason for this difference between species is unknown.

At two hours after feeding plasma methionine differed among treatments but the typical broken-line response reported for studies in rats, chicks, pigs, calves, and steers (Stockland et al., 1970; Zimmerman and Scott, 1965; Mitchell et al., 1968b; Williams and Smith, 1973, 1974a, b, c, 1975; Fenderson and Bergen, 1975) was not observed at the highest dietary concentration (175%; Figures 5 and 7). Morrison et al. (1961b) also reported a plate, but did not propose a cause. Since plasma amino acids (PAA) arise from the balance between rates of digestion, absorption, and protein synthesis, as well as tissue breakdown (Albanese, 1959; Gitler, 1964; Harper,

1968; Munro, 1970; McLaughlan, 1974), the increased scouring at 175% may have impaired absorption of methionine. The above is partly supported by the decreased digestibility for both DM and CP at the highest dietary level. Methionine is a potent stimulator of adrenocortical activity (Munro, 1970). Adrenocortical hormones create a less favorable N-balance (Leathem, 1964), by activation of control and defense mechanisms which increase gluconeogenesis (Yates et al., 1974). Moreover, inappropriately high levels of aldosterone stimulate reabsorption of sodium from luminal fluids which can lead to isotonic expansion of the extracellular fluid volume (Yates et al., 1974). Stimulated glucorcorticoid secretion is probably the more dominant effect in calves with diarrhea, because the nutrients are less well absorbed, and gluconeogenesis from amino acids release energy which becomes limiting. The increased PUN levels suggest more gluconeogenic activity at the highest methionine level (Figure 8). Williams and Smith (1973, 1975) found that plasma urea levels in calves stayed low once the methionine requirement had been met but their studies did not include high enough levels of methionine to create a severe excess (imbalance) as apparently occurred in our study. Dietary methionine is the most toxic amino acid, and dietary excess can cause severe growth depression and histopathologic changes (Harper et al., 1970). In the present study the

average free methionine supplemented on treatment E was 2.78 g per day. The young calf may be more susceptible than rats to methionine toxicity, especially if predisposed by bacterial infections of the gastrointestinal tract.

At the four lower dietary levels, plasma methionine increased almost linearly with level fed, and did not show the typical broken-line response. The broken-line response might be shown more clearly if smaller increments of dose of dietary methionine were used. For both the first and the last day of sampling, dietary methionine accounted for 45 to 49% of the variation in plasma levels when fitted to third degree polynomials and showed an approximately linear increase between 110 and 140% methionine. If the 110% level is assumed equal to the breaking point in an ordinary assay it indicates a requirement of 2.73 g methionine per 16 g N (Figures 5 and 7). McLaughlan and Illman (1967) also found an almost linear relationship between plasma and dietary methionine levels in rats. When they considered the requirement to be at the dietary level at which the post-feeding level was equal to the normal fasting level, the requirement was in close agreement with average values obtained by other methods. In the present study the differences between the linear prediction equation for plasma levels before feeding and the third degree polynomial for plasma levels

two hours after feeding were calculated for the first and the last day of sampling and plotted against the dietary methionine level (Figure 10). The curves so obtained cross the zero-difference line at 119 and 111% level for the first and the last day, respectively, and indicate a requirement of 2.95 and 2.75 g per 16 g N. Figure 10 also shows that post-feeding plasma methionine concentrations are lower than the fasting levels at the lowest dietary intake. This may be caused by increased insulin secretion after feeding and increased uptake of methionine by the tissues, i.e., muscle (Leathem, 1964; Munro, 1964c, 1970; Wool and Scharff, 1968).

Differences between fasting plasma methionine on the first and the last days of the experimental period, and the comparable differences between the post-feeding levels are small (Table 21). This and the close agreement in methionine requirements determined on the two days of sampling indicate that an experimental period of approximately 3 days is sufficient. This length of time is arrived at becamse blood collected on the first day of the experimental period was on the third day that calves received the experimental feed.

Originally we planned to analyze samples taken before feeding and 1, 2, 4, and 6 hours after feeding to determine the optimal time of sampling. Only about half of the samples 1, 4, and 6 hours after feeding were

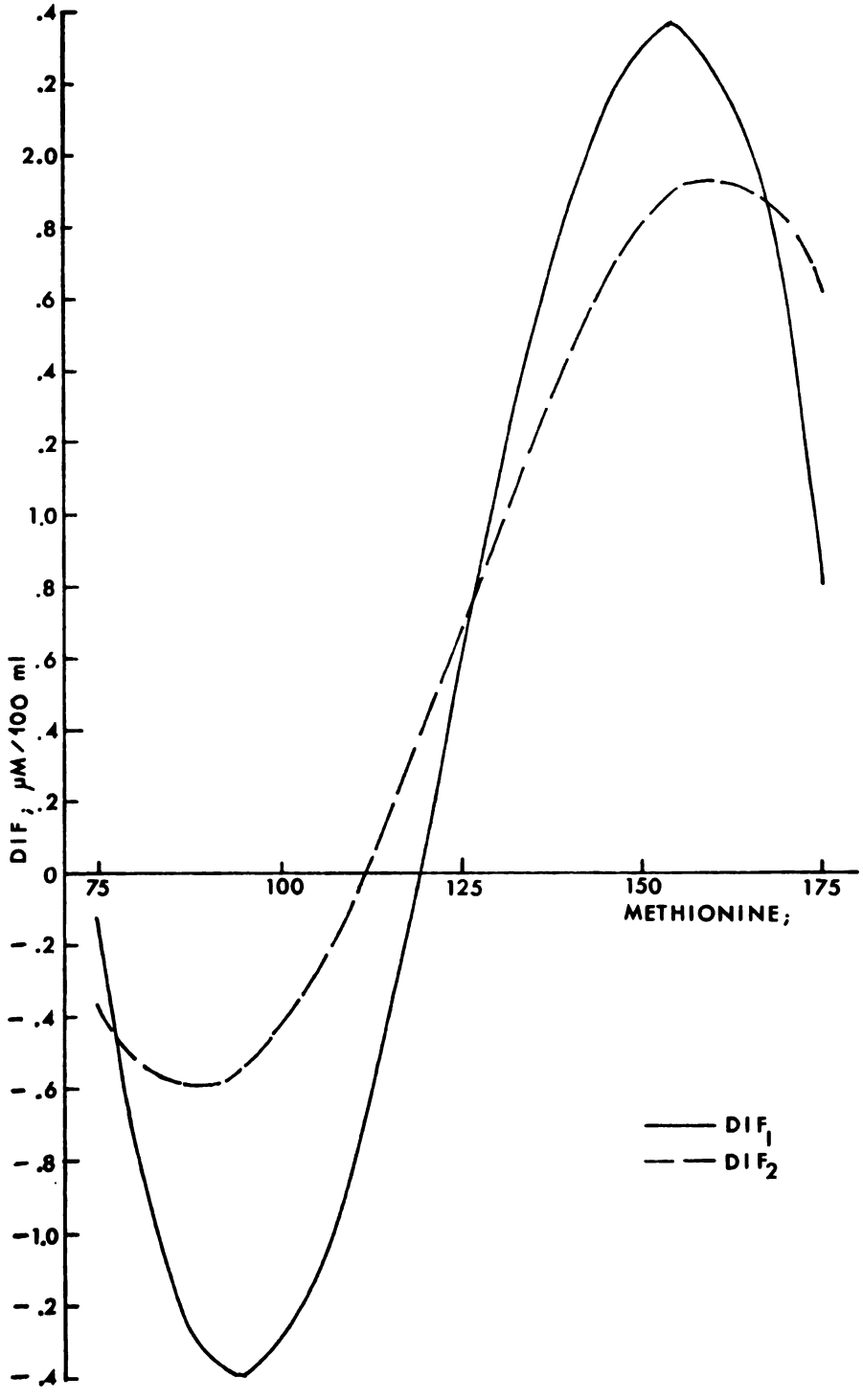

Fig. 10.--The relationship between dietary methionine levels and the difference (DIF) between the linear model for before feeding and the cubic model for two hours after feeding on the first (1) and the last (2) day of sampling in calves.

analyzed because of the large number and the cost of amino acid analyses. Because the plasma levels did not differ between periods, the total data set was pooled and unadjusted observations were analyzed by multiple regression to determine the relationship between plasma and dietary methionine levels at various time intervals after feeding. The prediction equation accounted for 30% of the variation in plasma methionine:

$$\hat{Y} = 7.771 - .2096\ X_1 + .2199\ (10^{-2})\ X_1^2 - .0640\ (10^{-4})\ X_1^3 + .4697\ X_2 - .6033\ (10^{-1})\ X_2^2;\ R = .55$$

where $\hat{Y}$ = plasma methionine levels (μM/100 ml),

X_1 = dietary methionine levels (%), and

X_2 = time of sampling (hours after feeding).

A maximum was found at 3.9 hours after feeding. Perhaps better results could have been obtained had the four hour sample been used instead of that taken two hours post-feeding, but little difference between two and four hours was noted. Similar observations were reported in calves (Williams and Smith, 1973, 1975) and rats (Stockland et al., 1970). Stockland et al. (1970) found that plasma lysine levels in rats were lower 6 hours after feeding with equal concentrations 1, 2, and 4 hours after feeding.

Plasma Urea Nitrogen.--On the last day of the experimental period plasma urea nitrogen (PUN) levels were higher for treatments A and E than for others and a

minimum was observed at the 125% level; suggesting a methionine requirement of 3.10 g per 16 g N. The increased PUN at the highest methionine level was unexpected because it has been found that PUN remains at a low level after the requirement is reached (Williams and Smith, 1973, 1975). The increased PUN levels may be related to increased gluconeogenesis as a protective mechanism against toxic levels of methionine and/or diarrhea.

The higher PUN levels at the lowest dietary methionine may be due to deamination of amino acids in excess of the relative methionine supply, and is in agreement with results in calves by Williams and Smith (1973, 1975) and in pigs by Brown and Cline (1974).

The difference between PUN levels on the first and the last day of the experimental period and the increased PUN at the highest methionine level indicate that this measurement cannot be used satisfactorily until the calves have been on full treatment for more than three days. It may also become less useful in scouring calves.

Hematocrit.--The hematocrit levels demonstrated a parabolic relationship with dietary methionine levels with a minimum at 100 to 105%; suggesting a methionine requirement of 2.48 to 2.60 g per 16 g N. The cause of the parabolic relationship is unknown, but may be related to a tendency towards more scouring at the highest treatment levels. Although the absolute hematocrit tended to

be lower in period 2 than 1, the observed trend was the same for both days of sampling, suggesting that hematocrit values give an early sign of dehydration.

Sulfur Amino Acid Requirement in Baby Calves.--In rats, chicks, turkeys, and pigs it is generally accepted that the total requirements for sulfur amino acids can be furnished by methionine alone or by a mixture of as low as 45% methionine and as high as 55% cysteine (NRC, 1971a, 1972a, and 1973a). In the present study, methionine requirement in baby calves (determined by various methods) ranged from 2.50 to 3.10 g per 16 g N (Table 26). Most of the methods had an optimum at 2.75 to 2.95 g per 16 g N, and the average for all methods was 2.76 g. The determinations were done in the presence of a constant content of 1.05 g cysteine per 16 g N in all diets. Therefore, the determined requirement of methionine only, or total sulfur amino acids (methionine plus cysteine) is 3.80 to 4.00 g per 16 g N. The validity of the assumption that cysteine can furnish 55% of the total sulfur amino acid requirement in calves needs further investigation since Patureau-Mirand et al. (1973) did not find any interaction between graded levels of both methionine and cystine.

The total sulfur amino acid requirement of the young calf determined in this experiment, and expressed per kg $BW^{\cdot 73}$ per day, is in good agreement with the requirement determined by Williams and Smith (1975) but

Table 26.--Methionine needs of the baby calf determined by various methods.

Method	Methionine need g/16 g N
Average daily gain	2.85
N-balance	2.80
Digestibility:	
Dry matter	2.50
Crude protein	2.90
Plasma methionine:	
First day[a]; before feeding	--[d]
First day[a]; two hours after feeding	2.73
Last day[b]; before feeding	--[d]
Last day[b]; two hours after feeding	2.73
First day[a]; fasting level[c]	2.95
Last day[b]; fasting level[c]	2.75
Plasma urea nitrogen:	
First day[a]	--[d]
Last day[b]	3.10
Hematocrit:	
First day[a]	2.48
Last day[b]	2.60

[a]First day of the experimental period.

[b]Last day of the experimental period.

[c]Difference between prediction equations for levels before and two hours after feeding.

[d]Could not be determined.

less than half of that suggested for only methionine by Patureau-Mirand et al. (1973) (Table 27). However, Patureau-Mirand et al. (1973) also expressed it as 3.50 g methionine per 16 g N when the calves were fed milk with 26.4% protein. This value is in close agreement with that determined in the present study, because cysteine was not included. The amino acid requirement is constant as a percent of crude protein when the protein content of the diet for chicks is increased from 14 to 23% (Boomgaardt and Baker, 1973).

If a total sulfur amino acid requirement of .26 g per kg $BW^{.73}$ per day is accepted, then whole milk would supply a sufficient amount, because an average calf would receive .30 g when fed whole milk at 10% of BW.

Jacobsen (1957) determined the amino acid content in the 40 week old calf fetus, and Williams et al. (1954) suggested that the requirement of other amino acids may be calculated by multiplying the relative values for the tissue content by that of the determined requirement of the amino acid under investigation. Requirement values so calculated are given in Table 28. They can only function as a temporary guide until actual determinations have been made for the other amino acids, and may be a slight underestimation since the cysteine content in the calf was not reported.

Table 27.--The requirement of total sulfur amino acids in the young calf as estimated by several workers.

Source	Total sulfur amino acids			
	g/16 g N	g/kg $BW^{.73}$/d	g/day	% of DM
Present study[a]	4.00	.26	3.85	.70
Patureau-Mirand et al., 1973[b]	3.50	.58	--	.9
Williams and Smith, 1975[c]	--	.23 - .26	3.9-4.5	--
Tzeng, 1974[d]	--	--	--	1.15-1.65

[a]Fed 1/2 of daily feed in two equal meals 12 hours apart.

[b]Diet contained 26.4% protein; five 2-week periods, avg. daily gain one kg.

[c]Fed equal amounts twice daily at 10.00 and 17.00 hours.

[d]On day of bleeding fed 1/6 of morning feed in six hourly feedings, and bled one hour after the last feeding.

Table 28.--Calculated requirement of essential amino acids in the young calf.

Essential amino acid	Amino acid content[a] g/16 g N	Calculated requirement[b] g/kg BW$^{.73}$/d
Arginine	6.75	.75
Histidine	1.47	.16
Isoleucine	2.94	.33
Leucine	6.53	.73
Lysine	7.00	.78
Methionine	1.72	.19[c]
Phenylalanine	3.24	.36
Threonine	3.70	.41
Tryptophane	1.1	.12
Valine	4.20	.47

[a]From Jacobsen (1957); amino acid content in the calf fetus at 40 weeks. The cysteine content was not determined.

[b]Determined from the content of other amino acids relative to methionine content in the calf and the determined methionine requirement. (Total sulfur amino acids (.26) minus the content of cysteine (.07).)

[c]Total sulfur amino acid requirement = .26 g.

CONCLUSIONS

The requirement of total sulfur amino acids in the baby calf was determined to be .25 to .26 g per kg $BW^{.73}$ per day between 9 and 27 days of age. It was assumed that 55% of the total sulfur amino acid requirement can be furnished by cysteine, but this needs further investigation since it has been reported that cysteine does not have a sparing effect on methionine in calves. The methionine requirement was estimated by average daily gain, N-balance, and plasma methionine and urea nitrogen levels. When poor health due to factors other than treatments was encountered, average daily gain, N-balance, and plasma urea nitrogen were less sensitive to diet than plasma methionine. These data suggest that three days on the experimental feed are sufficient to estimate the amino acid requirement in calves by plasma amino acid levels. Because of the high cost of crystalline amino acids, this short period makes the method even more attractive. Plasma methionine was minimized at 3 to 4 hours after feeding but whether this sampling hour might improve the estimate needs further investigation.

Poor health due to bacterial infections of the gastrointestinal tract was a problem, but the severity of scouring could not be related to dietary methionine in general. However, the severity tended to increase at the highest methionine intake. At this dietary level it was also found that plasma methionine tended to plateau instead of increasing linearly as theoretically expected. The cause is unknown but is suggested to be due to decreased methionine absorption, since the digestibilities of both dry matter and crude protein also decreased linearly with increased defecation; or to increased deamination of amino acids for gluconeogenesis as indicated by the increase in plasma urea nitrogen levels. Whether gluconeogenesis from amino acids is stimulated because energy becomes limiting for vital functions in scouring calves, or is brought about by a direct stimulation of glucocorticoid secretion by high level of free methionine in the diet is not known.

The requirements of other amino acids were calculated from the knowledge of the amino acid content in the newborn calf and the determined requirement of methionine. The requirement so determined should serve only as guidelines, prior to direct determination of the requirement of these amino acids.

APPENDIX

APPENDIX

Table A1.--Analysis of variance for initial and final body weight, average daily gain, and fecal score of calves fed graded levels of methionine.

	Mean squares				F-ratio[a]		
	Calves	Periods	Treat-ments	Error	Calves	Periods	Treat-ments
Degrees of freedom	19	1	4	15	19	1	4
Body weight							
Initial	45.90	11.85	1.234	1.098	41.79***	10.79**	1.12
Final	53.27	1.429	3.031	1.011	52.71***	1.41	3.00[a]
Avg. daily gain	36,462	438,971	102,698	48,010	.76	9.14**	2.14
Fecal score	.8777	19.60	.6046	.5920	1.48	33.11***	1.02

[a]***: P < .001; **: P < .01; a: P < .10.

Table A2.--Analysis of variance for the relationship between dietary methionine levels (X, %)[a] and final body weight (BW, kg) average daily gain (ADG, g), and fecal score (FS), respectively. Adjusted observations.[b]

Source of Variation	d.f.	Mean Square	F-ratio	Significance level[c]
Dependent variable: BW				
β of X	1	7.4689	.26	NS
β of X^2	1	.8377	.03	NS
β of X^3	1	10.6339	.37	NS
Error	36	28.5973	---	--
Dependent variable: ADG				
β of X	1	202,626	3.98	**
β of X^2	1	274,704	5.40	*
β of X^3	1	170,927	3.36	**
Error	36	50,861	---	--
Dependent variable: FS				
β of X	1	.4986	.42	NS
β of X^2	1	2.1224	1.78	NS
β of X^3	1	1.1793	.99	NS
Error	36	1.1935	---	--

[a]Relative methionine concentration: 100 = 2.48 g Met/16 g N.

[b]Adjusted observation = Observation - (Observed treatment mean - Adjusted treatment mean).

[c]*: $P < .05$; **: $P < .10$; NS: non significant.

Table A3.--Analysis of variance for the relationship between fecal excretion (F, kg) and average daily gain (ADG, g), and the estimated regression. Unadjusted observations.

Source of variation	d.f.	Mean Square	F-ratio	Significance level[a]
β of F	1	1,288,383	60.57	***
β of F^2	1	51,510	2.42	NS
β of F^3	1	157,002	7.38	*
Error	36	21,272	--	--

Estimated regression:

$\widehat{ADG} = 120.1 - 40.94\ F;\ R = .75***$

[a] ***: $P < .001$; *: $P < .05$; NS: non significant.

Table A4.--Analysis of variance for the relationship between fecal excretion (F, kg) and urine excretion (U, liters), and the estimated regression. Unadjusted observations.

Source of variation	d.f.	Mean square	F-ratio	Significance level[a]
β of F	1	1,173.45	6.29	*
β of F^2	1	.06	<.01	NS
β of F^3	1	82.84	.44	NS
Error	36	186.50	--	--

Estimated regression:

$\hat{U} = 33.2397 - 1.236\ F;\ R = .38*$

[a] *: $P < .05$; NS: non significant.

Table A5.--Analysis of variance for the relationship between urine excretion (U, liters) and average daily gain (ADG, g), and the estimated regression. Unadjusted observations.

Source of variation	d.f.	Mean Square	F-ratio	Significance level[a]
β of U	1	463,601	13.84	***
β of U^2	1	577,957	17.26	***
β of U^3	1	15,639	.47	NS
Error	36	33,487	--	--

Estimated regression:

$\widehat{ADG} = -791.5 + 43.12\ U - .4825\ U^2$; $R = .68^{***}$

[a] ***: $P < .001$; NS: non significant.

Table A6.--Analysis of variance for dry matter (DM) content (%) in milk replacer, milk weigh back, feces, and digestibilities of DM and crude protein (CP) in calves.

Source of variation	Mean squares				F-ratio[a]		
	Calves	Periods	Treat-ments	Error	Calves	Periods	Treat-ments
Degrees of freedom	19	1	4	15	19	1	4
DM concentration:							
Milk replacer	.0788	.3842	.0282	.0300	2.62*	12.80**	.94
Milk weigh back	3191.	.0308	1700.	1257.	2.54*	<.01	1.35
Feces	58.75	1377.	19.93	43.33	1.36	31.79***	.46
Digestibility, %:							
Dry matter	69.48	292.7	165.0	68.45	1.02	4.28†	2.41†
Crude protein	214.7	1518.	401.4	153.9	1.40	9.86*	2.61†

[a]Superscripts: †: P < .10; *: P < .05; **: P < .01; ***: P < .001.

Table A7.--Analyses of variation for the relationship between dietary methionine levels and the digestibility of dry matter (DM) and crude protein (CP) in calves. Adjusted observations.[b]

Source of variation	d.f.	Mean square	f-ratio	Significance level[c]
Dependent variable: DM				
β of X	1	140.2	1.79	***
β of X^2	1	36.20	.46	NS
β of X^3	1	298.5	3.80	**
Error	36	78.46	--	--
Dependent variable: CP				
β of X	1	367.2	1.61	***
β of X^2	1	606.5	2.67	***
β of X^3	1	1090.	4.80	*
Error	36	227.3	--	--

Estimated regressions:

$\hat{DM} = 79.32 + .8835\ (10^{-1})\ X - .0288\ (10^{-4})\ X^3$; R = .24

$\hat{CP} = 35.74 + .4425\ X - .1078\ (10^{-4})\ X^3$; R = .33**

$\hat{CP} = 354.3 - 7.965\ X + .7010\ (10^{-1})\ X^2 - .1969\ (10^{-3})\ X^3$; R = .45*

[a]Relative methionine level: 100 = 2.48 g per 16 g N.

[b]Adjusted observation = Observation - (Observed mean - Adjusted mean)

[c]*: P < .05; **: P < .10; ***: P < .25; NS: non significant.

Table A8.--Analyses of variance for the relationship between fecal excretion (X, kg) and the digestibility of dry matter (DM) and crude protein (CP) in calves.

Source of variation	d.f.	Mean square	F-ratio	Significance level[a]
Dependent variable: DM				
β of X	1	1221.	21.61	***
β of X^2	1	6.936	.12	NS
β of X^3	1	37.91	.67	NS
Error	36	56.49	--	--
Dependent variable: CP				
β of X	1	5061.	39.76	***
β of X^2	1	31.15	.24	NS
β of X^3	1	169.4	1.33	NS
Error	36	127.3	--	--

Estimated regressions:

$\hat{DM} = 89.03 - 1.260\ X;\ R = .61^{***}$

$\hat{CP} = 76.92 - 2.665\ X;\ R = .73^{***}$

[a] ***: $P < .001$; NS: non significant.

Table A9.--Analysis of variance for N-balance data.

Source of variation	Mean squares				F-ratio[a]		
	Calves	Periods	Treatments	Error	Calves	Periods	Treatments
Degrees of freedom	19	1	4	15	19	1	4
Volumes							
Milkreplacer, g	372,345	2,031	14,188	6,036	61.69***	.34	2.34
Milk weigh back, ml	11,542,518	1,119,237	8,106,820	5,894,616	1.96†	.19	1.38
Urine, ml	318,740,890	336,341,830	134,522,470	67,123,493	4.75**	5.50*	2.00
Feces, g	14,305,608	255,070,452	17,606,419	11,418,143	1.25	22.34***	1.54
N-concentration, %							
Milkreplacer	.0066	.0068	.0049	.0017	3.89**	3.97†	2.89†
Milk weigh back	.0196	.0002	.0209	.0128	1.53	.01	1.63
Urine	.0338	.0587	.0554	.0354	.95	1.66	1.56
Feces	.3610	8.3979	.0812	.2975	1.21	28.23***	.27
N-content, g							
Milkreplacer	350.384	2.825	9.613	9.497	36.89***	.30	1.01
Milk weigh back	60.549	40.874	45.971	34.939	1.73	1.17	1.32
Urine	361.330	3.035	425.088	401.584	.90	.01	1.06
Feces	209.504	2,892.539	291.650	136.793	1.53	21.15	2.13
N-balance, g	687.187	2,251.524	1,781.691	745.105	.92	3.02	2.39†

[a]***: $P < .001$; **: $P < .01$; *: $P < .05$; †: $P < .10$.

Table A10.--Analysis of variance for the relationship between urine excretion (U, liters) and urine N-concentration (NU, %), and the estimated regression. Unadjusted observations.

Source of variation	d.f.	Mean square	F-ratio	Significance level[a]
β of U	1	.7613	73.91	***
β of U^2	1	.2880	27.96	***
β of U^3	1	.0356	3.46	†
Error	36	.0103	---	---

Estimated regressions:

$\hat{NU} = 1.314 - .7821\ (10^{-1})\ U + .1721\ (10^{-2})\ U^2 - .1250\ (10^{-4})\ U^3$;

$R = .86^{***}$

$\hat{NU} = .9283 - .3483\ (10^{-1})\ U + .3406\ (10^{-3})\ U^2$; $R = .85^{***}$

[a] ***: $P < .001$; †: $P < .10$.

Table A11.--Analysis of variance for the relationship between fecal excretion (F, kg) and the fecal N-concentration (NF, %) for all observations, and for period 1 (NF_1) and for period 2 (NF_2), respectively, and the estimated regressions. Unadjusted observations.

Source of variance	d.f.	Mean square	F-ratio	Significance level[a]
Dependent variable: NF				
β of F	1	10.35	91.30	***
β of F^2	1	4.21	37.17	***
β of F^3	1	1.39	12.27	**
Error	36	.11	--	--
Dependent variable: NF_1				
β of F	1	1.669	44.64	***
β of F^2	1	.5898	15.77	***
β of F^3	1	.2035	5.44	*
Error	16	.0374	--	--
Dependent variable NF_2				
β of F	1	4.2887	22.12	***
β of F^2	1	1.0983	5.66	*
β of F^3	1	.0964	.50	NS
Error	16	.1939	--	--

Estimated regressions:

$\hat{NF} = 2.716 - .7181\ F + .7312\ (10^{-1})\ F^2 - .2304\ (10^{-2})\ F^3;\ R = .89^{***}$

$\hat{NF}_1 = 2.148 - .4614\ F + .4219\ (10^{-1})\ F^2 - .1243\ (10^{-2})\ F^3;\ R = .90^{***}$

$\hat{NF}_2 = 2.840 - .7779\ F + .6409\ (10^{-1})\ F^2;\ R = .79^{***}$

[a] ***: $P < .001$; **: $P < .01$; *: $P < .05$; NS: non significant.

Table A12.--Analysis of variance for N-balance data expressed as g N/day per 100 kg initial body weight.

Source of variation	Mean squares				F-ratio[a]		
	Calves	Periods	Treatments	Error	Calves	Periods	Treatments
Degrees of freedom	19	1	4	15	19	1	15
Milkreplacer	3.6549	6.7078	.7606	1.3452	2.72*	4.99**	.57
Milk weigh back	6.2343	4.3943	4.8358	3.7211	1.68	1.18	1.30
Urine	38.6758	1.0208	39.8321	37.9828	1.02	.03	1.05
Feces	29.5284	250.0871	30.0516	15.0417	1.96†	16.63***	2.00
N-balance	79.0335	234.0127	184.2274	81.4554	.97	2.87	2.26

[a]***: $P < .001$; **: $P < .01$; *: $P < .05$; †: $P < .10$.

Table A13.--Analysis of variance for the relationship between dietary methionine levels (X,[a] %) and N-balances expressed as total for the experimental period (NB_1, g), g N daily per 100 kg initial BW (NB_2), and g N daily per kg $BW^{.73}$ (NB_3), respectively. Adjusted observations.[b]

Source of variation	d.f.	Mean square	F-ratio	Significance level[c]
Dependent variable: NB_1				
β of X	1	1443.	1.83	NS
β of X^2	1	1111.	1.43	NS
β of X^3	1	8752.	11.23	**
Error	36	779.5	---	--
Dependent variable: NB_2				
β of X	1	151.3	1.73	NS
β of X^2	1	105.4	1.20	NS
β of X^3	1	910.0	10.38	**
Error	36	87.70	---	--
Dependent variable: NB_3				
β of X	1	.2713	4.10	†
β of X^2	1	.0189	.29	NS
β of X^3	1	.8574	12.97	**
Error	36	.0661	---	--

[a] Relative Met conc: 100 = 2.48 g Met/16 g N.

[b] Adjusted observation = Observation - (Observed mean - Adjusted mean).

[c] **: $P < .01$; †: $P < .10$; NS: non significant.

Table A14.--Analysis of variance for plasma concentrations of methionine, valine, leucine, and isoleucine.

Source of variation	Mean squares				F-ratio[e]		
	Calves	Periods	Treatments	Error	Calves	Periods	Treatments
Degrees of freedom	19	1	4	15	19	1	4
Methionine							
First day, T-0[a]	1.9289	.0672	.8590	1.9951	.97	.03	.43
First day, T-2[b]	3.3109	.2772	13.3068	1.9445	1.70	.14	6.84**
Last day, T-0[c]	1.3551	.4862	2.6000	1.0579	1.28	.46	2.46†
Last day, T-2[d]	4.3374	.8821	14.3256	2.0761	2.09†	.42	6.90**
Valine							
First day, T-0	213.64	137.94	183.51	204.81	1.04	.67	.90
First day, T-2	35.64	12.94	15.56	26.74	1.33	.48	.58
Last day, T-0	27.48	150.78	32.58	21.96	1.25	6.86*	1.48
Last day, T-2	29.74	37.81	37.39	20.56	1.45	1.84	1.82
Leucine							
First day, T-0	32.23	26.70	22.90	32.90	.98	.81	.70
First day, T-2	8.179	6.281	6.141	5.516	1.48	1.14	1.11

Table A14.--Continued.

Source of variation	Mean squares				F-ratio[e]		
	Calves	Periods	Treatments	Error	Calves	Periods	Treatments
Last day, T-0	7.870	20.98	10.92	5.636	1.40	3.72†	1.94
Last day, T-2	8.247	1.739	5.221	4.349	1.90	.40	1.20
Isoleucine							
First day, T-0	6.377	5.336	2.560	7.383	.86	.72	.35
First day, T-2	21.95	1.336	15.61	14.81	1.48	.09	1.05
Last day, T-0	11.24	45.05	20.05	11.17	1.01	4.03†	1.79
Last day, T-2	12.92	13.75	13.74	10.33	1.25	1.33	1.33

[a]First day of the experimental period. Blood collected before feeding.

[b]First day of the experimental period. Blood collected two hours after feeding.

[c]Last day of the experimental period. Blood collected before feeding.

[d]Last day of the experimental period. Blood collected two hours after feeding.

[e]**: $P < .01$; *: $P < .05$; †: $P < .10$.

Table A15.--Analysis of variance for the relationship between dietary methionine (X,[a] %) and the plasma methionine concentration (M μM/100 ml). Adjusted observations.[b]

Source of variation	d.f.	Mean square	F-ratio	Significance level[g]
Dependent variable: M-10[c]				
β of X	1	5.3572	2.95	†
β of X^2	1	.0344	.02	NS
β of X^3	1	.0060	<.01	NS
Error	36	1.8141	--	--
Dependent variable: M-12[d]				
β of X	1	52.8385	17.79	***
β of X^2	1	.2905	.10	NS
β of X^3	1	32.0197	10.78	**
Error	36	2.9702	--	--
Dependent variable: M-20[e]				
β of X	1	14.8264	14.17	***
β of X^2	1	.0396	.04	NS
β of X^3	1	1.6217	1.55	NS
Error	36	1.0460	--	--
Dependent variable: M-22[f]				
β of X	1	86.4282	32.72	***
β of X^2	1	.0179	.01	NS

Table A15.--Continued.

Source of variation	d.f.	Mean square	F-ratio	Significance level[g]
β of X^3	1	5.0220	1.90	NS
Error	36	2.6413	--	--

[a]Relative methionine concentration: 100 = 2.48 g Met/16 g N.

[b]Adjusted observation = Observation - (Observed mean - Adjusted mean).

[c]First day of the experimental period. Blood collected before feeding.

[d]First day of the experimental period. Blood collected two hours after feeding.

[e]Last day of the experimental period. Blood collected before feeding.

[f]Last day of the experimental period. Blood collected two hours after feeding.

[g]***: P < .001; **: P < .01; †: P < .10; NS: non significant.

Table A16.--Analysis of variance for the relationship between dietary methionine (X,[a] %) and the plasma methionine concentration (M, μM/100 ml). Group E deleted. Adjusted observations.[b]

Source of variation	d.f.	Mean square	F-ratio	Significance level[g]
Dependent variable: M-10[c]				
β of X	1	2.542	1.47	NS
β of X^2	1	.0673	.04	NS
β of X^3	1	.0636	.04	NS
Error	28	1.734	--	--
Dependent variable: M-12[d]				
β of X	1	55.52	19.41	***
β of X^2	1	17.30	6.04	*
β of X^3	1	4.338	1.52	NS
Error	28	2.860	--	--
Dependent variable: M-20[e]				
β of X	1	4.861	5.74	*
β of X^2	1	.6172	.73	NS
β of X^3	1	.6548	.77	NS
Error	28	.8470	--	--
Dependent variable: M-22[f]				
β of X	1	54.49	25.21	***
β of X^2	1	2.423	1.12	NS

Table A16.--Continued.

Source of variation	d.f.	Mean square	F-ratio	Significance level[g]
β of X^3	1	1.541	.71	NS
Error	28	2.1618	--	--

[a]Relative met conc: 100 = 2.48 g met/16 g N.

[b]Adjusted observation = Observation - (Observed mean - Adjusted mean).

[c]First day of the experimental period. Blood collected before feeding.

[d]First day of the experimental period. Blood collected two hours after feeding.

[e]Last day of the experimental period. Blood collected before feeding.

[f]Last day of the experimental period. Blood collected two hours after feeding.

[g]***: $P < .001$; *: $P < .05$; NS: non significant.

Table A17.--The relationship between dietary methionine levels (X, %)[a] and plasma urea nitrogen (PUN) in calves on the first (1) and the last (2) day of the experimental period.

Source of variation	Degrees of freedom	Mean square	F-ratio	Significance level[b]
Dependent variable: PUN_1				
β of X	1	10.95	1.49	#
β of X^2	1	11.69	1.60	#
β of X^3	1	14.91	2.03	#
Error	29	7.328	--	--
Dependent variable: PUN_2				
β of X	1	.6251	.06	NS
β of X^2	1	28.24	2.57	#
β of X^3	1	.4514	.04	NS
Error	30	11.00	--	--

Estimated regressions:

$\hat{PUN}_1 = -28.47 + 1.025\ X - .9085\ (10^{-2})\ X^2 + .2578\ (10^{-4})\ X^3$; R = .39#

$\hat{PUN}_2 = 22.78 - .2200\ X + .8773\ (10^{-3})\ X^2$; R = .28#

[a] Relative methionine level: 100 = 2.48 g methionine per 16 g N.

[b] #: P < .25; NS: non significant.

Table A18.--The relationship between dietary methionine levels (X, %)[a] and hematocrits (HEM) in calves on the first (1) and the last (2) day of the experimental period.

Source of variation	Degrees of freedom	Mean square	F-ratio	Significance level[b]
Dependent variable: HEM_1				
β of X	1	192.9	6.22	*
β of X^2	1	113.4	3.65	†
β of X^3	1	3.400	.11	NS
Error	29	31.03	--	--
Dependent variable: HEM_2				
β of X	1	144.4	3.77	†
β of X^2	1	53.12	1.39	#
β of X^3	1	29.05	.76	NS
Error	26	38.27	--	--

Estimated regressions:

$\hat{HEM}_1 = 56.05 - .3852\ X + .1827\ (10^{-2})\ X^2;\ R = .52^*$

$\hat{HEM}_2 = 48.57 - .2506\ X + .1251\ (10^{-2})\ X^2;\ R = .40^\dagger$

[a]Relative methionine level: 100 = 2.48 g methionine per 16 g N.

[b]*: $P < .05$; †: $P < .10$; #: $P < .25$; NS: non significant.

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