STUDIES ON THE NUTRITION OF THE RUMINANT

A thesis submitted to the University of Glasgow in accordance with the regulations for the degree of Doctor of Philosophy in the Faculty of Science.

by

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INTRODUCTION

The mortality rate of young calves in Great Britain is extremely high. A study of 335 herds in England and Wales (Lovell & Hill, 1940) has shown that 6% of female calves die before reaching six months of age. In Scotland the figure may rise to as high as 22% (Jordon, 1933), although a more recent survey in the Dumfriesshire area places the mortality rate at 7% (Hector & Rowat, 1948). Reports from India (Minett, 1946), New Zealand (Ward, 1945) and America (Atkeson, 1931; Angels & Cannon, 1936; Weaver, Horwood & Smiley, 1949) all indicate that a comparable and, indeed sometimes a much higher loss of life takes place in these parts of the world. These figures refer only to mortality rates. The morbidity rates must be considerably higher.

Bacterial diseases are cited by most workers as the primary cause of the mortality and only rarely is malnutrition cited as a direct or indirect cause. That faulty nutrition of the young calf plays an important role in mortality is shown however, both from laboratory studies and from recorded field cases of deficiency diseases in calves. Thus vitamin A deficiency of the calf results in blindness, failure to grow, pneumonia and finally death. In experimental studies, vitamin E deficiency results in muscular dystrophy followed by prostration and eventually death (Blaxter, Watts & Wood, 1951). On the other hand, if the heart is affected in this disease sudden death occurs with little sign of clinical abnormality. Similar signs and deaths have been reported in practice (Vawter & Records, 1947; Stamp & Blaxter, 1951). Phillips, Lundquist & Boyer (1941) have claimed that calf mortality from scours and pneumonia can be reduced by feeding large doses of vitamins A, D, thiamin, riboflavin, nicotinic acid, pantothenic acid and choline to calves from birth to four weeks of age. A similar experiment (Norton, Eaton, Loosli & Spielman, 1946), however, did not confirm these results. This

more recent experiment was carried out with animals from a well managed university herd, and the results may indicate that, although these vitamin deficiencies do not occur in animals treated well, they may do so in calves maintained under poorer conditions. Of the minor mineral deficiencies which occur, calves may die from copper pining (Jamieson & Allcroft, 1950) and magnesium tetany (Duncan, Huffman & Robinson, 1935). Little is known regarding other minerals, though Archibald, Kucinsky, Brook & Greeman (1938) have found a nutritional anaemia in practice which responded to iron treatment.

Of the nutritional deficiencies which occur in farm animals, it is well known that by far the most economically important and widespread are the deficiencies of protein and energy. Such deficiencies do not result in the spectacular series of signs which accompany minor element and vitamin deficiencies, but they are associated with a slow decline in vigour, possibly rendering the animal more susceptible to bacterial infection. Despite their considerable importance, the deficiencies of protein and energy are not widely recognised, in fact as far as the young calf is concerned, little is known regarding even its requirement for these nutrients. The excellent early work of Soxhlet (1878) and of Fingerling (1908, 1912) has been used as the basis for the computation of protein and energy requirements of the calf by a number of workers during the last 50 years, often with widely divergent results. Armsby (1917) sums up the position excellently.."..there has been a persistent tendency to substitute for the study of the principles of nutrition a series of exercises in applied arithmetic".

To remedy this deficiency in our knowledge, experiments on protein and energy metabolism were carried out with calves, and the results are embodied in this thesis.

The word protein was derived from the Greek 'proteios' meaning, 'of primary importance' by Mulder (1839) after a consideration of the experiments of

Magendie (1816) who showed that dogs which were given only nitrogen free foods such as olive oil, sugar or butter, died in a month or less. He found that death could be prevented by feeding protein containing substances (or as he termed it - "azotised" foods). Boussingault (1857), Prout (1855) and Liebig (1843) each considered protein as the single principle in nutrition, thereby relegating all other nutrients to minor roles. The present understanding of the relationships between protein, fat and carbohydrate and of the individuality of the proteins is based on the inspired work of Carl Voit and his students (Rubner, Atwater, Müller, E., Voit, Cremer, Lusk and Cathcart) and of his contemporary, Pflüger. In the light of modern knowledge protein nutrition is now known to be amino-acid nutrition. The classical work of Osborne and Mendel and of Rose has shown that certain of the amino-acids are 'essential' - that is they cannot be synthesised in the body, whereas others are 'non-essential', or can be synthesised from other amino-acids in the body.

The adult ruminant has a considerable synthesising micro-flora in its rumen and so it is comparatively independent of an external source of amino-acids provided it is supplied with minerals, roughage, a source of nitrogen and those vitamins which it, or its micro-flora cannot synthesise. As early as 1891 Zuntz and Hagemann put forward the hypothesis that micro-organisms of the rumen might synthesise protein from non-protein nitrogenous compounds, and that subsequently this bacterial protein might be digested and utilised by the host animal. Experiments leading to the proof of this theory have been reviewed by Krebs (1937) and by McNaught & Smith (1947). Before the rumen of a young calf develops, however, the animal is rather unusual. By species it is a ruminant but its rumen is not functional and digestion takes place in a manner similar to that in simple stomached animals such as the dog, rat or man. During this pre-ruminant stage, the young calf should be just as dependent on

an external source of amino-acids as are these animals.

The problems dealt with in this thesis then, are divisible into two categories; firstly, those concerned with the requirement of the calf for nitrogen for a particular function and secondly, the demonstration of the differences in the nutritive value between proteins to meet these requirements. Basic information on the nitrogen metabolism of the calf, such as the endogenous losses and the losses during starvation are not known however, and were therefore characterised before the main problems were undertaken. The technique used throughout this study was that of nitrogen balance using simplified diets.

In Part I, details are given of the calves used and of their experimental treatment. The methods of chemical analysis are also outlined.

Part II is concerned with the nitrogen metabolism of the calf. To characterise its basic metabolism the first experiments describe losses which occur during total starvation and during specific protein starvation. From these experiments, an estimate of the maintenance requirements of the calf for total calories and for protein is made. The next experiment is concerned with factors which affect the determination of the quality of a protein. Some results of this experiment were confirmed using whole milk as the diet. The results of these two experiments indicated that whole milk was deficient in total calories compared with protein calories. Consequently, an experiment was carried out using gelatinised starch as a supplement to whole milk. Taking into consideration the knowledge and experience acquired in the previous experiments, the last experiment which is included here, is concerned with the demonstration of the difference in nutritive value between gelatin, casein and the proteins of dried skim milk. The final section compares data obtained in all these experiments. After a discussion of the digestibility of the diets and of the urinary excretion of nitrogen contained in various fractions, an estimate of the protein requirement of the calf is calculated.

PART I - METHODS

A. GENERAL TREATMENT OF ANIMALS WITH SPECIAL REFERENCE TO BALANCE TECHNIQUE

1. Animals

Ayrshire bull calves were used in these experiments. When possible the animals were obtained from the Hannah Dairy Research Institute farm or adjacent farms; otherwise they were chosen from Ayr Cattle Market where they were selected for bright: :ness and freedom from apparent abnormality.

2. Housing

Calves were housed in heated rooms, in which the thermostats were set at 65°F. Maximum and minimum temperatures were recorded daily and these showed that maximum gradients of 10°F over 24 hours occurred.

3. General pre-experimental treatment

On arrival at the Institute, the animals were isolated when possible in pens littered with straw. They were given a drink of warm water and were later given 25ml. "Welcome" b. coli anti-serum by subcut: :aneous injection. This was to ensure that the calves received antibodies whether or not they had previously been given colostrum. For the first feed one litre of whole milk was given. The quantity was increased slowly to two litres per feed the progression depending on the condition of the animals. Feeding was carried out twice daily making the intervals between meals as near to 12 hours as possible. At all feeding times the milk was warmed to 102°F. When the animals had received whole milk for one week the experimental diet was gradually introduced, until after four days the animals were receiving their full complement for the experiment. This general proced: :ure was, of course, modified according to the condit: :ion of the animal. If diarrhoea occurred, extra water was added, sometimes with salt. If scouring

was extreme, the diet was replaced by water and Phthalylsulphathiazole ('Thiazole') was given orally. When the condition had improved the diet was again gradually re-introduced. Meanwhile the animals were changed to metabolism cages, which are described below, for the separation and collection of urine and faeces. About four days were allowed for the animals to accustom themselves to the cages and collection procedure. Collections of urine and faeces for the determination of a nitrogen balance were then started. Roughage was specifically excluded from the diets of the animals.

4. Composition of diets

The animals were given diets resembling milk. The composition of the diets was altered according to the particular experiment, but they consisted essent: :ially of fat homogenised into a protein solution. Lard was used as the source of fat, and dried skim milk was usually used as a source of protein. The dried skim milk also supplied lactose, minerals, water soluble vitamins and a small amount of fat and assoc: :iated vitamins. Table 1 gives typical figures for the composition of dried skim milk from which the theoretical composition of the semi-synthetic diets was calculated.

TABLE 1

Typical figures for the Composition of Dried Skim-Milk

	Composition (%)
Moisture	3.8
Protein	33.5
Fat	1.1
Lactose	53.5
Ash	8.0
Calcium	1.3
Phosphorus	1.0

TABLE 2

Mineral Mixture used to Supplement the Diets of Experimental Calves

Macro constituents	Quantity (g.)
$CaHPO_4$ $2H_2O$	200
K_2HPO_4	350
$CaCl_2$	100
MgO	40
$Na_2HPO_4.12H_2O$	150
NaCl	80
$CaCO_3$	100
Citric acid	150
Fe citrate	20.
Micro constituents	**Quantity (g.)**
$MnSO_4.4H_2O$	0.5
$ZnCl_2$	0.5
$CuSO_4.5H_2O$	0.5
$CoCl_2$	0.1
Kl	1.0
NaF	0.5

If the percentage of dried skim milk in the diet was low or nil, additional balanced minerals were added, including the essential minor elements. The components of the supplementary mineral mixture are given in Table 2.

In Table 3 its composition is compared with that of whole milk.

TABLE 3

The Major radicles supplied by the Mineral Mixture compared with those supplied by Cow's Milk

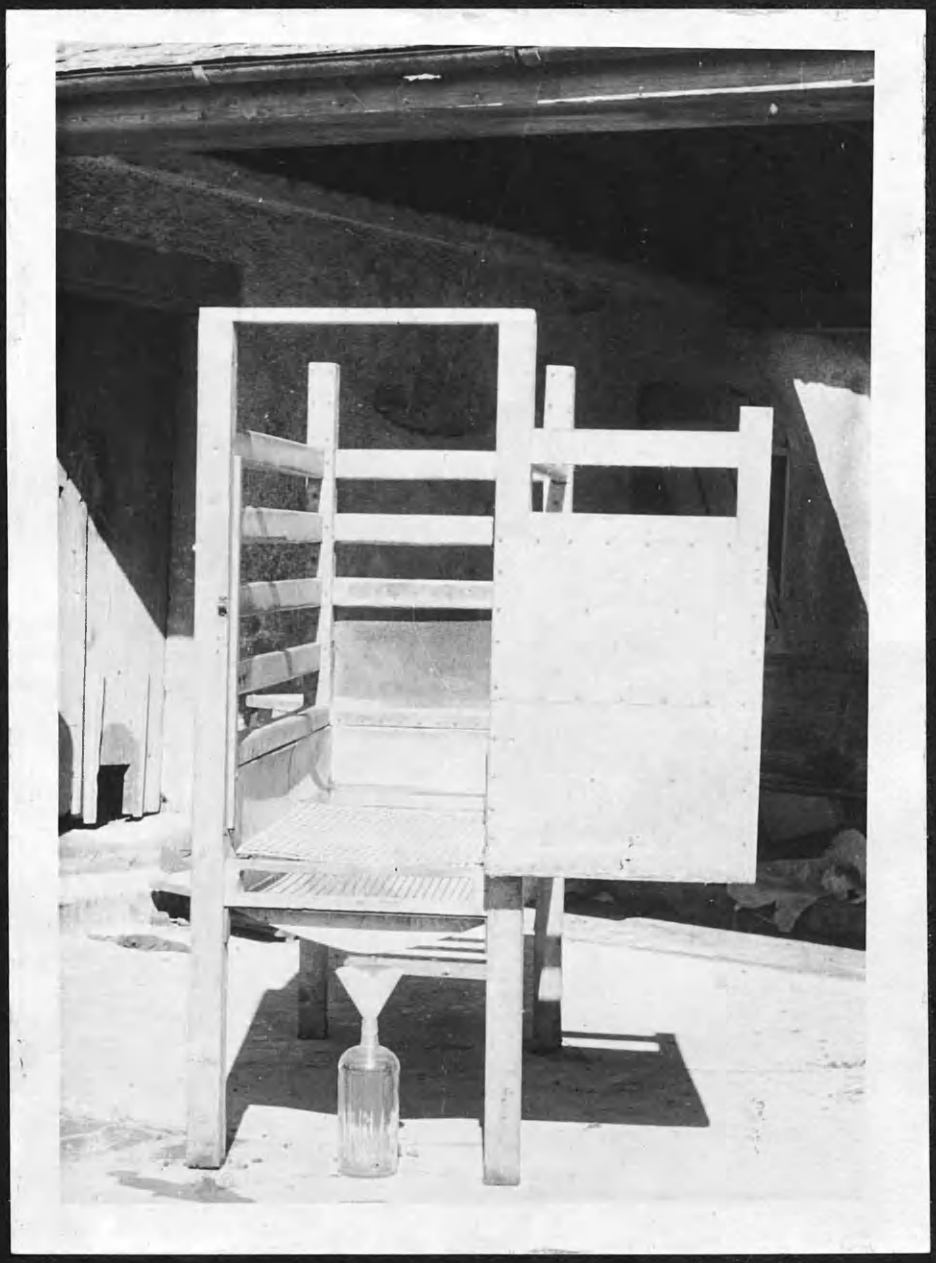
	Typical figures for cow's milk	Mineral mixture used in the present work
	(mg. /100ml. milk)	(mg. in 1.38 g.)
Calcium	120	122.
Phosphorus	100	110.
Sodium	50	50.
Potassium	150	157.
Chlorine	110	112.
Magnesium	12	24.

It will be seen that the composition of the supple: :mentary mixture was adjusted to resemble the composition of the ash of milk with the exception that the magnesium content was doubled to prevent hypomagnesaemic tetany (Duncan, Huffman & Robinson, 1935). When dried skim milk was not used in the diet vitamins of the B complex were provided by a yeast extract, prepared according to the method of McCrae, El Sadr & Sellers (1942). Vitamins A and D were provided by homogenising into the diet either veterinary cod-liver oil or the pure vitamins dissolved in arachis oil. (2000 I.U. vitamin A and 200 I.U. vitamin D per ml.)

5. Preparation of diets

The dried skim milk or other protein, fat and glucose were accurately weighed into buckets. The dried skim milk powder and glucose were dissolved with

Plate I.



Metabolism crate used for the separation of urine and faeces.

the aid of an electric stirrer in cold water contained in a churn. The churn and contents were then heated by immersion in hot water and maintained at 65°C for 15 min. Meanwhile the fat was melted by placing the bucket in a boiling water-bath. About half the volume of hot reconstituted dried skim milk was mixed with the melted fat and vitamin A and D supplement. The constituents were well mixed and then homogenised at a pressure of 3500-4000 lbs./sq. in. pressure. The concentrated homogenate was re-homogenised and returned to the original churn con: :taining the remainder of the reconstituted dried skim milk. The bulk was then homogenised at the same pressure. Minerals were added and the diet made up to volume with cold water. The diets were stored in an immersion cooler maintained at 5°C until required for use. Under these conditions of storage it was necessary to make up diets twice weekly.

6 General balance technique

(a) Diet

All synthetic milks were either weighed or measured accurately before feeding. A fresh sample of milk was taken for the determinations of total nitrogen and total solids and an aliquot then preserved with 40% formaldehyde for subsequent determinations of fat and minerals.

(b) Urine collection

Plate 1 shows one of the cages in which quantitative urine collections were made. The wooden frame was covered with sheet metal on the inside for ease of cleaning and also to prevent the animal from chewing the wood, The floor of the cage was essentially of heavy gauge 1" wire netting, through which the urine passed on to a sheet metal floor below. The latter sloped towards a hole near the front of the cage where the urine was collected by means of a funnel and Winchester bottle. Approximate: :ly 5 ml. glacial acetic acid per litre of urine were used as a preservative when nitrogen distributions

Plate 2.

Animal fitted with harness and bag for the quantitative collection of faeces.

were to be carried out.

Collection of excreta for the day started imm: :ediately after the morning feed and finished at the same time the following morning. Urine volumes were measured every day and an aliquot taken for the determination of total nitrogen. Another aliquot was placed in the refrigerator for the nitrogen fraction: :ations which were carried out at two-day intervals. A further 10% aliquot of the total urine volume was taken for the determination of minerals.

(c) Faeces collection

One of the main difficulties in carrying out balance studies with calves was found to be the quantitative collection of faeces, especially when alimentary disturbances were present. Plate 2 shows an animal fitted with the harness and bag which was designed to permit a quantitative collection of faeces.

Faeces bags were constructed from light weight car inner tubes cut to shape from a metal pattern. The bottom of the tube was sealed and four metal 'D' pieces fastened to the top by means of rubber strips as shown in Plate 2. At first rubber solution was used as an adhesive, but as leakage difficulties occurred, the seams were later vulcanised. The weight of each bag was marked on a metal disc attached to one of the 'D' pieces. A hole was made at the top of the bag and then bound with rubber so as to fit the tail fairly tightly but without chafing.

The harness to keep the bag in position on the animal may also be seen in Plate 2. It was constructed from light webbing and consisted of a girth band behind the shoulders maintained in position by another band passing over the chest. The chest band was fitted with four slides of webbing to each of which was attached a metal 'D' piece. The bag was attached to the harness by means of spring curtain wire covered with rubber tubing. Removal of the bag from the harness was facilitated by spring clips.

When the calf was producing normal faeces this method of collection was satisfactory. If, however, the faeces were very liquid loss from the bag could be high. This loss was found to occur in two ways; either through the tail hole when the animal defaecated, or between the bag and the hocks of the animal when it was lying down. The first type of loss was effectively stopped by fitting a thin sheet of rubber tightly over the tail before the faeces bag was placed in position. The only method of stopping loss from the sides of the bag was to change it at frequent intervals.

Collections for the determination of nitrogen balance entailed changing the faeces bag daily before the morning feed. The bag was then weighed and the difference between the empty and full bag was taken as the weight of faeces excreted. The contents were well mixed and an aliquot weighed into a ground glass stoppered jar. The same percentage aliquot was added the second day. Analyses were carried out every two days on these pooled samples which were stored in the refrigerator if they were not analysed immediately.

(d) Body weight, pulse rate and general notes

During the experiments the animals were weighed to the nearest 100g. every two days before the morning feed.

Pulse rates were taken before each feeding time, and general notes on the well being of the calf were made at the same time.

7. Respiratory exchange determinations

Oxygen consumption and carbon dioxide product:ion of the calves were determined using the apparatus and technique of Blaxter & Howells (1951). Briefly, the procedure was as follows:-

The calf was connected by means of a rubber mask to a large spirometer of maximum volume 220 litres. The volume of air expired in 30 minutes was measured and

an aliquot taken for the determination of oxygen and carbon dioxide.

B. METHODS OF CHEMICAL ANALYSIS

The methods used are grouped under determinations in milk, urine and faeces respectively. Those methods which are applicable to all, viz:- dry matter, total nitrogen and mineral determinations are presented first. Unless otherwise stated, all methods which were used are to be found in the usual text books of quantitative analysis (Peters & Van Slyke, 1932; Hawk, Oser & Summerson, 1947).

1. General determinations

(a) Determination of dry matter

Faeces were dried to constant weight in an oven maintained at 100°C. Milk was first evaporated on a water-bath and then dried to constant weight in the oven.

(b) Determination of total ash

This was carried out on the same sample used for the determination of dry matter. Organic matter was first burnt off and then the sample incinerated in a muffle furnace at 500°C overnight or until the ash was white.

(c) Determination of total nitrogen

Kjeldahl method.

An aliquot of milk, urine, or faeces containing 10-25 mg. Nitrogen was digested with concentrated sulphuric acid and potassium sulphate, using copper sulphate and selenium as catalysts. The ammonia was liberated with sodium hydroxide and distilled into boric acid solution containing the double indicator methyl red and brom-cresol green. It was then directly titrated with standard acid.

For micro-Kjeldahl determinations the same reagents were used but the Markham apparatus was used for the distillation.

(d) Mineral determinations

i. Ashing procedure

Milk and faeces were ashed for the determination of calcium, magnesium, sodium and potassium. Urine was ashed for the determination of potassium, but calcium, magnesium and sodium were determined on the original material. Chloride and phosphorus were also determined on the original material.

Organic matter was burnt away slowly at low temperature over the bunsen and the residue was then incinerated overnight at 450-500°C. The ash was moistened with nitric acid and taken to dryness. It was again taken to dryness with concentrated hydrochloric acid and dissolved in a minimal quantity of N hydrochloric acid. The solution was then made up to 50 ml.

ii. Determination of Calcium

Modified method of McCrudden (Peters and Van Slyke, 1932).

Calcium was precipitated as the oxalate whilst hot from the solution buffered with ammonium acetate and acetic acid. The precipitate was filtered off, washed and titrated with standard permanganate solution.

iii. Determination of Magnesium

Modified method of McCrudden. (Peters & Van Slyke, 1932).

The filtrate from the calcium determination was buffered with sodium citrate. Ammonium phosphate and concentrated ammonia were added to precipitate magnesium as the magnesium ammonium phosphate. The precipitate was washed, dried with alcohol and ether and weighed as the hydrated salt.

iv. Determination of Phosphorus

Method of Fiske & Subbarow (Hawk, Oser and Summerson, 1947).

Organic matter was destroyed by digestion with sulphuric acid and hydrogen peroxide. Acid molybdate solution was added to form phosphomolybdic acid which was subsequently determined colorimetrically by the addition of 1;2;4-amino-naphthol sulphonic acid.

v. Determination of Sodium

Butler and Tuthill's modification of the Baber and Kothoff method (Peters & Van Slyke, 1932).

Phosphate was removed with lime and the sodium precipitated as the uranyl sinc sodium acetate compound. This was dried with alcohol and ether and weighed.

vi. Determination of Potassium

Method of Sideris (1937, 1942).

Potassium was precipitated as the cobaltinitrite in centrifuge tubes. After centrifuging and washing the cobalt in the precipitate was determined colorimetrically as the nitroso-R-salt complex.

vii. Determination of Chloride

Method of Volhard (Peters & Van Slyke 1932).

Chloride was precipitated by a known amount of silver nitrate. The excess silver nitrate was back-titrated with standard thiocyanate solution using ferric alum as indicator.

2. Milk analyses

(a) Determination of total fat

i. Method of Werner-Schmidt

Alcohol and ammonia were added to the milk sample which was then extracted three times with a 50/50 mixture of ether and light petroleum. The solvent was distilled off and the fat weighed.

ii. Method of Gerber

The routine determination was used for cow's

whole milk only.

3. Urine analyses

(a) Distribution of nitrogen in the urine

i. Determination of Allantoin

Method of Young & Conway (1942)

Allantoin was hydrolysed to allantoic acid by alkali which was in turn hydrolysed to glyoxylic acid by heating with acid. A red colour was produced with glyoxylic acid when phenylhydrazine hydrochloride and potassium ferrocyanide were added.

ii. Determination of Amino-Nitrogen

Method of Albanese & Irby (1944).

A copper-amino-N complex was formed between copper in a buffered copper phosphate suspension and the amino-acids in urine. The soluble copper complex was filtered off, and the copper in the filtrate determined iodometrically.

iii. Determination of Creatinine and Creatine

Method of Folin (Hawk, Oser & Summerson, 1947).

Creatinine was determined by the orange colour produced with alkaline picrate (Jaffe reaction), and the colour was read in the Spekker absorptiometer using green filters.

Creatine was first hydrolysed to creatinine by autoclaving at 115°C. for 20 min.

iv. Determination of protein

Urine was heated with glacial acetic acid to precipitate any protein in solution. The precipitate was filtered off together with any skin and hair debris. This was washed and the nitrogen content determined by Kjedahl's method.

v. Determination of Purine bases

Modified method of Krüger & Schmidt (Hawk, Oser & Summerson 1947).

Purine bases, together with uric acid were precipitated by boiling the urine with copper sulphate and sodium bisulphite. After filtering and washing

the precipitate its nitrogen content was determined by Kjeldahl's method. Uric acid was determined separately by a colorimetric method (see below).

vi. Determination of Urea and Ammonia

Method of Van Slyke & Cullen (Hawk, Oser & Summerson, 1947).

Ammonia was determined by aspiration from urine made alkaline with sodium carbonate, into boric acid solution, where it was titrated directly with standard acid.

Urea was converted into ammonia by incubation with Jack-Bean meal which provided the enzyme urease.

vii. Determination of Uric acid

Modified method of Benedict & Franke (Hawk, Oser & Summerson, 1947).

The blue colour produced between uric acid and sodium cyanide + arsenophosphotungstic acid was determined quantitatively in the Spekker absorptiometer using orange filters. Urea was added to the cyanide solution to make it more stable (King, 1946).

(b) Distribition of Surphur in the urine

i. Total Sulphur

Method of Benedict (Hawk, Oser & Summerson, 1947).

The urine was evaporated and ignited with Denis' reagent (copper nitrate, ammonium nitrate and sodium chloride) to destroy organic matter. All sulphur was oxidised to sulphate which was then precipitated as the barium salt, filtered, washed, ignited and weighed.

ii. Inorganic Sulphur

Method of Folin (Hawk, Oser & Summerson, 1947).

Barium chloride solution was added to cold acidified urine. The precipitated barium sulphate was washed, filtered, dried and weighed.

iii. Ethereal Sulphur

Method of Folin (Hawk, Oser & Summerson, 1947).

The filtrate from the determination of inorganic sulphur was boiled gently to hydrolyse conjugated sulphates. The precipitated barium sulphate was washed and weighed as before.

iv. Neutral Sulphur

This was obtained by difference:- Total S - (Inorganic S + Ethereal S) = neutral S.

(c) Determination of Total Ketone Bodies

Method of Van Slyke (Peters & Van Slyke, 1932).

Ketone bodies which may be present include acetone, aceto-acetic acid and B hydroxybutyric acid. Aceto-acetic acid was converted to acetone by hydrolysis and hydroxybutyric acid was oxidised to acetone by refluxing with potassium dichromate. Acetone was then precipitated as the basic mercuric sulphate compound which was filtered off and weighed. Glucose and other interfering compounds were first removed by precipitation with copper sulphate and calcium hydroxide.

4. Analysis of Faeces

(a) Sampling

The aliquots representing the excretion for two days were well mixed in the glass container and all analyses other than for mineral constituents were carried out on the wet material. Weighing was done rapidly and accurately on a small filter paper, or by difference from a weighing bottle depending on the consistency of the particular sample.

(b) Determination of Total 'Fat'

Modified method of Saxon (Peters & Van Slyke, 1932).

The sample of faeces was acidified and heated to liberate fatty acids from their soaps. Alcohol was then added and the fat and fatty acids extracted three times with equal quantities of ether and light petroleum. The solvent was evaporated off and the residue weighed.

Note. This method does not give a true figure for total fat since neutral fat, free fatty acids, free fatty acids from soap and non-saponifiable residue are included. It was not found possible to determine these fractions every two days because of the time taken by the method.

(c) Fractionation of Faecal Lipids

Method of Tidwell & Holt (1936).

Neutral fat, free fatty acids and non-saponifiable residue were directly extracted from the faeces. Free fatty acids were titrated with standard alkali; non-saponifiable residue was obtained by saponifying the extract and then re-extracting; and the true neutral fat was obtained by difference. Soaps were determined by acidifying the residue from the first extraction and then re-extracting.

(d) Determination of Starch

Method of Nielsen (1943, 1945).

The starch present in the faeces sample was dissolved in perchloric acid and the colour produced between starch and iodine determined quantitatively.

5. Digesta analysis

(a) Steam volatile fatty acids

The sample was made normal with respect to sulphuric acid and steam distilled. 500ml. fractions of distillate were collected until the titration was 1ml. or less of N/10 alkali. The results were expressed in terms of N/10 acid.

6. Blood analysis

(a) Fractionation of serum proteins

Modified method of Majoor (1946, 1947).

Various fractions were precipitated by increas:ing the concentration of anhydrous sodium sulphate. " γ " globulin was precipitated with a concentration of 159 g. / litre; " α + β" globulins with a concentration of 270 g. / litre; and albumin with a concentration above 270 g. / litre.

Precipitation and filtration was carried out in an incubator at 37°C, and the nitrogen content of each precipitate determined by the micro-Kjeldahl method.

7. Gas analysis

(a) Oxygen and carbon dioxide

The composition of air expired by the calves was determined using a Haldane gas analysis apparatus.

(b) Methane and hydrogen

A known volume (about 200 litres) of expired air was passed through concentrated sulphuric acid to dry it and then through soda-lime to remove respiratory carbon dioxide. The methane and hydrogen were ignited at 700°C to form water and carbon dioxide. These were absorbed in weighed bottles containing concentrated sulphuric acid and soda-lime respectively. From the water and carbon dioxide produced the quantities of hydrogen and methane were calculated.

PART II. - NITROGEN METABOLISM OF THE YOUNG AYRSHIRE CALF.

A. THE METABOLISM OF THE CALF DURING STARVATION AND SUBSEQUENT REALIMENTATION.

In order to interpret the results of experiments on the effect of quality of protein in the diet of the calf it was necessary to study factors affecting the nitrogen metabolism in this animal. As part of this study, an experiment was carried out to determine the metabolism of nitrogen, sulphur, minerals and energy during starvation since during starvation the body materials are specifically drawn upon to maintain the life processes of the body.

A further, more practical interest of this experiment was its relation to calf diarrhoea. When a calf is affected by acute infantile diarrhoea -"scouring"- there is a marked fall in the nutrients which are absorbed from its digestive tract. This fall in the food supply to the tissues is sufficient to cause such negative nitrogen and energy balances that in severe cases the calf approaches a state of complete inanition. In farm practice a common and effective method of controlling this type of diarrhoea is to substitute boiled water for the calf's milk allowance, until the faeces become normal in appearance and then to commence realimentation very slowly. Such a method of control has the effect of substituting complete inanition for the partial inanition which results from scouring.

Much has been published on the effect of starvation in mature animals, more especially man, but comparatively little study has been made of the effect of starvation on the metabolism of the really young animal. In the mature ruminant, energy metabolism has been studied by Braman (1924), Benedict & Ritzman (1927) and Ritzman & Benedict (1938); while extensive studies of the nitrogen metabolism of the fasting steer or cow have been made by Carpenter (1927), Hutchinson & Morris (1936 a,b) and Morris and Ray (1939).

The energy metabolism has been studied in detail by Benedict & Ritzman (1931), Brody (1932), Ritzman, Washburn & Benedict (1936), Blaxter (1948) and Marston (1948). There is therefore much information available on the effect of starvation on the mature ruminant which can be used for comparative purposes.

2. Plan of experiment

Two calves were used in this experiment and each was subjected to two periods of starvation. The original plan was to study starvation and recovery in each animal when at a normal weight for its age and also following a period of undernutrition when its weight would be only 50% of that expected from its age. The object of this design was two-fold. Firstly, it has been shown by Marston (1948) that the minimal level of energy metabolism is reached more quickly when animals are starved following low levels of food intake than when starved following long periods of over or optimal nutrition. Secondly, scouring in calves tends to be chronic before an acute stage is reached, and thus, undernutrition for a period before almost complete inanition, is common. A study of inanition following undernutrition therefore seemed desirable.

Details of the calves and their experimental treatment are given in Table 4.

TABLE 4

Details of calves and their experimental treatment

Calf No.	Body-weight (kg.)	Age at commencement (days)	Experimental periods (days)					
			Normal intake of diet (see Table 6)			Reduced intake of diet (see Table 6)		
			Preliminary	Starvation	Recovery	Preliminary	Starvation	Recovery
4	33.5	6	12	4	10	12	4	12
5	34.6	6	14	4	12	--	-	--

Unfortunately one calf (No.5) became ill during the course of the pre-starvation period for the low

plane of nutrition and this section of the experiment was abandoned.

The composition of the semi-synthetic diet is given in Table 5 and the amounts given to each calf in Table 6. During periods of starvation water was given instead of milk, in quantities sufficient to maintain urine volume at the pre-starvation level.

TABLE 5.

Composition of the experimental diet

	Composition (g. / litre)
Dried skim milk	77.6
Lard	38.7
Cod-liver oil	3.3
Glucose	14.0
Minerals	1.3

TABLE 6.

Quantity of milk diet given to each of the calves

Calf Nº	Amount of milk (kg.) fed daily during	
	Preliminary and recovery periods	
	Normal intake of diet	Reduced intake of diet
4	3.8	3.0
5	4.0	3.2

The following analyses were carried out:-

Diet

Dry matter, total nitrogen and total fat on each sample.

Urine

Total nitrogen(determined daily), urea, ammonia, creatine, creatinine, uric acid, allantoin, purine bases, protein, total acetone bodies, total sulphur, inorganic sulphur, ethereal sulphur and

neutral sulphur (determined every two days), chloride, sodium, potassium, calcium, magnesium and phosphorus (on certain samples).

Faeces

Total nitrogen, dry matter, total ash, total fat and soaps (determined every two days).

Respiratory exchange

Determined during periods of starvation only.

3. Results

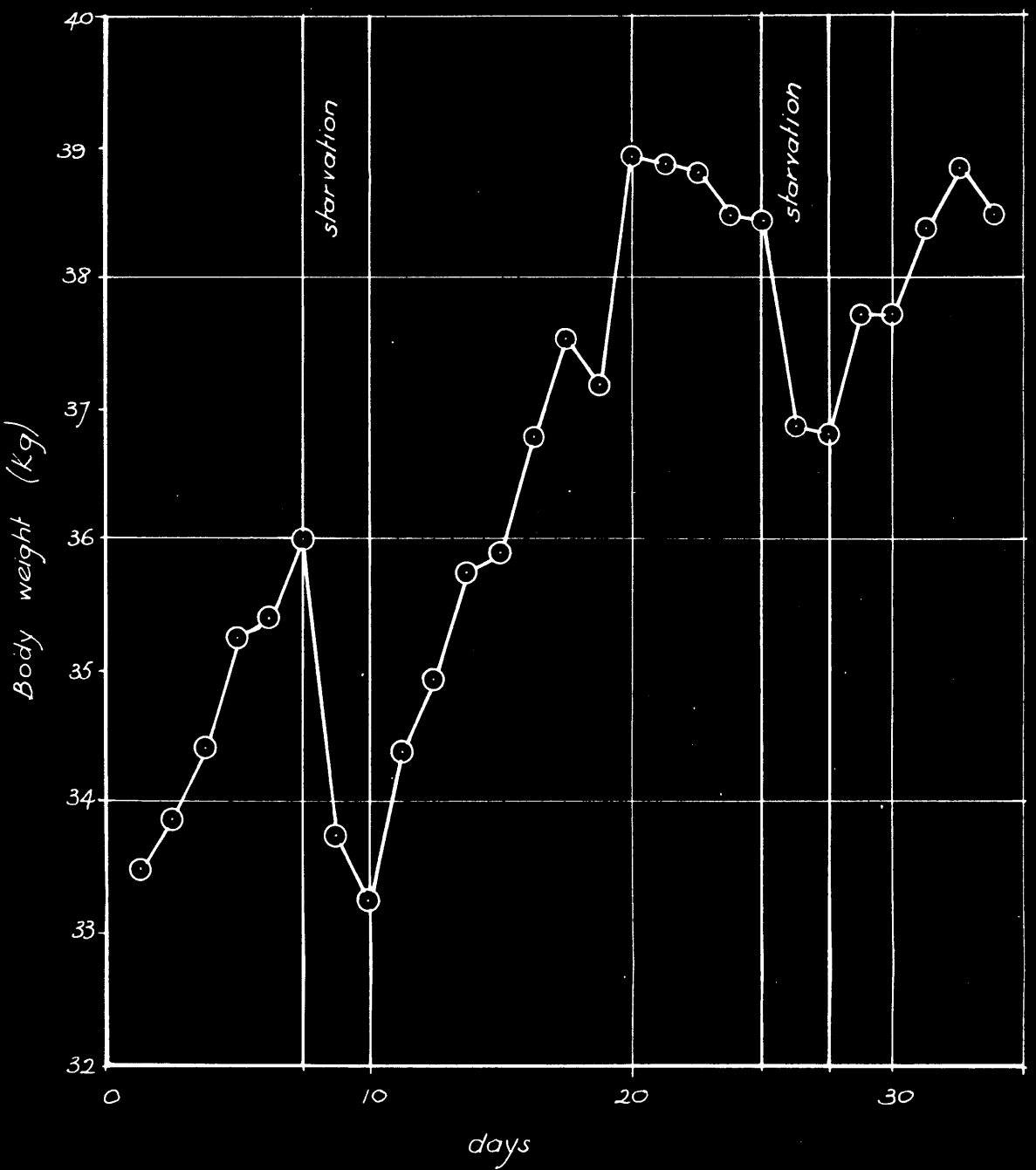
(a) General behaviour of the animals during fasting and when given sub-normal quantities of milk

During the preliminary period, when the calves were given quantities of diet commensurate with normal gains in body weight, both were very lively and tended to be highly excitable, especially at feeding times. They would stand and play with their harness or tails for quite long periods. During the first two days of starvation this normal behaviour continued, the excitability at feeding times still persisting although they were given nothing but water. Later they became lethargic, but could hardly be called weak. When they were again given normal quantities of food they recovered within a few days. During the period when the subnormal quantity of feed was given, however, activity declined markedly and the animals developed a craving for roughage - a dietary component excluded from their diets. When calf 4 was starved on the second occasion chewing of the walls of the metabolism cage and his harness increased and on one occasion he swallowed the whole of the rubber straps supporting his faeces bag. This caused no distress and in the subsequent recovery period he was noted to have been ruminating on fragments of rubber. During this period rubber appeared in the faeces, invalidating the determinations of carbohydrate by difference methods.

The behaviour of the animals on realimentation is of some interest. Realimentation of calf 4 was slow; he was given only half his normal quantity of

figure 1.

The effect of starvation on the growth of calf no. 4.



diet on the first day following starvation and it was noted that his urinary nitrogen remained elevated. It was thought that this might be due to the sub-normal feeding, so calf 5 was given the full quantity of diet immediately following the starvation period. The result was profuse diarrhoea, which lasted for several days. The same type of reaction in a mild form was shown by calf 4 during the second period of realimentation when two-thirds of the normal allowance was given on the day following starvation. Such alimentary disturbances after fasting are common in man (Lusk, 1931).

(b) Bodyweight

During the starvation periods, the calves were weighed every morning instead of every second morning. The growth curve of calf №4 is shown in Figure 1 while Table 7 summarises the data for both calves.

TABLE 7.

Mean daily gains (+) or losses (-) in weight by the calves calculated from regression annalysis of individual weights.

Calf №	Gain or loss of weight (g. / day)					
	Normal intake of diet (see Table 6)			Low intake of diet (see Table 6)		
	Prelim: inary	Starva: tion	Recov: ery	Prelim: inary	Starva: tion	Recov: ery
4	307±47	- 685	307±42	105±52	- 525	130±82
5	302±14	- 525	306±26	-	-	-

The weight of calf 4 on commencement of the second period of starvation was 38kg. If the rates of gain in the preliminary period are regarded as normal, then this animal should have reached that weight at the age of 27 days. In actual fact the calf was 45 days old and was therefore retarded in growth by 18 days in 45. This retardation of growth due to undernutrition was in accordance with the plan

of the experiment. There appeared to be relatively little adaptation to starvation as judged by economy in body gain following it. The normality of the gains in weight of the calves when receiving the normal intake of diet may be judged from the data compiled by Brody (1945). In the first month after birth Ayrshire calves grow at the rate of about 17 lb. a month or 257 g. / day, whereas in the second month the growth rate increases to 30 lb. a month or 450 g. / day. On this basis the gains of the calves during the adequate intake of diet were normal and when the reduced intake of diet was given, subnormal. In each case the loss of weight during starvation was severe and there appeared to be no significant difference between the weight losses in the two periods. The mean daily loss in weight during three experiments was thus 578 ± 53 g. / day.

(c) The digestibility of the diet and the excretion of faeces during starvation.

The digestibility of the dry matter, fat and total nitrogen of the diet was determined before and after starvation. The results are shown in Table 8.

TABLE 8

Mean coefficients of apparent digestibility of the diets before and after starvation.

Calf Nº	Level of feeding	Period	Apparent digestibility (%)		
			Total dry matter	Total fat	Total N
4	Normal	Preliminary	95.7	98.8	89.9
		Recovery	98.3	97.5	98.2
4	Low	Preliminary	96.7	97.1	95.1
		Recovery	94.8	93.5	93.2
5	Normal	Preliminary	95.3	93.2	90.4
		Recovery	92.3	88.7	87.4
Mean of 3 experiments		Preliminary	95.9	95.4	91.8
		Recovery	95.1	93.2	93.0

There was no significant change in the apparent digestibility of the diet following the first experimental starvation period. With calf Nº4 on the normal level of feeding the digestibility of the

diet increased, but the remaining results showed a decline in digestibility associated with slight diarrhoea on realimentation. A comparison of these figures with the digestibility of whole milk by the calf will be given later (Page 109).

Faeces continued to be excreted during the fasting periods and the amounts collected were surprisingly large. The mean daily excretion of dry matter is shown in Table 9.

TABLE 9.

Mean daily excretion of dry matter in the faeces during starvation and during feeding.

Period	Calf 4 normal ration	Calf 4 reduced ration	Calf 5 normal ration	Mean
	Weight of dry matter in faeces (g. /day)			
Preliminary	22.1	13.3	25.2	20.2
Starvation	8.1	13.7	10.1	10.7
Recovery	8.6	20.9	41.9	23.8

The starvation faeces tended to be firmer than normal faeces, but otherwise looked the same. The mean composition of faeces during feeding and fasting is shown in Table 10.

TABLE 10.

Mean composition of faeces during starvation and during feeding

	Normal faeces (%)	Starvation faeces (%)
Amount of dry matter	18.8	30.7
Composition of dry matter:		
Total lipids	39.6	39.6
Soaps	25.0	23.1
Neutral fat, free fatty acids and unsaponifiable residue	14.6	16.5
Ash	20.4	19.9
Total nitrogen	5.4	5.1

TABLE 11.

Mean excretion and ingestion of N and N balance

Calf №	Level of feeding	Period	Days	Nitrogen (g. / day)			
				Intake	Excretion in		Balance
					Urine	Faeces	
4	Normal	Preliminary	12	16.53	6.46	1.67	+8.40
		Starvation	4	Nil	9.41	0.49	-9.90
		Recovery	4	17.20	9.47	0.43	+7.30
		Recovery	8	17.76	7.85	0.25	+9.66
4	Low	Preliminary	12	13.35	7.39	0.65	+5.31
		Starvation	4	Nil	10.19	0.60	-10.79
		Recovery	4	12.42	8.88	0.60	+2.94
		Recovery	8	13.81	8.31	0.94	+4.56
5	Normal	Preliminary	14	17.27	7.33	1.65	+8.29
		Starvation	4	Nil	7.11	0.49	-7.60
		Recovery	4	15.57	10.36	2.62	+2.59
		Recovery	8	17.91	7.83	2.25	+7.83

The higher percentage of dry matter in starving faeces is shown in this table and it will be noted that the composition of the faecal dry matter was comparable in both periods. This suggests three possibilities; firstly, that the starvation faeces were in fact undigested food residues, or secondly that they were entirely metabolic products secreted into the gut during starvation. It is more likely, however, that the starvation faeces consisted of both food residues and metabolic products but the magnitude of each cannot be assessed from the present results.

(d) Nitrogen balances

Table 11 summarises the nitrogen balance results. The excretion of nitrogen in the urine of calf №4 increased during both periods of starvation. With calf №5 there was no spectacular change in urinery excretion of nitrogen during fasting. In this calf however, the first few days of realimentation were associated with slight diarrhoea and urinary nitrogen rose markedly. Table 12, besides summarising the results with calves, includes results obtained with other animals for comparison.

TABLE 12.

The loss of body nitrogen during starvation in the young calf compared with that observed in other animals

Animal	Loss of nitrogen (mg. / kg. body-weight per day)	Reference
Young calf	259	Present work
Sheep	152	Morris & Ray (1939)
Goat	162	Morris & Ray (1939)
Pig	60	Voit (1901)
Cow	90	Hutchinson & Morris (1936a) Morris & Ray (1939)
Steer	69	Carpenter (1927)

The table shows that the fasting nitrogen catabolism of the young calf is more than twice as

TABLE 13

Mean daily excretion of nitrogenous metabolities in the urine.

	Calf N°4 Normal diet			Calf N°4 Reduced diet			Calf N°5 Normal diet		
	Prelim: inary	Starva: tion	Recov: ery	Prelim: inary	Starva: tion	Recov: ery	Prelim: inary	Star: vation	Recov: ery
No. of days anal: yses	8	4	10	6	4	10	10	4	8
Constit: uent	Amount excreted (g.nitrogen / day)								
Urea	4.62	7.34	6.11	4.68	7.10	4.74	4.80	5.00	3.69
Ammonia	0.393	0.904	0.893	1.017	0.582	1.183	0.662	0.546	1.273
Total urea + ammonia	5.01	8.25	7.00	5.69	7.69	5.92	5.46	5.55	4.96
Creatinine	0.365	0.379	0.285	0.232	0.236	0.229	0.467	0.379	0.441
Creatine	0.124	0.214	0.098	0.125	0.197	0.232	0.108	0.377	0.252
Uric acid	0.038	0.057	0.031	0.026	0.048	0.029	0.037	0.048	0.042
Purine base	0.131	0.148	0.193	0.216	0.206	0.252	0.146	0.138	0.211
Allantoin	0.606	0.520	0.373	0.476	0.569	0.583	0.605	0.507	0.645
Total purine	0.775	0.725	0.597	0.718	0.823	0.864	0.788	0.693	0.898
Residual	0.729	0.117	0.233	1.039	1.227	2.101	0.483	0.186	1.857

Graphs : showing the change in Urinary N distribution during starvation

figure 2 UREA N

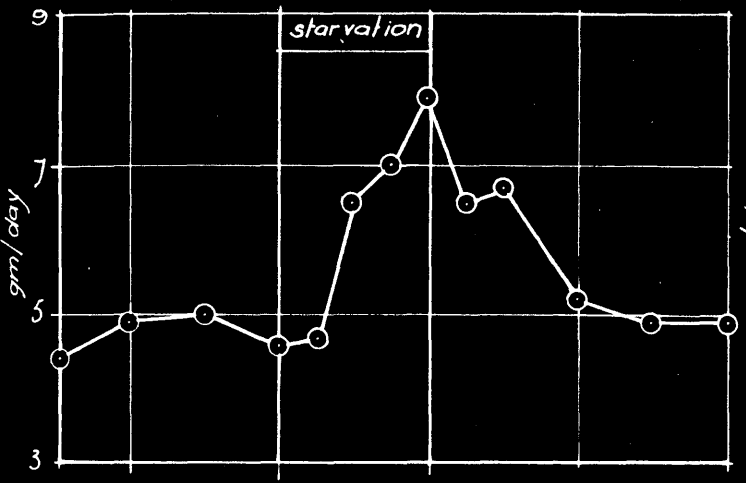


figure 3 AMMONIA N

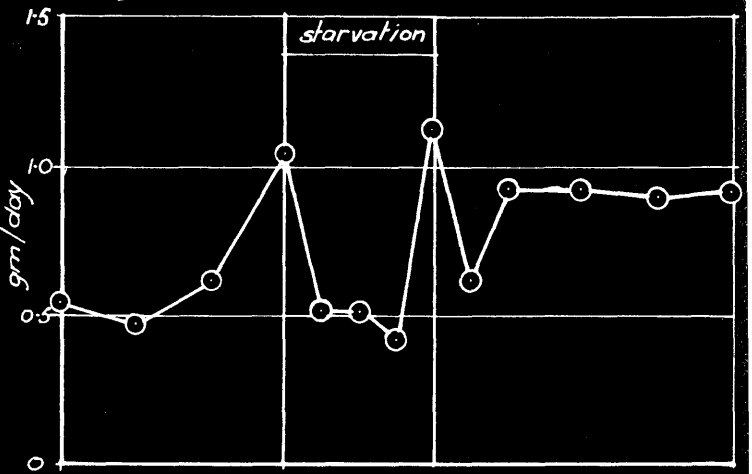


figure 4 CREATINE N

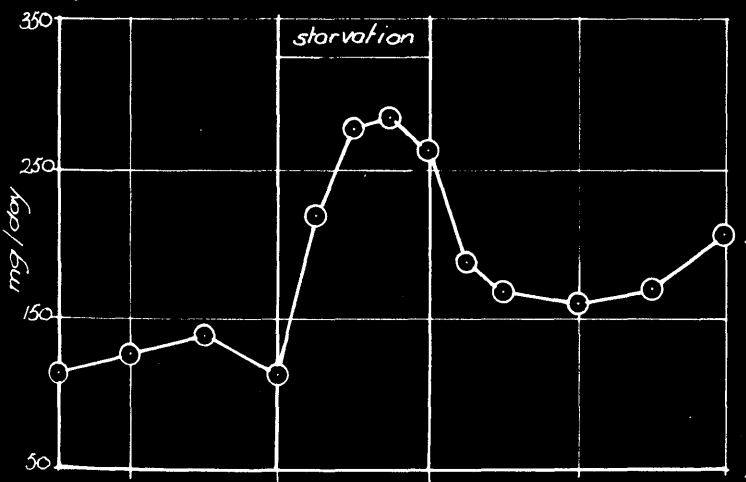


figure 5 ALLANTOIN N

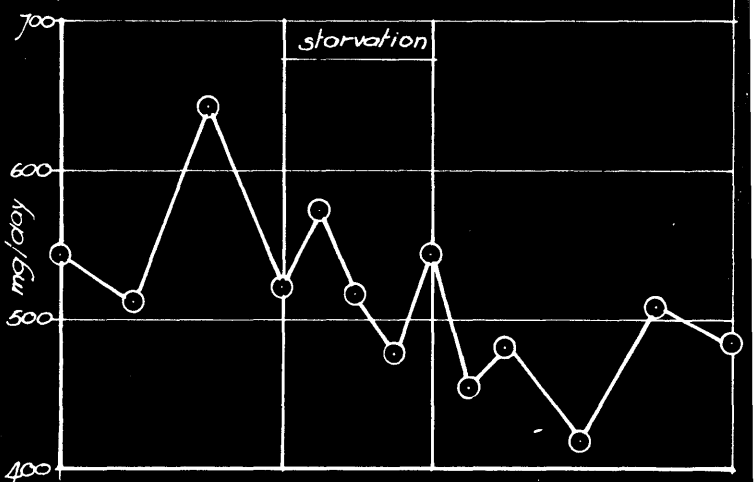


figure 6 PURINE BASE N

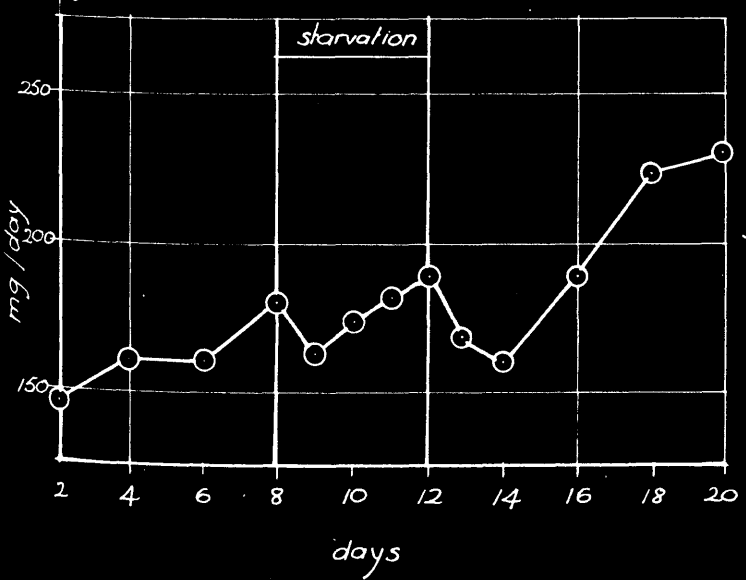
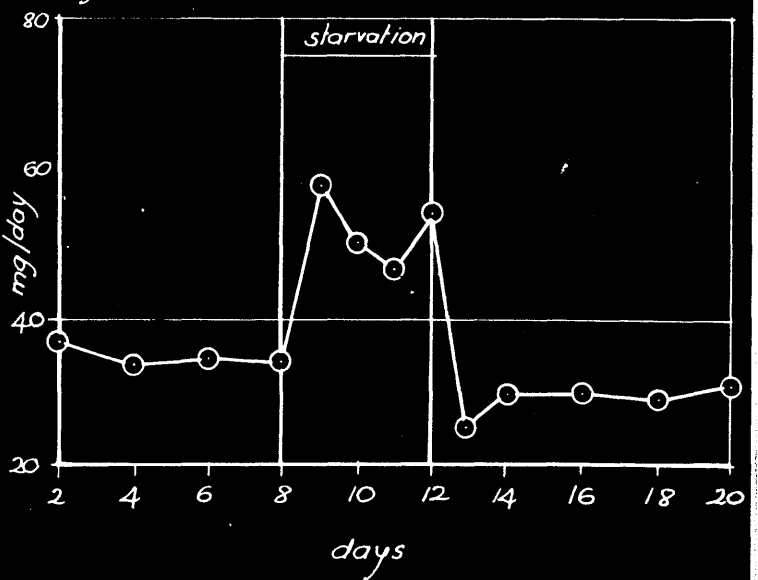


figure 7 URIC ACID N



intense as the fasting catabolism of the cow at maturity and is much greater than the fasting catabolism of the sheep and goat which are small ruminants of approximately the same body size as the young calf.

The first four days of realimentation which are shown separately in Table 11 indicate that the urinary nitrogen remained high and nitrogen retentions correspondingly low during this period. Part of this in two cases was probably due to the slight alimentary disturbances which occurred. In the remaining instance, calf №4 in the first period of starvation no diarrhoea occurred and thus such an explanation does not entirely account for this lowered nitrogen balance. It would appear therefore, that excessive deamination of amino-acids for meeting energy demands takes precedence over replacement of lost tissue nitrogen following a fast. Alternatively, fasting may result in preferential demands for one particular amino-acid in the subsequent period of realimentation.

(e) The distribution of nitrogen in the urine

The analytical results obtained for each calf are summarised in Table 13. Analyses were made at two-day intervals, and daily throughout the starvation periods. Figures 2, 3, 4, 5, 6 and 7 show the mean changes in more detail and are referred to later.

Table 13 and Figure 2 show that urea excretion increased during starvation. This was marked with calf №4 on both occasions but was negligible with calf №5, a result in agreement with the nitrogen balance results previously discussed. Figure 2 shows that urea excretion fell slowly following the fast, again in agreement with the total nitrogen metabolism results. Ammonia excretion was not grossly affected by the fast. This differs from the effect of starvation in man (Cathcart, 1907) where an increase in ammonia excretion meets the marked acidosis

TABLE 14.

Total urinary S excretion by the calves and the N/S ratios

Period	Total excretion of S (mg./day)			
	Calf 4 Normal diet	Calf 4 Low Calorie diet	Calf 5 Normal diet	Mean
Preliminary	323	413	326	354
Starvation	611	607	403	540
Recovery	476	394	229	366
N/S ratio in preliminary period	22.7	19.0	22.0	-
N/S ratio in starvation period	16.6	16.7	17.7	-

TABLE 15.

The mean results for the partition of the urinary S

Fraction	Excretion in preliminary period	Excretion during starvation	Increase
	Amount excreted (mg./day)		
Inorganic	210.4	382.2	+ 171.8
Ethereal	66.3	63.3	- 3.0
Neutral	93.8	95.1	+ 1.3
Total	370.5	540.6	+ 170.1

which occurs. The results are shown in Figure 3.

Creatinine excretion declined very slightly throughout, there being a slight fall in excretion in the recovery period of calf №4 during the first period of starvation, a change which is of doubtful significance. This is in agreement with Folin's contention (1905) of a constant endogenous metabolism and a constancy of creatinine elimination. Creatine excretion increased when the calves were starved, (Figure 6). This was true even of calf №5 which showed no pronounced change in total nitrogen excretion during fasting. The purine bases showed no large changes during starvation. Allantoin N excretion did not change but uric acid N excretion increased during starvation in both animals. The amount of nitrogen involved was, however, small. The total purine N excretion was not affected by starvation, the small variation in urinary metabolite excretion being the usual day to day variation met in such studies. The purine N excretion is shown in Figures 5, 6 and 7. This negligible change indicated that there was little loss of nuclear material during starvation.

The residual nitrogen, which represents largely amino-nitrogen with small amounts of nitrogen present as proteins and other compounds, was variable. Part of the variation was undoubtedly due to analytical errors, for the estimation of this fraction involves eight separate determinations of nitrogen or nitrogen-containing compounds.

(f) The excretion of sulplur in the urine and the partition of urinary sulphur.

The mean results of urinary sulphur excretion are shown in Table 14. It is clear that an increase in total sulphur excretion occurred during starvation, smaller in calf 5 than in calf 4 a result which is in agreement with the nitrogen metabolism results. The partition of the urinary sulphur recorded in Table 15 shows that the major part of the increase in excretion

occurred in the inorganic fraction and that the neu: tral and ethereal sulphate fractions remained constant.

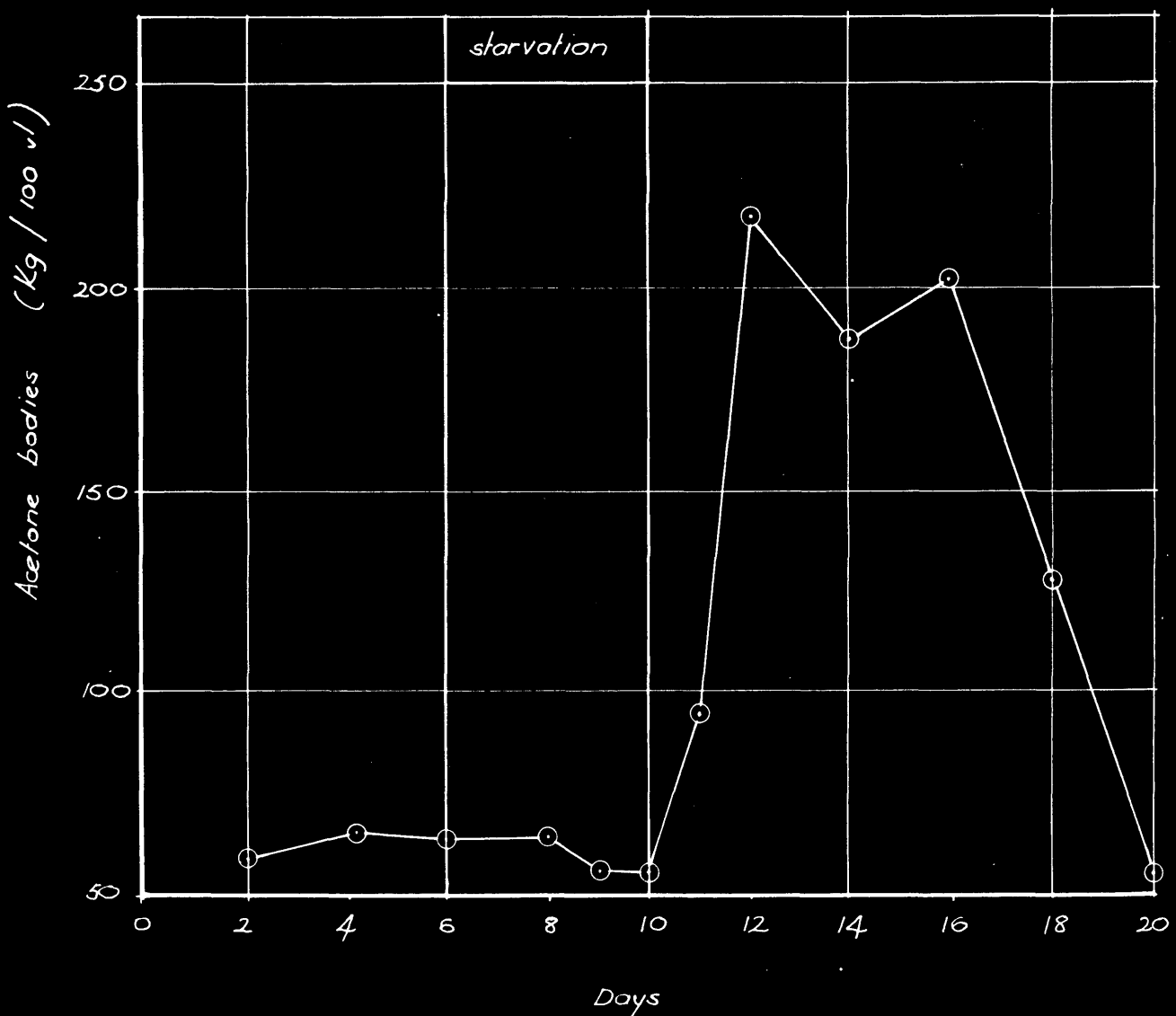
The constancy of excretion of the neutral sulphur fraction is in agreement with the contention that it is an endogenous fraction, though the experiments of Amann (1933) do not support this hypothesis, since in his experiments it increased markedly when very high protein diets were given. It was not expected that the excretion of ethereal sulphate would be constant, because this fraction is known to result from phenols formed by putrefaction of food in the gut. Thus during starvation a decline in the sulphate ester excretion would be expected. Possibly the continuing excretion of faeces during fasting indicates that there were sufficient feed residues present in the intestinal tract to give rise to phenols in comparatively large quantities.

The ratio of N/S in the urine of man during starvation varies between 15 and 17 (Lusk, 1931; Benedict, 1915; Cathcart & Green, 1913). The N/S ratio in muscle is 13.4 (Wilson, 1925). In the calf the N/S ratios are comparable to those found during starvation in man, but were higher than would be expected if muscle proteins and their constituent S-containing amino-acids were the scource of both the nitrogen and the sulphur. It is known that part of the nitrogen excreted in the urine is an inevitable loss - the 'endogenous nitrogen' excretion (see page 41). If it is assumed that the neutral sulphur fraction is a measure of the minimal sulphur excretion and the excretion of nitrogen above the endogenous level (page 54) is taken as representing catabolism of body protein, then the ratio of non-endogenous nitrogen to non-endogenous sulphur would be 13.8, a value in fair agreement with Wilson's value of 13.4 for the N/S ratio in muscle.

It may be concluded therefore, that there was an increase in sulphur excretion during fasting following a milk protein diet, the increase being

figure 8

The effect of starvation on the excretion of ketone bodies



entirely in the inorganic fraction. This would be expected if large quantities of body protein were being catabolised.

(g) Acetone excretion

The daily excretion of acetone is shown in Figure 8 and the mean results are summarised in Table 16.

TABLE 16

Daily excretion of ketone bodies

Period	Calf 4 Normal diet	Calf 4 Reduced diet	Calf 5 Normal diet	Mean
	Amount of ketone bodies excreted (mg. acetone / day)			
Preliminary	60.7	58.5	68.7	62.6
Starvation	49.4	54.3	89.2	64.3
Recovery	95.7	134.2	120.3	116.7

It is clear that no ketosis occurred on starvation of the young calf. This agrees with the results obtained in the mature ruminant by Sjollema & van der Zande (1923), Carpenter (1927) and Hutchison & Morris (1936b), none of whom observed any ketosis in cattle on starvation. This result in the young calf which, to all intents is a non-ruminant, is interesting since in man many grams of ketone bodies are excreted during fasting. The poorly nourished individual, however, excretes a smaller quantity of ketones (Deuel & Gulick, 1932) suggesting that in the calf, an animal possessing only small fat reserves, ketosis would not be a symptom of starvation. On the other hand infants and children evidently develop ketosis sooner during starvation than the adult (Gamble, Ross & Tisdall, 1923). Figure 8 shows that an increase in urinary acetone excretion occurred during realimentation. This suggests that there is a slight disturbance of carbohydrate metabolism at this time. It may be that the large demand for energy to replenish the depleted glycogen reserves of the animal results in an increase in fatty acid oxidation following

fasting.

(h) Urinary mineral excretion

Calcium, magnesium, sodium, potassium, chloride and phosphorus were determined on two-day samples of urine collected during the three periods of each experiment and the results are shown in Table 17.

TABLE 17

The daily excretion of mineral elements by the calves

Calf N°	Period	The amount of element excreted (mg./day)					
		Cl	P	Na	K	Mg	Ca
4 (Normal diet)	Preliminary	2467	227	914	2678	62	149
	Starvation	664	312	661	1493	34	75
	Recovery	2884	399	1398	2780	30	106
4 (Reduced diet)	Preliminary	2003	245	529	2027	13	135
	Starvation	274	143	330	1569	30	33
	Recovery	2614	345	876	3291	18	87
5 (Normal diet)	Preliminary	3057	182	338	3145	45	94
	Starvation	140	184	-	1315	8	22
	Recovery	2379	28	313	1496	18	71
Mean loss/ day	during Starvation	326	213	495	1456	24	43

Balances were not determined and it must be remembered that the sampling errors involved in obtaining these results may be high since the two-daily variation in the excretion of nitrogen was as high as ± 15% for calf N°4 when receiving 3.8 l. of diet per day.

It is clear that there was a reduction in the excretion of chloride, potassium, sodium and calcium during starvation and no consistent change in the excretion of the other elements. The mean daily loss during starvation may be used to indicate the extent

of the catabolism which occurred. If only muscle substance were broken down and if the potassium loss is assumed to have come entirely from muscle, the approximate excretion of other elements which could have come from muscle substance may be estimated. Due to the fairly large sampling error this can be only an approximation. The analytical figures for muscle are those determined on normal calf muscle (Blaxter & Wood, 1951) and the final results of the calculation are shown in Table 18.

TABLE 18

An approximation to the amounts of body protein catabolised based on the analysis of muscle tissue.

Element	mg. present in muscle when 1456 mg. of K are present	mg. of element excreted in urine	Conclusion
K	1456	1456	
Na	224	495	Loss of extra-cellular fluid
Cl	181	326	
Ca	21	43	Little loss of minerals from bone.
P	752	213	
Mg	69	24	Retention of essential enzyme systems of the cell.

It would appear from this table that there was little loss of bone minerals during starvation. This is not in agreement with some of the data obtained for man (Peters & van Slyke, 1931), but, as has been pointed out, part of the bone loss in the human may be due to accompanying acidosis. Hawk, Oser & Summerson (1947), however, state that long periods of starvation in the dog (up to 104 days) do not cause any marked loss of minerals from the bone.

The larger quantity of sodium and chloride present suggests a loss of extra-cellular fluid during

TABLE 19

Regression equations relating functions measured during fasting respiratory exchange determinations to time following the cessation of feeding with coefficients of variation estimated by analysis of variance of the regression.

Function	Equation D = days of fast	% decline / day	e^{2z}	C.V.
CO_2 production (litres/hr)	x = 10.117 - 0.600 D	5.93	736.4	1.4
O_2 consumption (litres/hr)	x = 12.997 - 0.755 D	5.81	233.2	2.0
Respiratory quotient	x = 0.784 - 0.006 D	7.96	3.7	1.8
Heat production (kg.cal./hr)	x = 61.67 - 3.76 D	6.10	502.4	1.4
Pulse rate/min.	x = 79.7 - 6.38 D	8.00	498.3	2.0
Respiratory rate/min.	x = 16.6 - 1.15 D	6.93	7.0	13.9
Minute volume of respiration (l.)	x = 4.40 - 0.032 D	7.36	17.8	13.8
Body weight (kg.)	x = 35.88 - 0.655 D	1.83	14.9	1.6

starvation. The smaller quantities of magnesium and phosphorus present on starvation suggest that there was no extensive loss of nucleo-protein material and that the enzyme systems in the cell were retained. This is in agreement with the results previously reported on the absence of an increase in purine metabolism during the fast.

(i) Energy metabolism and respiratory exchange.

(i) Accuracy of the determinations

In that the interpretation of the results of the respiratory exchange determinations depends largely on the accuracy with which the determinations were made, it is essential to have information on the variation associated with each determination. The method adopted for determination of respiratory exchange was very sensitive. Slight head movements of the calf in a duplicate run invariably could be detected in a higher oxygen consumption and carbon dioxide production. The accuracy of the method was determined by analysis of variance technique computing the coefficient of variation from the mean and the standard deviation of the residuals from a fitted linear regression. The results of calf N⁰4 which were quite typical are shown in Table 19. From these detailed results it is clear that the errors involved in the determinations are very small. For oxygen consumption, heat production and carbon dioxide production the errors expressed as a percentage of the mean are all less than 2%. The respiratory rate and ventilation rate per minute were slightly more variable but even so they were well within the range of varia: bility one might expect of a function under partial voluntary control.

(ii) The course of heat production during fasting with reference to the constancy of the basal metabolism of the calf.

From Table 19 it is clear that the linear regression relating heat production to days of starvation was very highly significant in each

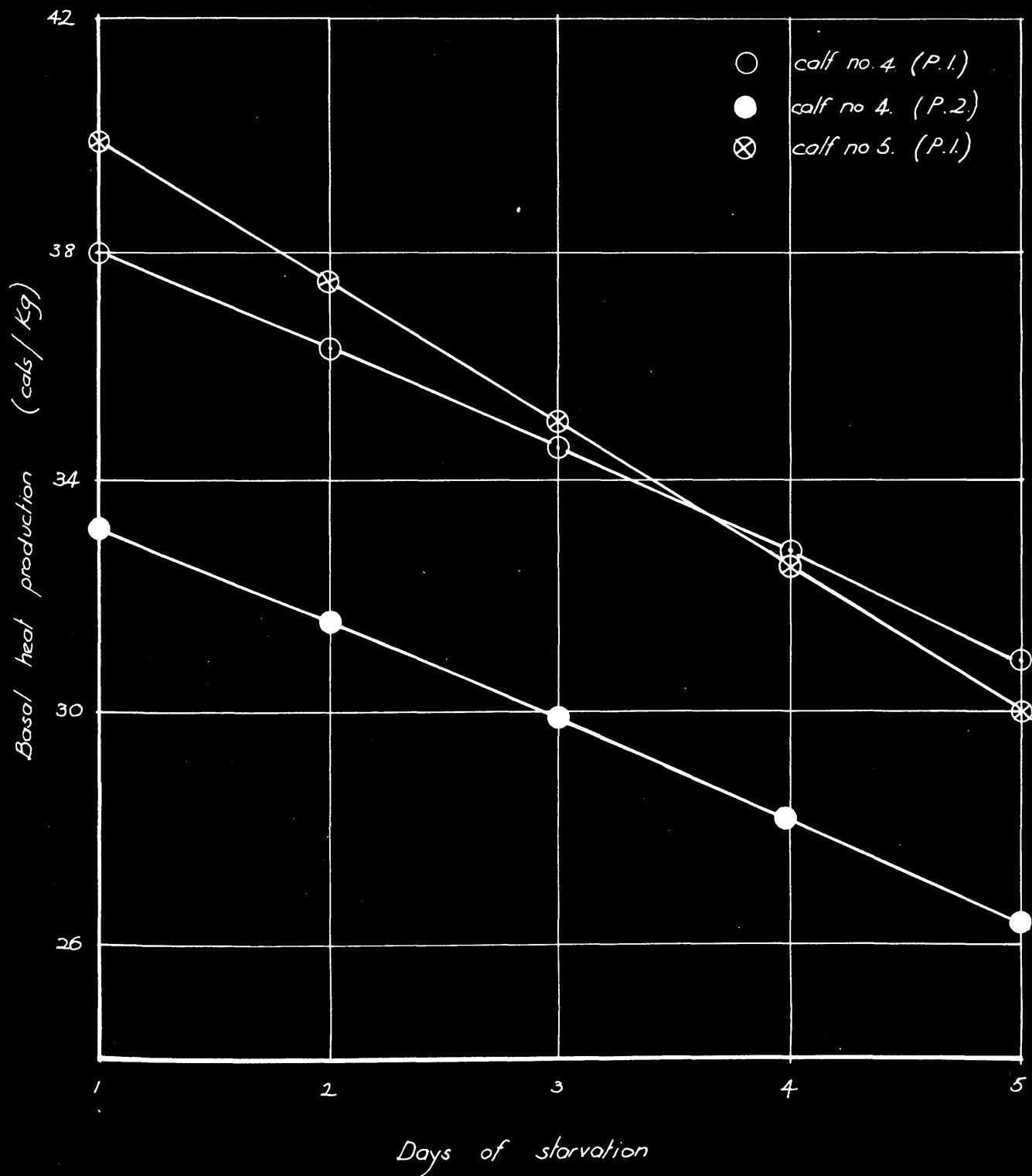
TABLE 20.

Regression equations showing the fall in heat production of the calf with continued starvation, its fall in O_2 consumption and its decline in body weight.

Calf Nº and diet	Function	Regression equation where D = days	% decline in function per day
4 Normal	Oxygen consumption (l./hr)	x = 13.00 - 0.600 D	5.81
	Heat production (kg.cal./hr)	x = 61.67 - 3.76 D	6.10
	Body weight (kg.)	x = 35.88 - 0.655 D	1.83
4 Reduced	Oxygen consumption (l./hr)	x = 13.28 - 0.454 D	3.42
	Heat production (kg.cal./hr)	x = 53.67 - 3.32 D	6.19
	Body weight (kg.)	x = 38.83 - 0.525 D	1.35
5 Normal	Oxygen consumption (l./hr)	x = 14.86 - 0.68 D	4.58
	Heat production (kg.cal./hr)	x = 64.58 - 4.36 D	6.76
	Body weight (kg.)	x = 38.89 - 0.315 D	0.81

figure 9

fall in heat production of the calves with continued starvation



particular case and that the residual variance was very small. In each calf heat production following starvation declined slowly over the whole four days of observation. The same was true of each function studied, - pulse rate, respiratory rate and minute volume. The data relating to each animal is shown in Table 20 and graphically in Figure 9. These show that there was no indication at any time that a constant level of metabolism had been reached, and the regression equations were not in any respect non-linear, as can in fact be inferred from the errors shown in Table 19. In every case the fall in oxygen consumption, heat production and bodyweight with continued fasting were all highly significant, P being always smaller than 0.01 and sometimes smaller than 0.001.

It will be seen that the decline of heat production was much greater than the decline in body-weight, which means that heat production / kg. body-weight also declined markedly during starvation.

It is clear that this fall in heat production of calves is not only highly significant in the single individual but, where these two calves are concerned, is reproducible between animals. These results do not agree with those obtained on man and mature animals. In the first place, whilst there is a fall in total heat production of man in long continued fasting, fasting over a period of a week does not result in a large fall in metabolism per kg. of body-weight, (Lusk, 1931; Benedict, 1907, 1915.) In the cow metabolism / kg. bodyweight falls during the first day of starvation, largely due to the long period needed to reach a post-absorptive state. The metabolism of the animal does not appear to decline markedly once this level has been attained (Benedict & Ritzman, 1927). In sheep the observations of Blaxter (1948) and Marston (1948) indicate that a plateau in metabolism occurs. In prolonged starvation of the rat, however, fasting reduces heat production

whether expressed per kg. of bodyweight or per sq. m. of body surface (Benedict and Fox, 1934).

Data relating to this decline in heat production in other species are given in Table 21.

TABLE 21

Regression of heat production (Cal./kg. bodyweight) on length of fast in days; a comparison of the calf with man and the steer.

Species	Reference	Regression equation	Length of observation (days)	Statistical significance
Calf	Present results	Cal.=37.03-1.98D	4	Very highly significant
Man	Benedict (1907)	Cal.=32.28-0.66D	5	N.S.
Man	Lusk (1928)	Cal.=30.97-0.39D	6	N.S.
Steer				
C	Benedict & Ritzman (1927)	Cal.=16.62-0.47D	6	N.S.
D		Cal.=17.12-0.58D	6	N.S.
E		Cal.=22.4 -0.04D	4	N.S.

D: days of starvation. N.S.: not significant.

Statistical analyses were made of the published results using the same methods as were used in dealing with the calf results.

It will also be noted from Table 20 and Figure 9 that the rate of decline of heat production was the same whether or not calf №4 had received an adequate or a reduced amount of diet and that the rate of fall in heat production of calf №5 was similar.

Thus, heat production of the calf following fasting is not affected to any appreciable extent by the prior nutritional level. Marston (1948) has shown that the heat production of sheep given a high plane of nutrition fell to a constant basal level more slowly (over a period of 7 days) than the heat production of animals fed a sub-maintenance diet which

reached constancy after only 2 days of fasting. Marston has attributed this difference in the shape of the curve of fasting heat production to the failure of the animals given the higher plane of energy to reach a post-absorptive state.

The reason for this decline in heat production in prolonged fasting in the calf is not certain. It might be supposed that a post-absorptive state had not been reached in the calf starved for four days. This is not supported by the results for the following reasons:-

1. The respiratory quotient did not show any marked decline during the fast. This was also true of the non-protein R.Q.
2. The peak of creatine excretion in the urine occurred early in the experimental period, indicating that the animal was very close to a post-absorptive state even on the first day of starvation.
3. There was no significant difference in the rate of decline in metabolism following different levels of food intake, as has been shown to occur in the sheep.
4. The experimental points showing a fall in heat production did not deviate from a linear regression. If the decline in metabolism was due to the metabolism of food residues an asymptote would be expected.

The decline in heat production was thus not due to failure to reach the post-absorptive state. This is reasonable in so far that the rat reaches a basal level of metabolism at approximately 14 hr. (Wesson, 1930-31) and man at about 12 hr.. It is only herbivora that take 72 hours or more to reach the post-absorptive state.

A possible explanation is that there is a marked reduction in muscular tonus and in the small involuntary skin and muscle movements during fasting in the calf.

4. Discussion

(a) The materials catabolised during fasting in the calf and the loss in body weight.

From the respiratory quotient of an animal (R.Q. = vol. CO_2 produced ÷ vol. O_2 consumed) it is possible to calculate the proportion of carbohydrate to fat catabolised in the body, since the R.Q. of glucose is 1.0 and that of fat 0.707. If only mixtures of fat and carbohydrate were catabolised the R.Q. would therefore lie somewhere between these two values. Protein is also catabolised, however, especially when fat reserves are low, as in the case of the calf and a correction must be made. From the excretion of nitrogen in the urine, the carbon dioxide derived from protein catabolism is obtained (g. urinary N x 9.35), as is the oxygen required to oxidise this protein (g. urinary N x 8.49). By difference from the total gas exchange, the oxygen consumption and the carbon dioxide production are obtained, which may be used to calculate the true non-protein R.Q. Using the factors given by Zuntz & Schumburg as modified by Lusk (1931) the proportion of fat to carbohydrate oxidised in the body may be obtained, together with the heat production of the animal. This method of computation has recently been criticised (Soskin & Levine, 1946) since reactions other than simple total oxidation of fat and carbohydrate are always occurring. As pointed out by Dewar & Newton (1948-9) over long periods of time this criticism is not valid, for the intermediate side reactions resulting in abnormal respiratory quotient will be cancelled out. In the present experiments the experimental periods were only 45 minutes in total duration (including preliminary periods) and abnormal R.Q.s might therefore be expected. In the first experiment with calf №4 the non-protein R.Q. remained high throughout, indicating that in the early stages of the fast 10-12% of the heat production was arising from complete oxidation of carbohydrate. In the remaining experiments R.Q.s were quite normal, varying

within small limits. On occasions, values below 0.707 were obtained, however, indicating either that complete collection of the CO_2 produced was not obtained owing to underventilation, or that momentarily the determination had coincided with a phase of intermediary metabolism resulting in a low apparent R.Q. The variation from the general trend of the R.Q. with time, however, was within the limits of ± 0.05.

It would appear, therefore, that the present results may be used to compute the fat and carbohydrate oxidised, and for reasons given above, the results of calf №4 period 1 have been excluded as far as a partition of the non-protein heat production is concerned. Table 22 summarises the results of the calculation of the materials catabolised.

TABLE 22

An estimate of the materials lost per day from the body of the calf during starvation;

Calf 4/period 2 and Calf 5/period 1.

Constituent of body	Daily loss (g.)
Body weight	525
Body fat	99
Body protein	54
Body carbohydrate	13
Extra-cellular water	39
Intra-cellular water	326
Total weight loss accounted for	531

The extra-cellular and the intra-cellular water losses are based on the molal quantities of sodium and potassium excreted in the urine during fasting using the equations of Gamble, Ross & Tisdall (1923). The table shows that by far the greatest loss to the body is water and that most of this is water from the cells rather than from the intercellular

spaces. The loss of extra-cellular water is small and indicates only a slight dehydration of the animals. The loss of body fat of nearly 100 g. per day was the mean loss on the second day of starvation. Towards the end of starvation, on the fourth day proportionally far more protein (up to 24% of the total calories in calf N№4) was lost to the body. In this respect it has to be remembered that not the whole of the urinary nitrogen is necessarily a reflection on the protein catabolism, since the endogenous loss is included in this fraction. Thus the protein value in Table 22 is probably too high an estimate, part of the loss of nitrogen being of body N.P.N. compounds.

The source of catabolised protein, fat and carbohydrate was not determined, but it has been shown that there is only a small loss of nucleic acid derivatives on fasting the calf. This result may be used to indicate the source of catabolised protein which occurs during fasting of the calf. Davidson (Davidson & Waymouth, 1944; Davidson, 1945) has shown that desoxyribonucleic acid (D.N.A.) is confined to the nucleus of the liver cells, whilst ribonucleic acid (R.N.A.) is a constituent of the cytoplasm of these cells. During starvation of the rat there is a loss of nitrogen and R.N.A. which results in a relative increase of D.N.A. of liver cells (Kosterlitz, 1944; Davidson, 1945). The number of cell nuclei remains constant. Davidson (1945) has suggested that the labile liver nitrogen is present in the liver as a ribonucleic-protein complex. If the large amount of nitrogen excreted by the calf during fasting originated from the liver, a large increase in the excretion of purine compounds from nucleic acid breakdown would be expected. This did not occur. The nucleic acids of skeletal muscle do not appear to have been studied in detail during starvation of an animal, but according to Schneider & Klug (1946) the R.N.A. content of muscle cytoplasm is only one tenth of that found in the liver cytoplasm. Whether R.N.A. is, or is not lost from the muscles during starvation,

it seems reasonable to assume that most of the nitrogen excreted during fasting of the calf originated from the cytoplasm of the muscle cells. Undoubtedly, smaller quantities also arose from the liver.

(b) Fasting the calf as a method for the control of diarrhoea.

From the above discussion it is clear that fasting the young calf causes marked losses of its body protein and fat and, because of its more intense metabolism than the older animal, its losses are more severe (see Tables 12 and 20). A higher percentage of the total heat loss of the calf arises from the degradation of body protein than in the mature animal. This is in accord with the classical work of Voit (1901) who showed that the amount of protein metabolism in starvation depends on the amount of fat in the body. The calf has little body fat at birth or in the early stages of growth (Armsby & Moulton, 1925) and the effect of starvation is therefore intense. If calves are affected by diarrhoea and fasting is used as the method of control, starvation follows a period in which depletion of reserves has already occurred. Realimentation after starvation, if too rapid, may lead to an exacerbation of symptoms as shown by the behaviour of calf №5 during the first period of starvation. Lastly, there is no indication that following realimentation, efficiency of food utilisation is enhanced, and the loss in weight, body protein and body fat can only be restored by establishing a higher plane of nutrition than that existing before fasting began.

B. THE RELATIONSHIP BETWEEN ENDOGENOUS NITROGEN AND BASAL METABOLISM, AND THE MAINTENANCE REQUIREMENT OF THE CALF FOR NITROGEN.

1. Introduction

The results of the previous experiment show that starvation results in a considerable loss of tissue constituents which are drawn upon to meet the demand of the body for energy. Part of the nitrogen lost during starvation is an inevitable loss from the body, since even when an animal consumes a nitrogen-free diet in amounts sufficient to cover its energy requirements, a loss of nitrogen occurs. This is known as the endogenous nitrogen excretion, first characterised by Folin (1905). McCollum & Steenbock (1912) showed for the pig, and Mitchell (1923-4) for the rat, that nitrogen equilibrium could be attained when animals digested sufficient amounts of protein of high quality to provide nitrogen just sufficient to cover the endogenous losses in the urine. This inevitable loss has therefore been considered to be a measure of the minimal protein requirement of an animal for maintenance (Mitchell, 1926, 1929; McLellan & Hannon 1932). It has been pointed out, however, (Blaxter & Mitchell, 1948) that there is also an inevitable loss of nitrogen from the faeces in the form of digestive juices, cell debris etc. which must be made good by the animal from the feed. Thus, the endogenous faecal nitrogen, or metabolic faecal nitrogen excretion should also be included as a part of the maintenance requirement of an animal for protein.

It seems reasonable that there should be a relationship between minimal catabolism of the nitrogenous constituents of protoplasm and the rate at which energy is liberated in the enzyme systems involved, as measured by the basal heat production. Palmer, Means & Gamble (1914) showed that the daily excretion of creatinine nitrogen as an index of total endogenous nitrogen excreted daily by men and women, was proportional to their basal metabolic rates. In men the number of

TABLE 23

Published data relating to the urinary nitrogen excretion of cattle on nitrogen-low or nitrogen free diets.

Author quoted	Weight of animal (kg.)	Urinary nitrogen (g./day)	Endogenous nitrogen (g./kg.)	Computed basal metabolism (cal./kg.)	Ratio endogenous N/basal metabolism (mg./kg.)
Steenbock, Nelson & Hart (1915)	145	6.48	0.045	25.7	1.75
Honcamp, Koudela & Muller (1923)	385	16.32	0.042	14.9	2.81
Hart, Humphrey & Morrison (1912)	117	6.33	0.036	23.4	1.54
Honcamp et al (1923)	440	16.40	0.035	13.0	2.69
Buschmann (1907)	485	14.0	0.029	12.7	2.28
Hutchinson & Morris (1936)	?	12.70 20.91	0.039	13.0	2.93
Mean ratio					2.24

calories per mg. of creatinine excreted was 0.98 and for women 1.26. Terroine and Sorgmatter (1927) showed that the ratio of mg. urinary nitrogen + faecal nitrogen excretion on a nitrogen free diet, to calories of basal metabolism ranged from 2.2 to 2.6. This relationship was confirmed by Smuts (1935), who also showed that it applied to animals varying in size and species. For the mouse, rat, guinea-pig, rabbit and pig there was a loss of 2.0 mg. urinary nitrogen for every calorie of basal heat loss. In adult man lower values have been obtained (Bricker, Mitchell and Kinsman, 1945; Murlin, Edwards, Hawley and Clark, 1946) some as low as 1.4 mg. nitrogen per basal calorie.

Few estimates of the relation-ship are available for the ruminant; but from the results of experiments carried out by others for other purposes this relationship may be calculated. The results of relevant experiments have been summarised by Mitchell (1929) and Brody (1930) and are shown in Table 23. The basal metabolism figures have been calculated from equations given by Brody (1945). The mean value of 2.24 is in substantial agreement with the results obtained for other animals. It will be noted, however, that the lower values of the ratio are for the smaller animals.

There is little data available on the ratio in young animals. Sorgmatter (1928) studied the endogenous nitrogen excretion per calorie of basal heat loss in rats and cockerels of various weights and ages, and again the ratio fell within the narrow range of 2.20 to 2.53 mg., averaging 2.37 mg. On the other hand, Treichler (1939) has shown that in the rat, age has a marked effect - the ratio gives a maximum in early maturity, with low values for the young and for the mature animal. There are no data or reliable estimates available for the young calf.

This concept of an endogenous nitrogen excretion has more recently been criticised by Schoenheimer & Rittenberg (1940). These workers showed that

animals given amino-acids labelled with isotopic nitrogen, retained over half of the nitrogen in the tissues, the remainder being excreted in the urine as both 'endogenous' compounds and 'exogenous' compounds. It was concluded that no distinction was possible between the endogenous and exogenous metabolism of protein. Mitchell (Burroughs, Burroughs & Mitchell, 1940; Mitchell, 1948), however, has criticised this conclusion and has suggested that the endogenous metabolism of Folin represents the summation of irreversible reactions involving nitrogen. Whatever interpretation of this nitrogen excretion is made it remains a measure of the basal and inevitable loss of nitrogen from the body.

The following experiment was carried out to find whether the relationship between endogenous nitrogen and basal heat production was the same for the very young calf as it is for other species and older animals of the same species. It has already been shown that the starvation metabolism of the young calf is more than twice as intense as that found in the mature animal, and it was important to know whether this intense metabolism was associated with an equally intense endogenous nitrogen excretion. If the relationship between basal energy metabolism and endogenous nitrogen excretion holds for the calf, it would be possible to obtain an estimate of the endogenous nitrogen excretion by determining basal heat production - a much simpler and less time consuming procedure than the direct determination. Subsidiary objects in this experiment were to study the partition of urinary nitrogen during nitrogen inanition and to determine any changes in the faecal nitrogen excretion.

2. Plan of the experiment

Four periods were included as follows:-

1. A preliminary period of 14 days when a medium protein diet was given.
2. A nitrogen free period of 8 days.
3. A recovery or final period of six days on the

same diet as used in the preliminary period.

4. A period of starvation of 24 hr. at the end of which the basal energy metabolism was determined.

Details of the three Ayrshire calves which were used are shown in Table 24.

TABLE 24

Details of experimental calves

Calf Nº	Age at commencement of experiment (days)	Initial weight (kg.)	Notes	Diet (litres per day)
1	40	34.2	A thin, poorly nourished calf	3.6
2	5	27.6	Normal	2.8
3	5	33.2	Normal	3.4

Calf Nº1 had already been used as an experimental animal and had been given low calorie diets prior to the present experiment. Calves 2 and 3 had received the general treatment of whole milk feeding as previously detailed (p.).

The composition of the experimental diets is shown in Table 25.

TABLE 25

Composition of the experimental diets

Constituent	Normal diet Nº6	N-free diet Nº7
Dried skim milk powder (g./l.)	77.6	Nil
Lard (g. / l.)	35.9	38.7
Cod-liver oil (ml. / l.)	3.3	3.3
Glucose (g. / l.)	14.8	95.0
Mineral mixture (Table 2)(g/l.)*	1.3	13.0
Yeast extract (ml./calf)	Nil	20
Dry matter (g. / l.)	130	147
Energy value (Cal./l.)(Calculated)	740	750
Nitrogen (g./l.) (by analysis)	4.6-4.8 depending on sample	<0.1≠

* Magnesium supplied as the sulphate
≠ Varied slightly from calf to calf (35.6 mg.N received in the yeast extract).

Diet 6 was prepared by the method previously given (p. 7) and diet 7, the nitrogen free diet, was made by homogenising the fat directly into the glucose solution at a temperature of 85°C. With this diet emulsifying agents had to be used to give stable emulsions. After considerable trial on a small scale using sodium tauroglycocholate, cholesterol, lecithin, sodium oleate, sodium alginate and a proprietary preparation of sodium alkyl sulphates separately and in combinations, the following were found to be suitable for each 4 gallons (18 litres):- 10 g. sodium tauroglycocholate, 5 g. cholesterol and 8ml. of a saturated solution of sodium alkyl sulphates. Failure to homogenise the diet has been shown to cause profuse diarrhoea in the calf and a loss of hair (Bate, Dwight & Cannon, 1946). The yeast extract (Table 25) was prepared according to the method of McCrae, El Sadr & Sellers (1942). The diets were given to the calves in the quantities shown in Table 24, and the following analyses were carried out:-

Diet

Dry matter, total fat and total nitrogen (determined on each sample).

Urine

Total nitrogen (determined every day) urea, ammonia, creatine, creatinine, uric acid, allantoin (determined every two days).

Faeces

Dry matter, total ash, total nitrogen, "fat" and soaps (determined every two days). Non-protein nitrogen was determined on trichloroacetic acid or alcohol extracts of fresh faeces.

3. Results

(a) General appearance and behaviour of the animals throughout the experiment.

When diet 6 (Table 25) was given, the animals behaved normally with the exception of calf №3. This calf suffered from acute diarrhoea in the preliminary period and was treated by reducing its food intake and by slow realimentation over a period of four days.

In the nitrogen free period, however, difficulties arose in persuading the calves to drink their diet. This was not an immediate reaction - the calves consumed the diets normally for the first three days. On the fourth there was a reluctance to drink which increased throughout the remainder of the period. Similar disturbances of appetite on feeding low nitrogen diets have been observed by Miller (1937) and by Ferguson and Neave (1943). All calves suffered from diarrhoea at times when the nitrogen free diet was being given. This appeared to be due to a dietetic disturbance rather than to bacterial infection and resulted in the calves becoming dull and lethargic. Slight shivering was noted on several occasions, otherwise the calves were normal, and, despite the diarrhoea, seemed content.

(b) Bodyweight

Changes in bodyweight were not regular from weighing to weighing. This may be accounted for by differences in the contents of the bladder and the digestive tract which were not minimised by weighing at the same time every day. Table 26 shows the mean bodyweight changes per day obtained by regression analysis of the individual weights.

TABLE 26

Changes in bodyweight of the calves (g./day)

Period	Calf №1	Calf №2	Calf №3
Preliminary period	+ 215	+ 133	- 230
N-free period	- 63	+ 220	- 153
Final period	+ 213	+ 238	+ 238

With the exception of calf 3 in the preliminary period all calves gained in weight when they received diet №6. This loss of bodyweight in calf №3 was due to the severe diarrhoea from which it suffered. The loss in weight of Calves 1 and 3 when receiving the nitrogen free diet may also be explained in the same way.

TABLE 27

Mean daily excretion of water and dry matter in the faeces and digestibility of the dry matter ingested by the calves.

Calf №2	Preliminary period, diet №6	N-free period diet №7	Final period diet №6
Mean daily excretion of dry matter (g.)			
1	32.3	122.5	49.6
2.	21.0	112.4	18.3
3.	25.2	100.0	24.7
Mean	26.2	111.6	30.9
Mean daily excretion of water (g.)			
1	165	989	216
2	93	435	88
3	64	1366	87
Mean	107	930	130
Mean daily apparent digestibility of dry matter (%)			
1	93.2	77.3	89.6
2	94.4	73.2	95.1
3	94.3	80.4	94.5
Mean	94.0	77.0	93.1

TABLE 28

Mean percentage composition of faecal dry matter of the calves

Constituent	Calf №1		Calf №2		Calf №3	
	Prelim: inary	N-free	Prelim: inary	N-free	Prelim: inary	N-free
Dry matter in fresh faeces	17.6	11.4	20.1	20.6	19.5	6.8
Ash in dry matter	21.4	8.0	14.1	7.2	12.9	8.9
Total fat in dry matter	35.3	64.1	28.0	66.0	39.5	63.7
N x 6.25 in dry matter	37.1	12.6	58.4	10.2	47.2	12.7
Residual material, i.e. "carbohydrate'	6.2*	15.2	-0.5	10.2	0.3	16.7
Fat present as soaps (% total fat)	54.6	38.6	63.1	15.3	37.3	23.1
N present as non-protein N (% total N)	24.2	<4	21.0	12.1	22.4	14.7

* This animal had access to sawdust bedding for two days before the preliminary period began. Some was eaten and small quantities appeared in the faeces during the first 4 days of the preliminary period.

TABLE 29

Mean composition of the faeces of the calves and the digestibility of the diet given in the preliminary period and in the nitrogen free period.

	Preliminary period diet №6	N-free period diet №7	Difference	Significance
Ash in dry faeces (%)	16.1	8.0	8.1 ± 2.76	N.S.
Fat in dry faeces (%)	34.3	64.6	30.3 ± 4.04	$P \leq 0.02$
N in dry faeces (%)	7.6	1.9	5.7 ± 1.10	$P < 0.05$
Residual 'carbohydrate'	2.0	15.5	13.5 ± 2.38	$P < 0.01$
Fat excreted (g.)	10.5	74.9	64.4 ± 1.75	$P < 0.01$
Apparent digestibility of dietary fat (%)	91.7	44.9	46.8 ± 3.56	$P < 0.01$
Energy excreted in faeces (cal./day)	172	844	672 ± 36.9	$P < 0.01$
'Apparent digestibility' of dietary energy (%)	92.9	66.5	26.4 ± 2.52	$P < 0.01$

N.S.: not significant

(c) Digestibility of the diets.

Table 27 summarises the data relating to faecal water and dry matter excretion, together with the apparent digestibility of the dry matter. Although the day to day variation in faecal excretion of dry matter and particularly water was high, it is clear that there was a large increase in excretion when the calves were given the nitrogen-free diet. Analysis of variance showed that, statistically, the increase in water and dry matter excretion of the total diet and the fall in the digestibility were highly significant ($P = 0.001$).

(d) Composition of the faecal dry matter.

The unweighted mean composition of the faeces of each calf is shown in Table 28 while Table 29 summarises the statistical significance of the changes which occurred in percentage composition. It will be noticed that large and significant changes occurred in the fat content and the nitrogen content of faeces when the nitrogen-free diet was given, and that the increase in the residual matter, which was presumably carbohydrate, was highly significant. Since the dry matter excretion increased during the nitrogen-free period it is clear that the total daily excretion of fat and fat digestibility showed large changes. These are shown in the lower part of Table 29. Total fat excretion increased by seven times when there was no nitrogen given in the diet, and the digestibility of dietary fat dropped to less than half the normal value. The increase in dry matter excretion was therefore largely due to a decrease in fat absorption since 75% of the increased faecal dry matter excretion consisted of fat. Although there was a decrease in the percentage of soaps in the dry matter (Table 28) the amount of soaps excreted in g. / day, when the nitrogen free diet was given, was double that of the preliminary period. This suggests that absorption of fat is at fault rather than digestion of fat - probably due to a rapid passage of diet through the digestive tract.

TABLE 30

Nitrogen balance results for the calves (g. / day)

		Calf №1	Calf №2	Calf №3	Mean
Preliminary period diet №6	Intake	15.86	12.34	15.31	14.50
	Faeces excretion	1.35	2.02	2.17	1.81
	Urine excretion	7.87	6.69	9.42	7.99
	Balance	+6.74	+3.62	+3.72	+4.70
Experimental period N-free diet №7	Intake	0.04	0.04	0.04	0.04
	Faeces excretion	2.45	1.76	2.08	2.10
	Urine excretion	2.99	2.24	2.52	2.58
	Balance	-5.40	-3.97	-4.56	-4.64
Final period diet №6	Intake	15.13	11.77	14.29	13.73
	Faeces excretion	2.49	1.15	1.49	1.71
	Urine excretion	9.42	5.90	7.93	7.75
	Balance	+3.22	+4.72	+4.87	+4.27

This may have been caused in some measure by the large quantities of glucose which were present in the diet (see page 80). Whatever the cause, this large quantity of fat in the faeces implies a considerable reduction in the number of calories available to the animal. The faecal calories and the 'digestibility' of the ingested calories were calculated from the data on food and faeces composition, using factors of 9.1 cal. / g. for fat, 4.0 for carbohydrate and 5.6 for protein (N x 6.25). These results are also shown in Table 29, and their importance is discussed later (page 53).

(e) Nitrogen balance, metabolic faecal nitrogen and endogenous urinary nitrogen.

Statistical analysis of the nitrogen balance results which may be seen in Table 30 showed that there was no significant change in faecal nitrogen excretion, but that there was a highly significant ($P = 0.01$) change in urinary nitrogen excretion when the nitrogen-free diet was given.

As previously stated the nitrogen excreted in the faeces, when a nitrogen-free diet is given, is known as the metabolic faecal nitrogen. With rats and pigs, Schneider (1934, 1935) showed that metabolic faecal nitrogen could be divided into two fractions; a digestive fraction which varied directly with the quantity of dry matter consumed and a constant fraction which was probably of true excretory origin. In ruminants the metabolic faecal nitrogen is approximately 0.5 g. per 100 g. dry matter consumed (Blaxter & Mitchell, 1948) a value which is about five times as great as that observed for rats or man. This difference between ruminants and non-ruminants has been regarded as entirely due to the higher fibre content of the ruminant's diets since large quantities of fibre in the diet of the rat increased metabolic faecal nitrogen (Mitchell, 1926). It would therefore be expected that milk-fed calves would give much lower values than those obtained for the adult ruminant.

When, however, the nitrogen excretion during the nitrogen-free period was related to the dry matter intake of these calves, values of 0.45, 0.42 and 0.41g. per 100 g. dry matter ingested were obtained, values well within the range of those quoted by workers with adult ruminants. These results might suggest that there is a true species difference which is established at an early age. A more probable explanation, however, is that the digestibility of the dry matter of the diet influences metabolic faecal nitrogen excretion. This contention is supported firstly, by the fact that the nitrogen excretion of the calves was, on the average, higher during the nitrogen-free period than in the periods of normal feeding when the digestibility of the total diet was high. Secondly, Mukherjee (1946), working with Indian bullocks, has shown that the metabolic faecal nitrogen excretion is more closely related to dry matter excretion than to dry matter intake. Thus, it is probable that the metabolic faecal nitrogen excreted by the calf is in proportion to the dry matter excretion, which was high during the time that the nitrogen-free diet was given. From Table 27 and Table 30 the metabolic faecal nitrogen excretion was 1.9 g. / 100 g. dry matter excreted.

The distribution of nitrogen in the faeces excreted on feeding nitrogen-free diets was completely different from that produced on normal diets. As shown in Table 28, the N.P.N. content of the faeces was very small, and in fact was absent from some faeces samples. Selected faeces samples also showed other main differences. There were no nitrogenous substances extracted by 10% sodium chloride solution, compared with 40% of the nitrogenous substances so extracted during the preliminary period. Water soluble substances which were heat coagulable were not produced when the nitrogen-free diet was given although 10% of the faecal nitrogen was contained in heat coagulable substances during the preliminary period. Only 30% of the faecal nitrogenous compounds excreted when no nitrogen was being received by the calves was

figure 10

Effect of a nitrogen free diet on the urinary nitrogen excretion of the calves

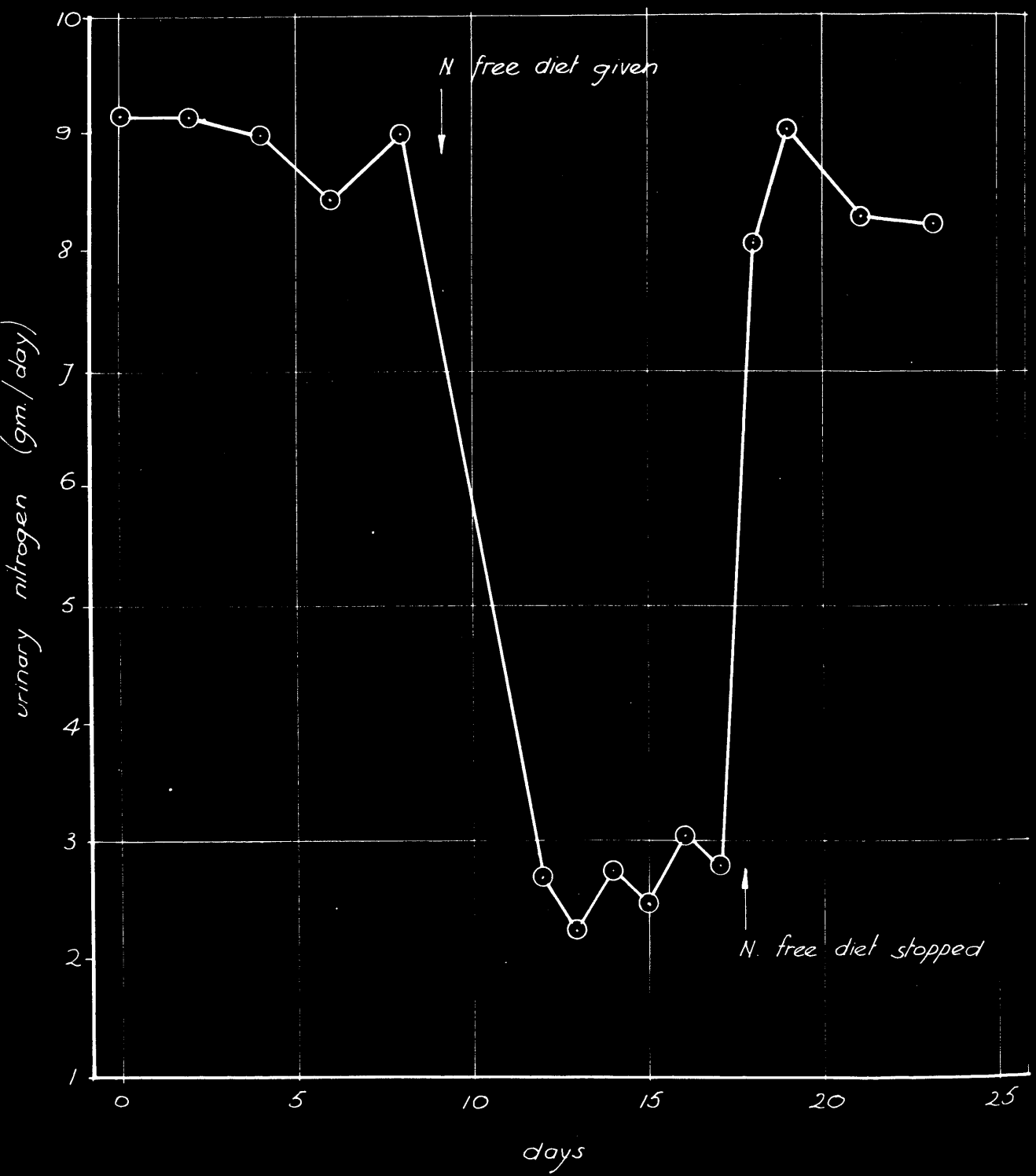


TABLE 31

Mean daily excretion of nitrogen in different nitrogenous metabolites in the urine of the calves

Metabolite (g./day)	Preliminary period diet №6				Experimental period N-free diet №7				Final period diet №6				Statistical significance of change during N-free period
	Calf №1	Calf №2	Calf №3	Mean	Calf №1	Calf №2	Calf №3	Mean	Calf №1	Calf №2	Calf №3	Mean	
Urea	4.55	4.20	5.35	4.70	1.04	1.02	1.07	1.04	6.38	3.66	4.95	5.00	$P<0.01$
Ammonia	0.98	0.68	1.82	1.16	0.34	0.21	0.46	0.34	1.12	0.76	0.65	0.84	$P<0.05$
Urea and Ammonia	5.54	4.88	7.17	5.87	1.39	1.22	1.53	1.38	7.50	4.41	5.60	5.84	$P<0.01$
Creatine	0.248	0.221	0.189	0.219	0.221	0.025	0.063	0.103	0.139	0.112	0.173	0.144	$P<0.05$
Creatinine	0.346	0.342	0.454	0.380	0.221	0.344	0.392	0.319	0.354	0.257	0.384	0.332	N.S.
Uric acid	0.050	0.029	0.054	0.045	0.048	0.028	0.047	0.041	0.069	0.026	0.086	0.060	N.S.
Allantoin	0.811	0.575	0.746	0.711	see text				see text				

soluble in N/10 sodium hydroxide, compared with 60% during normal feeding. This insolubility of faecal nitrogen must indicate that it is present either as compounds which are insoluble in, or compounds which are protected from the solvents in some way. This insoluble fraction may therefore consist of bacterial nitrogen, perhaps with some nitrogen from cell debris of the digestive tract. Thus, faeces excreted by the calves which received the nitrogen-free diet possibly consisted mostly of these fractions.

The urinary nitrogen excretion when the nitrogen-free diet was given represents the endogenous nitrogen excretion of the calf. The results are shown graphically in Figure 10. It will be noted that minimum constant levels of urinary nitrogen were reached within two days. The interpretation of this will be discussed later.

The negative nitrogen balances during the period of nitrogen inanition were approximately related to the body size of the calves. During the period of diarrhoea in Calf №3, the loss of nitrogen from the body per day was 13.5 g. despite the fact that food intake was only reduced to 67% over the period concerned. This loss indicates that protein was broken down for energy purposes, and shows that the calf has a very high requirement for calories.

(f) Distribution of urinary nitrogen

The distribution of urinary nitrogen for each calf is summarised in Table 31. It will be noted that the data relating to allantoin N are not complete. The method of Larson (1932) was used to determine allantoin during the preliminary period, and when this method was applied to urines excreted during the nitrogen-free feeding period, the method gave extremely high values - in fact the residual nitrogen fraction became greatly negative. Interference was traced to large quantities of reducing sugar present in the urine, which were not removed by the phospho-tungstic acid precipitation used. Stored

TABLE 32

Respiratory metabolism of the calves

Variable measured	Calf №1 Determination №		Calf №2 Determination №		Calf №3 Determination №	
	1	2	1	2	1	2
Environmental temperature (°F)	73	72	68	68	64	61
Respiratory rate /min.	16.8	15.5	16.0	14.8	19.4	18.5
Minute volume of respiration (l)	4.52	4.59	3.88	2.98	3.98	4.23
Tidal air (ml.)	269	309	243	201	205	229
Oxygen consumed (l./hr.)	13.17	12.69	10.70	10.27	13.06	12.96
Carbon dioxide produced (l./hr.)	9.80	9.43	7.95	7.21	9.67	9.13
R.Q.	0.74	0.74	0.74	0.71	0.74	0.71
Heat production (Cal./24 hr.)	1495	1440	1214	1155	1481	1457
Heat production (Cal./kg./24 hr.)	42.7	41.2	43.1	41.0	45.9	45.1

samples of urine were later analysed for allantoin by the method of Young & Conway (1942) and results were obtained which agreed with those values obtained in the preliminary period using Larson's method. The urinary excretion of sugar during the nitrogen-free period was probably an alimentary glycosuria comparable to the lactosuria noted in calves by Rojas, Schweigert & Rupel (1948). The concentration of sugar estimated by Benedict's method rose to over 2%. During the nitrogen-free period, the urine volume declined and the specific gravity rose- no doubt a reflection of the high faecal water loss at this time. In the last column of Table 31 the statistical significance of the mean changes in nitrogen distribution as a result of feeding the nitrogen-free diet are given. The fall in total nitrogen excretion was due almost entirely to a fall in the excretion of urea and ammonia with a smaller decline in the creatine elimination. Creatinine elimination was not significantly changed, nor was the excretion of uric acid. As mentioned above, there did not appear to be any marked change in the allantoin excretion when protein was excluded from the diet.

(g) Basal metabolism

Results of duplicate determinations of oxygen consumption and carbon dioxide production are given in Table 32. The agreement between duplicate determinations was again close and analysis of variance of the data showed that the coefficient of variation of heat production was less than 4%. These results confirm the intensity of metabolism which is found in the young calf, since when compared with the estimates of Brody (1945) for the adult (see Table 23), the basal metabolism of these calves per kg. of bodyweight is twice that found at maturity.

4. Discussion

(a) The relationship between endogenous nitrogen excretion and basal metabolism

Before any relationship between endogenous nitrogen excretion and basal metabolism can be calculated,

it is necessary to assess the validity of the determinations.

The conditions under which the determination of basal metabolism was carried out are compared below with the ideal conditions usually given for the determination of basal metabolism in man.

1. Relaxation prior to and during measurement.
   Muscular repose was maintained throughout the determinations and the animals were allowed 15 min. to accustom themselves to the procedure before an actual determination was made.
2. Post-absorptive state.
   Since there was a linear decline in basal metabolism with time (see page 33), it was considered that a most accurate estimate of basal metabolism would be obtained as soon after the post-absorptive state had been reached as possible, and yet it was necessary to be sure that this state had been attained. Twenty-four hours after feeding was chosen as the most suitable time.
3. Good nutritive condition - especially as regards energy and protein. The calves may be regarded as having been in good nutritive condition, since they were gaining in bodyweight at a rate of over 200 g. / day.
4. Environmental temperature of about 77°F.
   The environmental temperatures during the determination of basal metabolism are given in Table 32 from which it will be seen that the temperature at the time of the determinations was generally lower than 77°F. This may have had some effect on the results since the metabolism of calf №3 was higher than that of the other calves, and the environmental temperature was lower.

A reliable estimation of endogenous nitrogen is obtained when the following conditions are maintained:-

1. Previous feeding with a diet not containing excess protein.
   The experimental diet used during the preliminary period contained 20% of the dry matter as protein,

TABLE 33

Relation between the basal metabolism of the calves and their endogenous nitrogen metabolism.

Calf Nº	Weight (kg.)	Endogenous nitrogen metabolism		Basal metabolism		Ratio mg. N/Cal.
		(g./day)	(mg./kg./day)	(Cal./day)	(Cal./kg./day)	
1	35.7	2.99	83.8	1467	41.9	2.00
2	27.8	2.24	80.8	1185	42.0	1.93
3	31.0	2.52	81.0	1469	45.5	1.78
Mean	31.5	- --	81.9	----	43.1	1.90±0.07
Expected values in mature animals (Brody, 1945)	31.5	- --	55.6	----	28.1	---

compared with about 27% for whole milk. The result would only be affected if the tissues were saturated with labile nitrogen compounds immediately before the determination of the endogenous nitrogen excretion. This condition is unlikely to occur in a growing animal.

2. A diet adequate in energy.

Calculations of the metabolisable energy available to the animals from analytical data on urine and faeces in all cases gave values which were above the directly determined basal energy metabolism, and provided the heat increment of the diet is small - a reasonable assumption in an animal not converting a large portion of its dietary carbohydrate to lower fatty acids - these intakes of metabolisable energy should have been sufficient to meet basal requirements. A further point is, that with two of the calves, creatine disappeared completely from the urine by the end of the period while in the other it reached a very low level. It would appear that these nitrogen excretions are truly endogenous, and the figures may therefore be used for the calculation of the ratio - mg. N per basal calorie of heat production.

The endogenous nitrogen metabolism of the calves and their basal metabolism are shown in Table 33. The results suggest that the ratio of approximately 2 mg. nitrogen per basal calorie of heat production was the same in the calf as in mature animals of the same and different species. The table also shows the endogenous nitrogen and basal metabolism of mature animals of different species calculated for the same mean body weight as the experimental calves from the equations given by Brody (1945). It will be seen that the endogenous nitrogen metabolism of the young calf was far more intense per kg. of bodyweight than a mature animal of the same size or of the same species (compare Table 23). As previously shown, the same was true of the basal energy metabolism, so that the ratio of the one to the other appears to be the same in the young calf as in mature animals of

various species.

(b) The maintenance requirement of the calf for nitrogen

Table 33 shows that the mean endogenous nitrogen in mg. / kg. / day was 82. The metabolic faecal nitrogen (Page 49) was 1.9 g. / 100 g. dry matter excreted. These figures represent the inevitable loss of nitrogen which occurs from the body of the calf, and this loss is therefore considered to be the maintenance requirement of the animal in terms of metabolisable nitrogen (Blaxter & Mitchell, 1948; Lofgreen, Loosli & Maynard, 1951).

The mean loss of nitrogen during starvation was found to be 259 mg. / kg. bodyweight. Thus 32% of the nitrogen lost during starvation was an inevitable loss of endogenous nitrogen and the difference, 259 - 82 = 177 mg. nitrogen / kg. body-weight is the nitrogen from body protein catabolised for energy purposes.

C. MEASUREMENT OF 'PROTEIN QUALITY' FOR THE YOUNG CALF

1. Introduction

In the 19th century, it was considered that protein nutrition was essentially one of nitrogen nutrition. However, in 1832, Edwards & Balzac showed that a ration of bread and gelatin was insufficient for the nutrition of the dog, whereas one of bread, gelatin and a small amount of meat soup was sufficient for normal health. Also, in nitrogen balance experiments Voit (1872) showed that gelatin would not take the place of meat in the ration of a dog. These experiments led to the recognition that there was not one minimum protein requirement but that the requirement depended on the nature of the protein.

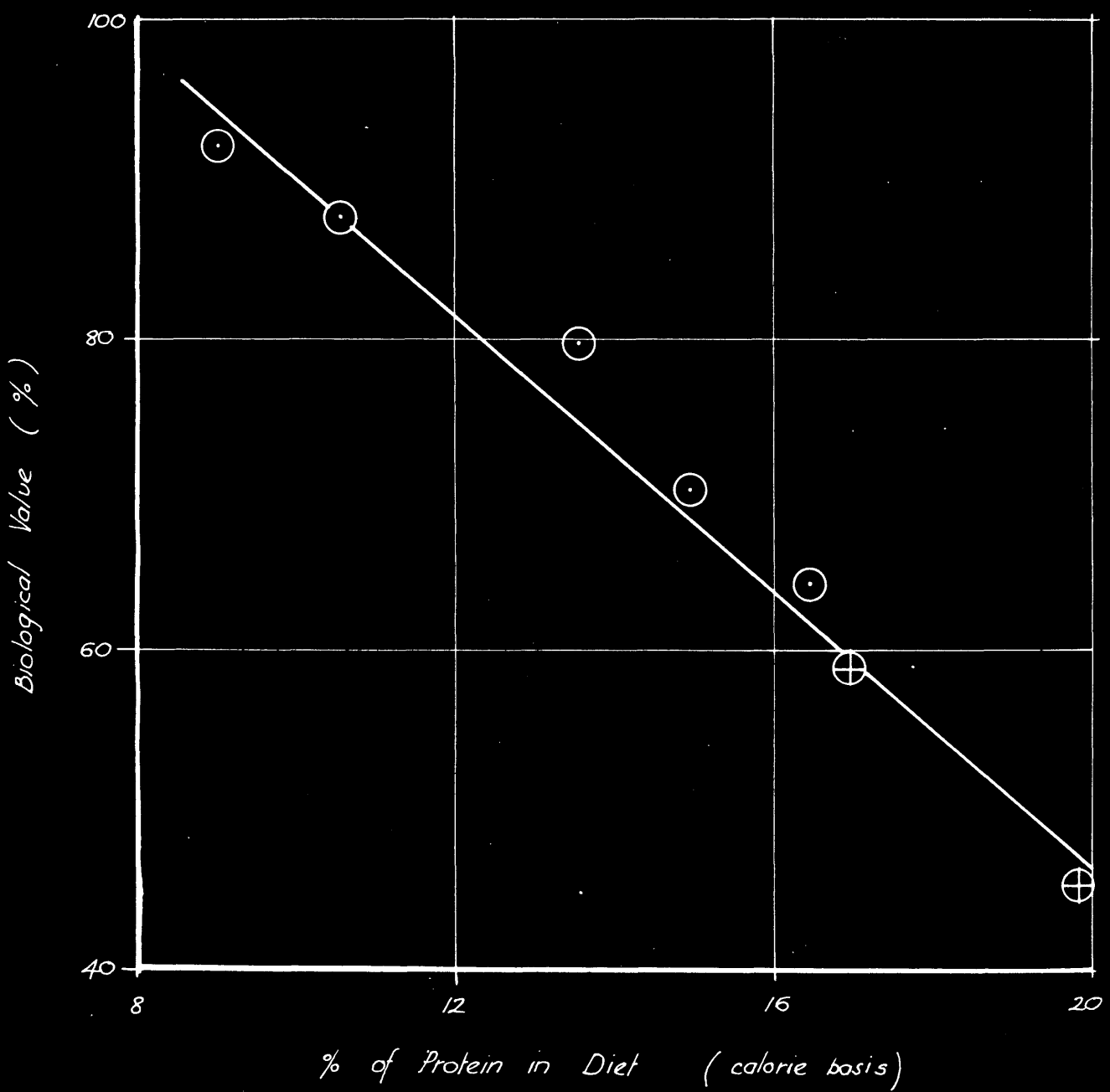
The classical studies of Osborne & Mendel on the nutritive value of purified proteins showed that when the intake of a protein was small, the efficiency of its utilization was limited because of relative shortages of one or more essential amino-acids. This remains the basis of current ideas on the nutritive value of proteins. The body requires a mixture of essential amino-acids (those it cannot itself synthesise) in certain definite proportions to replace the loss of both endogenous and metabolic faecal nitrogen and also for growth and production (milk, eggs). The nitrogen required for a given function then, is fixed and the amount of digested food protein which will satisfy the requirement varies according to its amino-acid content. No protein contains exactly the right mixture of amino-acids for a particular function except possibly those of dried whole egg for the growth of the rat and thus a portion of the amino-acids absorbed from the digested protein will not be required by the body. These will be deaminated and the carbon fragments utilised as a source of energy. The fewer amino-acids which are 'wasted' in this way, the higher will be the quality of the protein.

Of the methods which have been proposed to determine the quality of proteins in nutrition, two are

figure 11

The biological value of protein :

. as affected by the percentage present in the diet



○ whole egg (Hamilton)

⊕ dried skim milk (experiments A and B)

used extensively:-

(a) The Protein efficiency ratio. This is the number of grams of bodyweight gain for every gram of protein consumed. The original method of Osborne, Mendel & Ferry (1919) using progressively increasing percentages of the test protein has been modified, and most workers now test the protein at a constant level, usually 10% of the diet. Determinations of protein efficiency ratios have been criticised by Mitchell (1944). The main points in his criticism are as follows:-

(i) The differences between the proteins are exaggerated. Feeding in this method is ad libitum and the better the protein, the more food is eaten, but the greater the amount of food eaten, the higher becomes the ratio.

(ii) No account is taken of the digestibility of the protein. It is therefore impossible to conclude from the results why one protein is better than another.

(iii) It is assumed that bodyweight growth is of constant composition regardless of the ration on which it was obtained. This is, in fact not the case, as the work in this thesis will later show.

(b) The Biological value of a protein determined by nitrogen balance methods is the number of grams of nitrogen stored for every 100 g. of nitrogen absorbed. (Mitchell, 1923-4). This procedure also yields data on metabolic utilisation and digestibility. The results are affected if -

(i) the experimental period is not long enough to obtain a true estimate of nitrogen balance and the pre-experimental period is not long enough to ensure that equilibrium is obtained with the diet.

(ii) By far the most important influence on the result is the percentage of protein in the diet (Mitchell, 1923-4; Mitchell & Beadles, 1926-7; Hamilton, 1938). Figure 11 shows the results obtained by Hamilton (1938) on rats fed differing

percentages of protein when the source of protein was dried whole egg. Dried egg has a very high biological value in the rat when fed at the 8% level. This graph also includes results computed from nitrogen balance data obtained during experiments A and B in the present work. For this purpose endogenous nitrogen excretion was estimated by using the factor of 80 mg. endogenous nitrogen per kg. of bodyweight, while metabolic faecal nitrogen was calculated using the factor of 2.0 g. metabolic faecal nitrogen / 100 g. dry matter excreted. It is of interest that the values for the biological value of the proteins of dried skim milk in the calf almost coincide at lower levels of N intake with those for rats given whole egg. In growing rats the biological value of the proteins of dried skim milk is approximately 84 (Fairbanks and Mitchell, 1935; Sumner 1938; Swaminathan, 1937 a,b; Henry, Houston, Kon & Osborne, 1939; Henry, Kon, Lea & White, 1948). In these experiments with young rats the level of protein was 8%, whilst in adults percentages as low as 4-5 were used. It is therefore clear from Figure 11 that maximum biological values of the dried skim milk were not obtained with calves since the level of protein was too high. Only when the protein content of the diet is such that the demand by the tissues for amino-acids is greater than the supply will maximum biological values be obtained. This inevitably entails partial protein deficiency in the animal and a rate of growth which is lower than normal.

Experiments designed to study the biological value of dietary protein in the calf during the first few weeks of life do not appear to have been conducted, and information regarding the protein content of the diet which would render such experiments critical is not available. Preliminary observations have been presented, and, to obtain further information, a series of nitrogen balances were determined on calves given diets containing varying percentages of protein.

TABLE 34

Composition of the experimental diets

Constituent	Diet Nº		
	9	11	10
Dried skim milk (g./l.)	83.9	69.7	55.5
Lard, pure (g./l.)	41.8	41.9	42.1
Cod-liver oil (ml./l.)	3.3	3.3	3.3
Glucose (g./l.)	- -	15.4	30.8
Mineral mixture (g./l.)	- -	1.7	3.3
Lecithin (g./l.)	- -	0.05	0.1
Calculated composition			
Calories/l.	772	744	716
Fat (%)	4.6	4.6	4.6
Protein in dry matter (%)	22.0	18.0	14.0
Protein calories as percentage of total calories	20.5	17.0	13.5

TABLE 32

Arrangement of balance experiments and animals used

Diet		Calf №
Type and №	Amount given (l./day)	
High protein 9	2.4	11
	2.6	12
	3.8	7
	4.2	8
Medium-protein 11	3.4	11
	3.8	8
	4.2	9
	6.0	9
	9.0	9
Low-protein 10	2.4	12
	2.6	11
	3.8	9
	4.2	7

2. Plan of experiment to determine factors affecting the biological value of protein for the calf.

Five calves were used and at least one nitrogen balance was determined on each.

The composition of the diets used is shown in Table 34 and some details of the experiments completed are shown in Table 35. The following determinations were carried out:-

Diet

Total nitrogen, total fat and dry matter (determined on each sample).

Urine

Total nitrogen (determined every day).

Faeces

Total nitrogen, dry matter, ash and 'fat' (determined every two days).

The results of the series of thirteen experiments, each lasting 12 days, permitted several direct comparisons of one diet with another. Results with very high intakes of diets Nº 9 and 10 were not, however, obtained and the results with diet Nº11 are therefore treated separately.

3. Results

(a) General observations

It will be seen from Table 35 that the diets contained 4.6% of fat which is higher that that previously used. When these diets were substituted for whole milk in the usual manner all the animals suffered from profuse 'scouring'. Water was given for a short time and the animals were gradually accustomed to the diets over a period of ten days before the experiment commenced. It was concluded that the calf was unable to assimilate such large quantities of fat when only a few days old.

As the experiment progressed some of the animals became ill, showing symptoms which are described elsewhere (Blaxter, Watts & Wood, 1951). The nitrogen balance results of affected animals are not included here.

TABLE 36

Apparent digestibility for calves of diets Nos.9 (high protein) and 10 (low protein) at four levels of intake.

Amount given (l./day)	Dry matter		Total fat		Total N	
	Diet №9 (%)	Diet №10 (%)	Diet №9 (%)	Diet №10 (%)	Diet №9 (%)	Diet №10 (%)
2.4	93.1	86.8	89.6	81.5	87.8	64.6
2.6	95.7	90.8	96.3	86.9	91.3	84.0
3.8	92.9	91.5	92.4	87.7	86.2	82.6
4.2	93.8	95.1	91.9	91.6	91.7	91.5
Mean	93.9	91.1	92.6	86.9	89.3	80.7
Mean difference with its standard error (three degrees of freedom)	2.83±1.73		5.63±2.34		8.58±5.08	

(b) Digestibility of the diets.

Table 36 summarises the data obtained on the digestibility of the total dry matter, fat and total nitrogen of diet №9 (high protein) and of diet №10 (low protein). The mean digestibility of the dry matter, fat and protein tended to be higher when the diet high in protein was given. Statistical analysis of these results showed, however, that the differences were not significant. Table 37 summarises the results when diet №11 (medium protein) was given.

TABLE 37

Apparent digestibility for calves of the medium-protein diet (№11) at five levels of intake.

Amount given (l./ day)	Dry matter (%)	Total fat (%)	Total N (%)
3.4	90.9	87.9	86.0
3.8	93.0	86.9	87.2
4.2	97.3	96.7	94.5
6.0	96.6	95.7	93.3
9.0	96.5	95.6	94.1

It shows that the apparent digestibility of the total nitrogen declined at the lowest levels of intake. This conclusion may also be made from the results with the low protein diet given in Table 36. The percentage of nitrogen in the faeces tended to decline with increasing intake, but this was not statistically significant. Differences between the diets in percentage of nitrogen in the faecal dry matter were, however, statistically significant. The percentage of nitrogen in the dry faeces may be related to the percentage of protein in the diet by the equation

$$N \text{ in dry faeces} = 1.2 - 0.3\,P,$$

where P is the percentage of the total dietary calories present as protein. The intercept of this equation should represent the percentage nitrogen in the faeces when there is no nitrogen in the diet, that is the

TABLE 38

Nitrogen balances of the calves (g./day)

Diet given (l./day)	Intake	Excretion		Balance
		Faeces	Urine	
Diet №9 (high-protein)				
2.4	11.48	1.40	7.63	+ 2.45
2.6	13.23	1.15	7.85	+ 4.23
3.4	-- --	- --	- --	- --
3.8	18.19	2.51	8.46	+ 7.22
Diet №11 (medium-protein)				
3.4	13.77	1.93	6.17	+ 5.67
3.8	15.48	1.98	7.14	+ 6.36
4.2	17.80	0.99	6.82	+ 9.99
6.0	24.18	1.63	8.22	+14.34
9.0	37.68	2.21	11.47	+23.99
Diet №10 (low-protein)				
2.4	7.69	2.73	5.76	- 0.80
2.6	8.62	1.66	4.46	+ 2.50
3.8	12.20	2.11	3.97	+ 6.11
4.2	12.61	1.07	4.61	+ 6.93

metabolic faecal nitrogen. The value of 1.2 g. / 100 g. faecal dry matter differs from the value of 1.9 g. as directly determined (page 49). The errors involved, especially the assumption of linearity of the regression are, however, large and it is doubtful whether the difference is in fact real. From the variability of the nitrogen excretion in the faeces of these calves ranging from 0.99 g. by calf №9 ingesting 4.2 litres of the medium protein diet №11 to 2.73 g. by calf №12 ingesting 2.4 litres of the low protein diet №10, it is clear that in the young calf nitrogen excretion in the faeces is not so constant as it is in cattle and sheep given standard rations. This is largely due to varying degrees of alimentary disturbance in calves ranging from acute diarrhoea, when up to 60% of the ingested nitrogen appears in the faeces, to mild digestive upsets, and it would appear that these digestive upsets are sufficient to prevent the demonstration of even comparatively large differences in the digestibility of the diets unless many calves are used.

(c) Urinary nitrogen and nitrogen balance

Table 38 summarises the nitrogen balance data. This table shows that for each diet an increase in the amount ingested was associated with relatively little change in the urinary excretion of nitrogen, but with a marked change in nitrogen balance. The relation between the intake of nitrogen expressed as apparently digested nitrogen and the urinary nitrogen was examined by analysis of covariance. The observation on the animal in negative nitrogen balance when receiving the low protein diet was omitted. The analysis of variance is given in Table 39.

The mean regression between urine nitrogen excretion and apparently digested nitrogen was very highly significant and there were no statistically significant differences from this mean regression due to the protein percentage of the diet. There was, however, a large difference between the urinary

TABLE 39

Analysis of variance of the urinary nitrogen excretion of the calves expressed in g. / day, including the covariance of urinary nitrogen on apparently digested nitrogen.

Component	Degrees of freedom	Estimated variance	Variance ratio ($_e 2z$)
Joint regression of urinary N on apparently digested N intake	1	17.252	103.7 ***
Differences between regressions	2	0.035	N.S.
Differences between means	2	7.433	44.6 ***
Error	5	0.166	---
Total	10	----	---

-*** Significant at $P < 0.001$

N.S.: not significant

TABLE 40

Analysis of variance of the nitrogen balances of the calves expressed in g. / day , including the covariance of nitrogen balance on the nitrogen apparently digested.

Component	Degrees of freedom	Estimated variance	Variance ratio($_e$2z)
Joint regression of N balance on apparently digested N.	1	269.253	636.6 xxx
Differences between regressions	2	1.447	N.S.
Differences between means	2	5.061	12.0 x
Error	6	0.423	---
Total	11	----	---

xxx Significant at P 0.001 x Significant at P 0.05

N.S.: not significant

nitrogen excretion of the calves on the low, medium and high protein diets at the same level of intake. The three equations relating urinary nitrogen to apparently digested nitrogen were:-

$$UN_H = 0.21\ ADN_H + 5.30 \quad \ldots\ldots (1a)$$

$$UN_M = 0.21\ ADN_M + 3.71 \quad \ldots\ldots (1b)$$

$$UN_L = 0.21\ ADN_L + 2.13 \quad \ldots\ldots (1c)$$

where UN represents urinary nitrogen excretion in g. per day, ADN the apparently digested nitrogen and the subscripts H, M and L refer to the high-, medium-, and low protein diets.

The intercepts, 5.30, 3.71 and 213 represent the urinary nitrogen excretion when no nitrogen was given. These values do not represent the endogenous excretion of nitrogen since if the calves received no nitrogen with these particular diets they would not be receiving any diet at all, and therefore the urine nitrogen would reach the high value found during starvation. These regressions therefore cannot be linear, especially in the region of negative nitrogen balance. As the data used in the present study were all obtained in the region of positive balance, however, it is possible that the intercepts represent both an endogenous component and a constant 'basal deamination component', that is an amount of urinary nitrogen reflecting the higher amount of deamination that occurs on diets high in protein. This quantity should be independent of the dietary source of nitrogen.

The nitrogen balances were related to the nitrogen apparently digested in a similar analysis of variance, presented in Table 40. In this instance the data for the animal in negative balance were included. The differences between the individual regressions were not significant but the mean differences between the intercepts of the equation were significant. This is in agreement with the results obtained for urinary nitrogen, despite the inclusion of the one value in which the calf was in negative balance. The equations relating nitrogen balance

TABLE 41

Storage of body nitrogen by the calves following ingestion of different amounts of nitrogen in diets with high, low and medium levels of protein (g. / day).

Type of diet	Storage of N when equal quantities of apparently digested N are given		Storage of N when equal quantities of energy are given	
	Nitrogen given		Gross energy given	
	10 g.	20 g.	2500 cal.	3500 cal.
Low protein	5.00	13.13	4.59	7.68
Medium protein	4.01	12.04	5.53	9.43
High protein	2.51	10.64	6.16	10.88

to apparently digested nitrogen were:-

$$NB_H = 0.81\ ADN_H - 5.64 \quad \ldots\ldots (2a)$$

$$NB_M = 0.81\ ADN_M - 4.22 \quad \ldots\ldots (2b)$$

$$NB_L = 0.81\ ADN_L - 3.13 \quad \ldots\ldots (2c)$$

where NB represents nitrogen balance in g. per day and the other terms have the same significance as in equation 1.

When the observation on the animal in negative nitrogen balance was omitted the regression coefficient of nitrogen balance on nitrogen intake apparently digested was 0.79 and the values for the intercept - 5.30, - 3.71 and - 2.31, that is the same as for the equation relating UN to ADN, except that the signs are changed. The equations based on all the data, however, have been used in the following calculations, as the justification for discarding the point which did not fit, cannot be tested at the present time.

From equations 2a, 2b and 2c the results in Table 41 were calculated. They show that if equal quantities of gross energy are supplied, the storage of nitrogen falls as the protein content of the diet is reduced. For equal amounts of nitrogen digested, however, the storage of nitrogen increases with decreasing protein content of the diet. This means that if a protein-free supplement is added to a basal diet, nitrogen retention will increase, the effect being to reduce the 'basal deamination component' associated with the higher protein content of the basal diet. These relationships can be inferred from the data of Table 38, where comparisons can be made between animals receiving the same quantity of diets high or low in protein.

The bodyweight gains of the calves reflect these differences in nitrogen retention. Thus in the three calves given 3.8 litres of each of the three diets, the daily gains in bodyweight were 360, 305 and 229 g. for the high-, medium- and low-protein diets respectively. In the calves given 2.6 litres of the

high- or the low-protein diet, the daily gains were 155 and 54 g. respectively, whereas with only 2.4 litres the calf on the low-protein diet lost 46 g. daily and the calf given the high protein diet gained 18 g. For approximately equal intakes of energy, gains were smaller when the diets contained less protein.

A comparison of bodyweight gains at equivalent protein intakes, irrespective of total calorie intake, can similarly be made. Calf №12 ingesting 2.6 litres of diet №9 was ingesting about the same quantity of protein as Calf №11 ingesting 3.4 litres of diet №11, and calf №9 ingesting 3.8 litres of diet №10. The daily gains in weight were 155, 275 and 305 respectively. Similarly the calf ingesting 3.8 litres of diet №9 gained 360 g. and the calf ingesting 4.2 litres of diet №11 gained 455 g. The intake of nitrogen of both animals was about the same. The bodyweight gains, however, are subject to greater errors of estimation than are the nitrogen balances.

(d) Biological values of the ingested protein

The biological value of a protein, as defined by Mitchell (1923-4), is given by the equation

$$B.V. = 100 \times \frac{NI - (UN - EN) - (FN - MN)}{NI - (FN - MN)} \quad \ldots \quad (3a)$$

where NI = nitrogen intake, UN = urinary nitrogen excretion, EN = endogenous nitrogen excretion, FN = faecal nitrogen excretion, MN = metabolic component of the faecal nitrogen, and BV = biological value. This equation may be re-arranged by substituting the nitrogen balance (NB) for the necessary terms in the numerator of the equation and replacing the term (NI - FN) by the term ADN (apparently digested nitrogen). This gives the modification:-

$$BV = 100 \times \frac{EN + MN + NB}{ADN + MN} \quad \ldots \quad (3b)$$

It has already been shown that the nitrogen balance may be related to the amount of apparently digested nitrogen by a simple linear equation, (see equations 2a, 2b and 2c). The biological value as defined by Mitchell (1923-4) can thus be determined by substituting

in the equation above, the linear equation relating ADN to NB. It is also necessary to include values for the excretion of metabolic faecal nitrogen. For the moment these may be regarded as constants of 2.5 and 0.6 g. nitrogen respectively.

The biological value of the proteins of dried skim milk for the calf can thus be estimated from the equation:-

$$BV = 100 \times \left(\frac{2.5 + 0.6 + (0.81ADN - x)}{ADN + 0.6} \right) \quad .. \quad (4)$$

where x represents the intercept of the regression on the nitrogen balance axis. From this equation it is clear that the biological value will depend on the intercept which has been shown to be related to the protein content of the diet. It will also be determined by the amount of apparently digested nitrogen taken in, the biological value being greater when more nitrogen is apparently digested. This relationship however, can only apply if the equation relating NB to ADN is linear. It has already been indicated that when the nitrogen balance is negative, and the urine nitrogen excretion is high due to a low intake of diet, the equations cannot be linear. When the nitrogen balance is positive, however, there is no indication that the regression deviates from the linear, even at very high intakes of diet (see page 71). It is shown that when sufficient whole milk is given to result in nitrogen balances of up to 25 g. per day (bodyweight increase 910 g. / day) and to an energy intake about 2.5 times the maintenance requirement, the linearity of the equation still holds.

The 'biological value' of a protein in an animal which can store large amounts of protein is thus a simple inverse function of the amount of diet consumed and a linear function of the intercept of the nitrogen balance equation - in other words, to the percentage of protein cals. / total cals. of the diet.

It may be expected therefore, that the biological values determined in the present experiment would be in agreement with the hypothesis presented

TABLE 42

Mean biological values of skim milk protein for calves determined by nitrogen-balance methods

Amount of diet given (l./ day)	Biological value (%)		
	High protein diet	Medium protein	Low protein
2.4	40.00	-----	51.2
2.6	54.8	-----	61.6
3.4	----	74.5	----
3.8	64.6	68.1	87.9
4.2	----	78.0	80.0
6.0	----	80.2	----
9.0	----	80.2	----

above, and this is in fact shown in Table 42. For the high protein diet, the biological values increased rapidly as the amount of diet given was increased from that allowing a daily gain of only a few grams to a quantity permitting a gain of 360 g. A similar increase is shown also for the diets of medium and of low protein content. Errors are, however, attached to these estimates, because the values for metabolic faecal nitrogen and the endogenous excretion of nitrogen would vary from animal to animal. To confirm this relationship between biological value and amount of diet ingested, the next experiment (D) was carried out, using various quantities of whole milk as the diet and making more accurate estimates of endogenous nitrogen and metabolic faecal nitrogen excretions.

4. Discussion

It is apparent that the biological value of a protein estimated by nitrogen balance methods is by no means a constant. In order to measure the biological value of a protein, it is essential that the animal should not deaminate amino-acids to supply energy for the support of maintenance or growth. A biological value, if it is to be a measure of the essential amino-acids making up a protein, must be determined under conditions where such a need for energy does not arise. Thus, despite the variability of the biological value, a maximum should be obtained under conditions where the possibility of deamination of the constituent amino-acids of the protein to provide energy is excluded. It is known that a low percentage of protein in the diet is necessary before maximal biological values are attained and the present work has shown that maximal values are attained only when the intake of diet is high. This is no doubt a reflection of the large requirement for calories which the young growing calf possesses.

Allison (1948) has recently reviewed work concerned with the relationship between nitrogen storage and absorbed food nitrogen. The nitrogen balance equation relating these two variables which

this worker has presented, is given below:-

$$NB = K(AN) - NE \quad \ldots\ldots \quad (5)$$

where NB = nitrogen balance, AN = absorbed food nitrogen, NE = endogenous nitrogen and K is the slope of the regression line. This equation may be compared with equations (2a), (2b) and (2c) which have previously been given (page 62). One of the main differences between the equation of Allison and those given in the present work, is that in equation (5), AN is the truly absorbed nitrogen whereas ADN in equations (2a, b and c) is apparently digested nitrogen. The other difference is, that since energy was provided in adequate amounts throughout the experiments of Allison (1948), NE in equation (5) is the truly endogenous nitrogen excretion whereas in equations (2a, b and c) the equivalent term includes a 'basal deamination component'. Thus, equation (5) is for a straight line in the region of negative nitrogen balance and, as previously discussed, equations (2a), (2b) and (2c) cannot remain linear as the nitrogen intake becomes small. On the other hand the equation of Allison becomes curvilinear in the region of positive nitrogen balance (Allison & Anderson, 1945). That equations (2a, b & c) apply to intakes above maintenance in the calf is a reflection of the intensity of its nitrogen metabolism, since in the young animal there is no indication that there is a limit to the storage of nitrogen at least up to a bodyweight gain of about 1 kg./ day. In mature animals the relationship of nitrogen balance to absorbed nitrogen is curvilinear above maintenance, because the capacity of the animals to store nitrogen is limited.

The slope of the nitrogen balance line (K) in equation (5) has been called the nitrogen balance index of the dietary protein, which is "some function of, but not necessarily equal to, the biological value of the protein source". (Allison, Anderson & Seeley, 1946). The slope must be the number of g. nitrogen stored for every g. intake of truly digested

nitrogen which, by definition (see page 56) should be the biological value of the dietary protein, expressed as a decimal. When the regression relating truly digestible nitrogen to nitrogen balance is linear therefore, there seems to be no reason why the slope, or the nitrogen balance index should not give an estimate of the maximum biological value which is attainable.

The criticism made by Allison (1948, 1951) is that nitrogen balance indices greater than 1.0 have been obtained. Two main possibilities which may give rise to an apparent storage of more nitrogen than is being absorbed suggest themselves. Firstly, there may be a temporary retention of endogenous nitrogen compounds or protein catabolism products and secondly, the estimation of metabolic faecal nitrogen may be too high. Either of these factors would also result in a biological value for the equivalent protein of over 100 when estimated by the Mitchell (1923-24) procedure. The criticism would appear to be one against the determination of protein quality by nitrogen balance methods rather than one against the nitrogen balance index. The nitrogen balance index of 0.81 shown in equations (2a), (2b) and (2c) has a similar interpretation, except that the metabolic faecal nitrogen has not been taken into consideration in these equations.

D. THE NUTRITIVE VALUE OF COW'S WHOLE MILK

1. Introduction

In the previous experiment it was suggested that biological values of proteins could be determined by regression analysis of nitrogen balance results determined when different quantities of the same diet were given. The object of the present experiment was to repeat this type of determination using cow's whole milk.

The energy and nitrogen metabolism of calves when given whole milk diets has been the subject of experiment for over 70 years. Soxhlet's work in 1878 involved simultaneous study of mineral, nitrogen and energy balances and these experiments, together with those of Fingerling (1908), Blackwood, Morris & Wright, (1936), and of Tomme & Taranenko (1939), appear to be the only ones which have not been complicated by the inclusion of roughage in the diet of the animals. No metabolism studies appear to have been made in which variation in nutritional plane has been employed. Several practical trials have, however, been carried out in which different quantities of whole milk have been given (New Zealand Department of Agriculture,1948) and in which higher planes of nutrition have been associated with greater growth, at least during the first five weeks of life.

In view of the paucity of information on the calcium and phosphorus metabolism and the energy exchange of the young calf in relation to nutritional plane, additional information on these aspects was sought.

2. Plan of experiment

The two bull calves which were used were five days old at the commencement of the experiment. Whole milk was given to each calf as its sole diet in quantities varying by 0.8 l. for three 12-day periods. Thus Calf №13 received 2.6, 5.0 and 5.8 litres in consecutive periods and Calf №14, 4.2, 3.4 and 6.6 litres. Ten days were allowed between each changeover to attain

TABLE 43

The gain in bodyweight of the calves and its statistical significance with regression analysis showing a difference between calves.

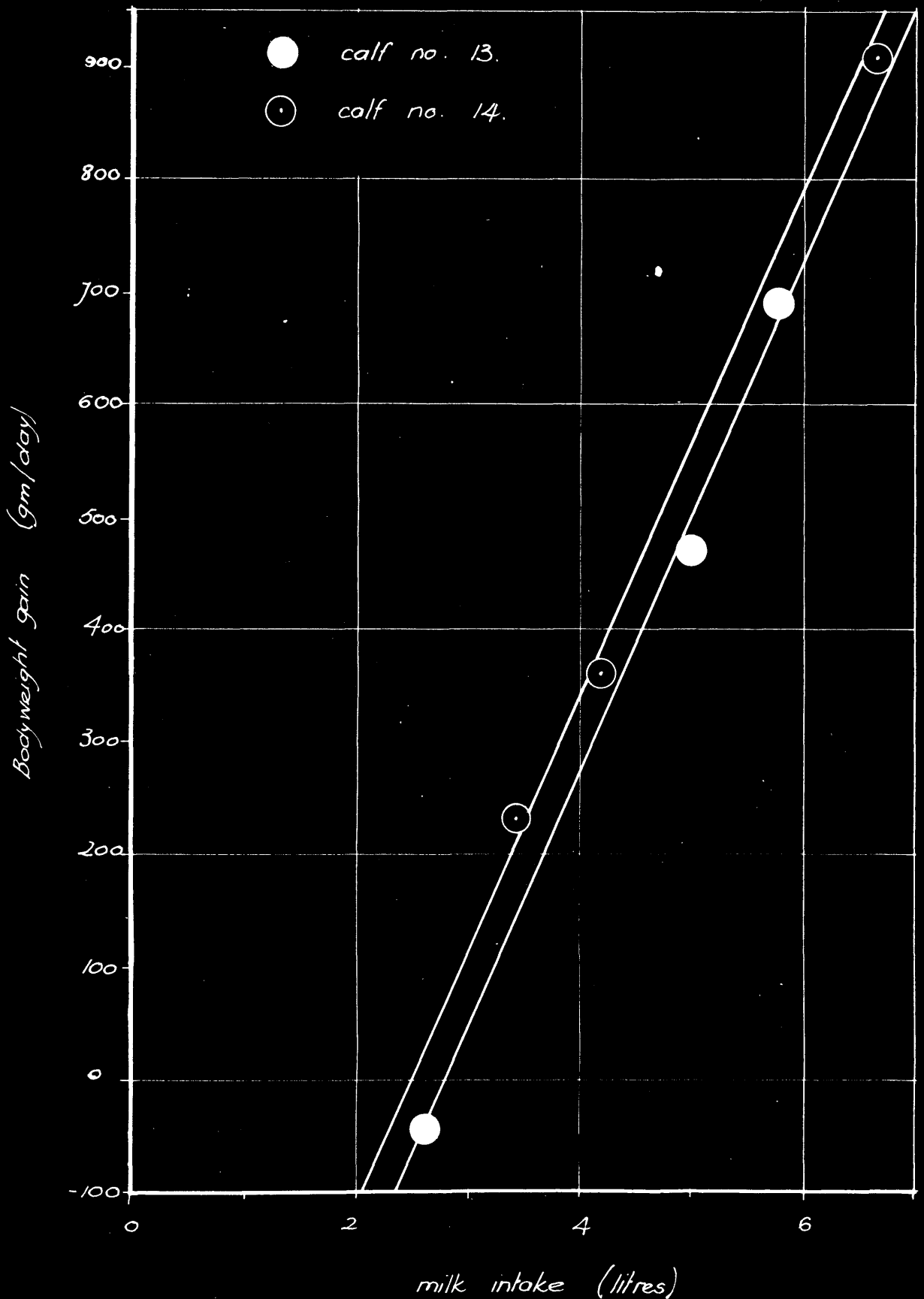
Calf №13		Calf №14	
Milk ingested (l./ day)	Bodyweight gain (g./day)	Milk ingested (l./day)	Bodyweight gain (g./day)
2.6	- 49	3.4	+ 230
5.0	+480	4.2	+ 355
5.8	+690	6.6	+ 910

Regression analysis of bodyweight gain (g./day)

Component	Degrees of freedom	Estimated variance	Variance ratio ($_e2z$)	P
Total error	4	-----	-----	-----
Between calves	1	8350.0	12.9	0.036
Pooled regression	1	550098.0	850.9	0.001
Deviations from regression	3	646.5	-----	

figure 12

The relation between milk intake and gain in bodyweight of the calves.



equilibrium. The whole milk used contained on the average 3.0 - 3.1% fat and was supplemented by a daily dose of 5ml. of a mineral mixture having the same composition per litre as the micro-constituents given in Table 2, except that 20 g. ferriccitrate and 60 g. hydrated magnesium chloride were included. The following determinations were carried out:-

Urine

Total nitrogen (determined daily); urea, ammonia, protein, amino-N, purine bases, uric acid, allantoin, creatine, creatinine (determined every two days); Calcium and phosphorus (determined on pooled aliquots representing the last 10 days of each period).

Faeces

Dry matter, total nitrogen, total ash, total 'fat' (determined every two days); mineral analyses as for urine.

Milk

Total solids, total nitrogen, total ash (determined every day); Total fat, calcium and phosphorus (determined on pooled samples)

Respiratory metabolism was determined once during each 12 day period.

3. Results

(a) General observations and bodyweight

Both calves were bright and in good condition at the commencement of the experiment. Calf №13, when receiving the lowest quantity of milk (2.6 litres/day) became rather thin, but still remained alert and active. Towards the end of the experiment this calf became lethargic and 'scoured' badly. Calf №14 was in excellent condition throughout the experiment. Calf №13 was more nervous and irritable than Calf №14.

Table 43 summarises the bodyweight gains per day calculated by regression analysis of the individual weights, and these values plotted against milk intake are presented in Figure 12. It will be noted that the relation between the rate of growth and milk intake was linear, even at the highest levels of feeding.

TABLE 44

Apparent digestibility of dry matter, nitrogen and fat

		Mean daily faecal excretion (g./day)			Coefficient of apparent digestibility (%)		
Calf №	Milk ingested (l./day)	Dry matter	Nitrogen	Fat	Dry matter	Nitrogen	Fat
13	2.6	12.8	0.88	5.0	96.3	94.1	93.4
14	3.4	19.6	1.83	4.0	95.4	90.7	96.1
14	4.2	15.2	1.50	4.4	97.0	93.8	96.4
13	5.0	11.3	0.85	3.2	98.2	97.1	97.9
13	5.8	38.8	2.70	13.0	94.4	91.7	92.8
14	6.6	19.5	1.64	6.5	97.5	95.6	96.8

These results were analysed statistically by computation of the pooled regression of the bodyweight gain on milk intake. The method used is shown in the second part of Table 43 and has been used in the analysis of all the results obtained. It permits an evaluation of the statistical significance of the regression of gain in body-weight on milk intake as well as of the mean differences between the two calves when given exactly the same amount of milk. The latter difference is the same as the difference in the intercept of the regression on the bodyweight axis. The two equations relating bodygain in g. / day (G) to milk intake in litres / day (M) were:-

Calf №13 $G = 223\ M - 622$ ..... (6a)

Calf №14 $G = 223\ M - 556$ ..... (6b)

As shown in the analysis of variance the pooled regression was highly significant statistically while the difference between calves on the intercept of the equation was also significant. From this it would appear that Calf №14 was a more efficient animal than Calf №13. The inverse of these two equations permits the estimation of the milk required for body-weight maintenance that is, the milk intake associated with no gain in bodyweight. For Calf №13 this was 2.8 litres per day, and for Calf №14 it was 2.5 litres. This difference was significant and the mean value of 2.65 ± 0.16 litres may be taken as an estimate of the requirement of the calf for bodyweight maintenance in terms of milk with a fat content of 3%.

(b) The apparent digestibility of the milk

From faeces collections and analyses, the coefficients of apparent digestibility of dry matter, nitrogen and fat were calculated as shown in Table 44. There were no statistically significant differences in the digestibility of any component due to either the effect of the amount of milk consumed, or to individual differences between the calves. Low apparent digestibility of the dry matter and nitrogen, and more particularly the fat occurred, however, when Calf №13

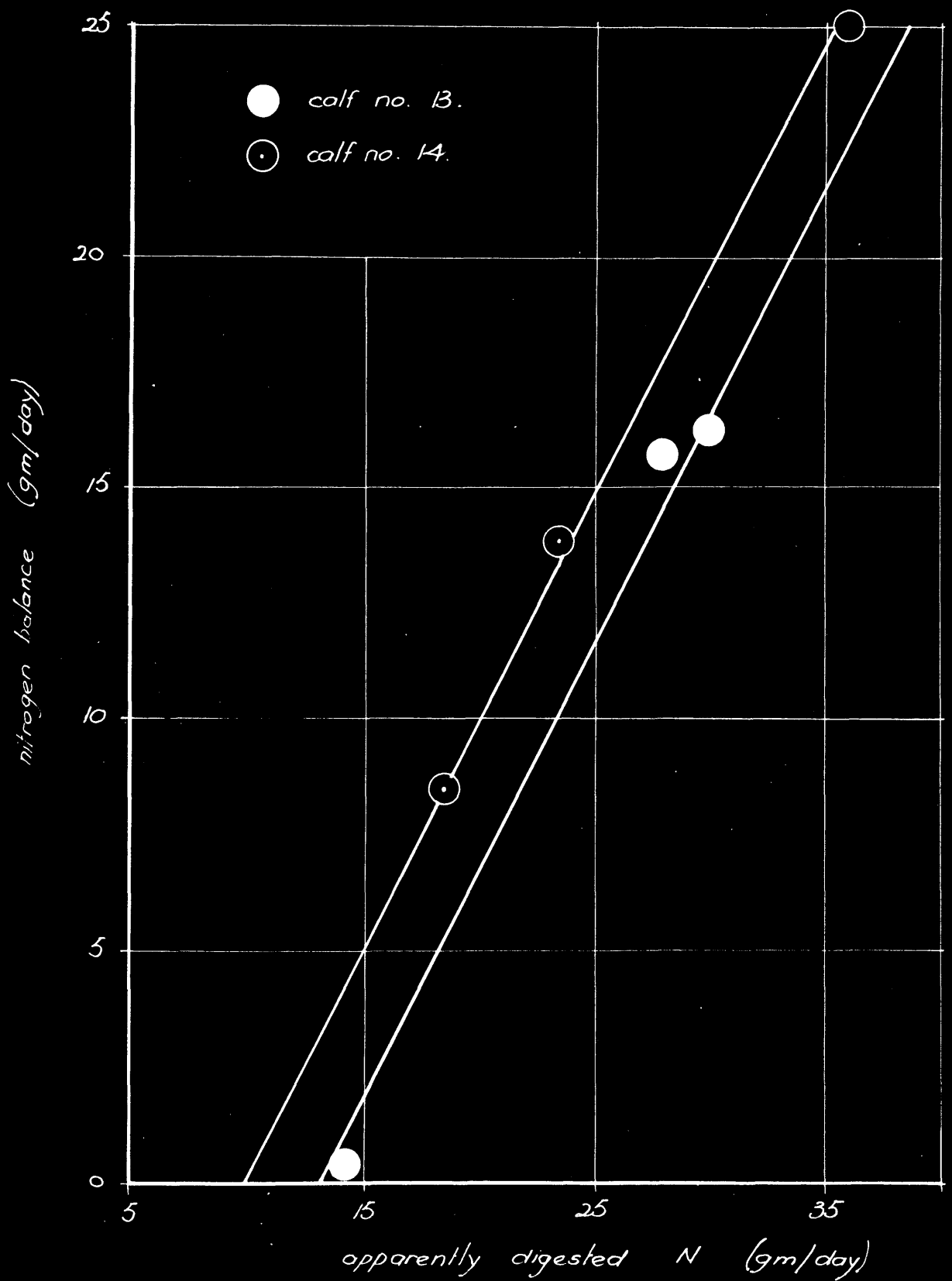
TABLE 45

Nitrogen metabolism in relation to milk intake

Calf Nº	Milk ingested (l./day)	Nitrogen intake (g./day)	Nitrogen apparently digested (g./day)	Urinary nitrogen (g./day)	Nitrogen balance (g./day)
13	2.6	15.0	14.1	13.5	0.5
14	3.4	19.8	18.0	9.5	8.4
14	4.2	24.1	22.7	8.7	13.9
13	5.0	29.1	28.2	12.6	15.7
13	5.8	32.4	29.7	13.6	16.1
14	6.6	37.0	35.4	10.3	25.1

figure 13

Relation between nitrogen balance and the nitrogen apparently digested by the calves



scoured as previously described. The mean apparent digestibility of nitrogen irrespective of calf or feeding level was 93.8% and that of fat 95.6%. These values are compared with the results obtained by other workers, and also with the digestibilities of semi-synthetic diets later (page 109). Except for Calf №13 when scouring occurred, the faecal excretion of fat remained very constant despite the wide range of dietary intake (from 75 g. to 205 g. / day). This suggests that most of the faecal fat is of body origin and does not represent unabsorbed dietary fat. Even during fasting the calf excreted 4.2 g. fat per day, a value close to the mean excretion of 4.6 g. observed in these experiments when one abnormal value was excluded.

(c) Nitrogen metabolism

Table 45 summarises the results from which the nitrogen balances may be calculated. When the nitrogen balances were plotted against the dietary nitrogen apparently digested as shown in Figure 13, it was seen that the calves differed in their nitrogen balances in the same way as they did in their bodyweight gain, Calf №14 storing more nitrogen than Calf №13 at comparable intakes. Employing the statistical method used in the analysis of bodyweight gain, it was found that the difference in intercept was statistically significant ($P = 0.01$), so that there were two equations relating nitrogen balance (NB) in g. / day to apparently digested nitrogen intake in g. / day (ADN)

Calf №13	NB = 0.97 (ADN) - 12.85	.....	(7a)
Calf №14	NB = 0.97 (ADN) - 9.2	.....	(7b)
Mean	NB = 0.97 (ADN) - 11.0	.....	(7c)

The intercepts of the equations represent the loss of body nitrogen when the nitrogen intake is zero and, as the independent variable is apparently digested nitrogen they also represent the urinary nitrogen excretion under the same conditions. The mean value of the intercept, 11.0 g. is higher than that found with diets containing various levels of dried skim milk proteins where these intercepts increased with varying level of dietary protein (page 62). It was suggested that

TABLE 46

Mean daily excretion of urinary nitrogenous metabolites

Nitrogenous constituent	Calf №13	Calf №14
	Nitrogen excreted (g./day)	Nitrogen excreted (g./day)
Urea	8.92	6.22
Ammonia	1.18	0.73
Amino-acids	0.34	0.23
Total urea, ammonia and amino-N	10.44	7.18
Purine bases	0.16	0.14
Uric acid	0.04	0.04
Allantoin	0.59	0.55
Total purine	0.79	0.73
Creatine	0.28	0.27
Creatinine	0.46	0.46
Protein	0.18	0.17

this intercept consists of two fractions, one endogenous and one largely determined by the higher levels of deamination which occur on high protein diets. The present result is in complete accordance with those results, for in cow's milk 29.7% of the total calories are present as protein, while in the previous experiments the maximum was 20.5%. Statistically, the regression of urinary nitrogen excretion on nitrogen intake was not significant. This is a reflection of the high biological value of whole milk protein, for the additional amounts of protein given were all stored. The difference between the calves in their urinary nitrogen excretion was, however, statistically significant ($P = 0.01$). The implications of this difference will be discussed later.

As may be inferred from the results for total urinary nitrogen excretion, only small changes in the distribution of nitrogen occurred as the result of a change in nutritional plane. Creatinine nitrogen increased ($P = 0.07$) with increasing intake of milk. There were, however, large and statistically significant differences between calves in their excretion of urea, ammonia and amino-N as shown in Table 46. An increase in the excretion of these three metabolites accounted for over 90% of the difference between the nitrogen excretion of the calves. The small difference between the calves in their excretion of purine nitrogen and creatinine nitrogen was not statistically significant. It may be concluded therefore that the higher nitrogen excretion by Calf №13 was the result of an increased protein metabolism, rather than an increase in its endogenous metabolism as indicated by purine and creatinine metabolism.

Biological values of the dietary nitrogen were

calculated as before and the values are shown in Table 47.

TABLE 47

Biological value of the nitrogen of whole milk at different levels of intake.

Calf №	Milk intake (l./day)	Absorbed nitrogen (g./day)	Biological value
13	2.6	14.3	40.0
14	3.4	18.3	63.8
14	4.2	22.9	76.6
13	5.0	28.0	70.0
13	5.8	30.4	70.3
14	6.6	35.8	83.6

The biological value of the whole milk nitrogen increased with increasing intake of milk in both calves. The regression of biological value on whole milk intake was statistically significant ($P = 0.03$) and this result confirms the results obtained with semi-synthetic diets which showed that, when the composition of the diet remains constant, the biological value of a protein in a growing animal varies with the total intake of food. If the regression coefficient of 0.97 x 100 (equations 7a, b, & c) gives an approximate estimate of the maximum biological value of whole milk for the calf, it is clear that maximum

TABLE 48

Respiratory exchange data of the calves

Variable measured	Calf Nº					
	13	14	14	13	13	14
Milk ingested (l./day)	2.6	3.4	4.2	5.0	5.8	6.6
Respiratory rate/ min.	26	21	28	43	39	25
Minute volume (l)	5.8	5.0	7.2	10.9	12.4	9.2
Oxygen consumption (l./hr.)	13.0	14.1	16.5	18.0	22.2	21.7
R.Q.	0.82	0.80	0.84	0.80	0.75	0.78
Heat production (cal./hr.)	63.1	67.6	80.2	86.3	105.1	103.9
Heat production (cal./sq.m./hr.)*	56.4	53.6	66.3	70.7	79.6	74.8

* Surface area, S estimated by the formula, $S = 0.15\ W^{0.56}$

where W = bodyweight.

biological values were not attained when calculated by the Mitchell (1923-24) method even when 6.6 l. of milk/day were given. This may be explained by the large excess of protein calories to total calories of whole milk, especially when the milk contained only 3% of fat, as in the present experiment.

(d) Respiratory exchange

Table 48 presents the results of respiratory exchange determinations. Heat production was calculated per square metre of body surface using the formula, surface area = 0.15 bodyweight $^{0.56}$. Statistical analysis by the method previously outlined showed that the regression of heat production per sq.m./hr. on milk ingested was statistically significant ($P = 0.01$). The difference between the calves could only be judged significant when $P = 0.088$. The regression equations for each calf were:-

Calf №13 $HP = 6.44\ M + 40.1$ ..... (8a)

Calf №14 $HP = 6.44\ M + 33.4$ ..... (8b)

Mean $HP = 6.44\ M + 36.8$ ..... (8c)

where HP is the heat production per square metre/hour.

The respiratory quotient was not appreciably affected by the plane of nutrition. The minute volume of the respiration and respiratory rate were increased at the higher levels.

The increase in the intake of milk was clearly associated with an increase in heat production. At a milk intake equivalent to bodyweight maintenance the mean heat production / sq. m./hr. was 53.8 cal.; when the milk intake was doubled the heat production was 70.9; when it was increased three times it was 88.0. It appears unlikely that this increase was due to unabsorbed milk nutrients remaining in the rumen and abomasum of the calf and reaching the small intestine many hours after the last meal, for it would entail the assumption of a very high heat increment of feeding for what must be small quantities of milk. Activity of each calf was minimal so that increased muscular activity does not afford an explanation. Part of the

TABLE 47

Calcium and phosphorus metabolism

		Calf №					
		13	14	14	13	13	14
Milk ingested (l./ day)		2.6	3.4	4.2	5.0	5.8	6.6
Calcium metabolism per day	intake (g.)	3.87	5.17	6.26	7.60	8.70	9.90
	urine excretion (mg.)	23	37	50	38	40	110
	faeces excretion (g.)	1.03	0.96	0.47	0.63	2.16	0.71
	Balance (g.)	2.82	4.18	5.74	6.93	6.50	9.08
Phosphorus metabolism per day	intake (g.)	2.55	3.50	4.11	5.15	5.80	6.60
	Urine excretion (g.)	0.80	0.86	0.94	0.94	1.27	1.07
	Faeces excretion (g.)	0.24	0.26	0.19	0.32	0.75	0.24
	Balance (g.)	1.51	2.38	2.98	3.88	3.78	5.29

increase may be explained by an increase in muscular tone, or a higher rate of muscular growth at the higher nutritional levels. In any case, the results suggest that the heat production of the calf determined under conditions comparable with those employed in the determination of the basal metabolism of the human, is not stable but is sensitive to changes in nutritional plane. That the effect is due to nutritional plane and not to age is shown by the results obtained with Calf №14. He was 14 days older when given 3.4 litres of milk than when given 4.2 litres, and his heat production was higher in the earlier period when the nutritional plane was high. Such an instability of heat production in relation to nutritional plane has been observed in the rat by Hamilton (1937) and by Treichler & Mitchell (1941). It has also been observed in adult cows by Ritzman & Benedict (1938).

(e) Calcium and phosphorus metabolism

Table 49 summarises the calcium and phosphorus metabolism results. Statistically the regressions of calcium and phosphorus balance on milk intake were highly significant ($P = 0.01$). In neither case was the difference between calves significant. When Ca = the calcium balance and P = the phosphorus balance in g. per day the regression equations were:-

$$Ca = 1.41\ M - 0.60 \quad \ldots\ldots \quad (9)$$

$$P = 0.86\ M - 0.64 \quad \ldots\ldots \quad (10)$$

There was no indication, even at the highest level of milk intake, that the capacity of the calf to retain calcium or phosphorus had been reached.

Urinary calcium excretion was low throughout the experiment, amounting to only 5% of the faecal calcium excretion. This is higher than the maximum value of 2% found by Blackwood et al. (1936) and does not justify the omission of the urinary calcium determination in experiments with calves as these workers inferred.

As previously discussed, Calf №13 when given 5.8 litres of milk scoured badly. The mean daily

excretion of ash and also of calcium in the faeces was exceptionally high during this period. The percentage of calcium in the total ash when the animal scoured was 27.0 which did not differ significantly from the mean percentage of 28.6 found during normal periods. Similarly, the faecal excretion of phosphorus also increased when the animal scoured but the percentage of phosphorus in the ash was 9.3 compared with a general mean of 9.6. Thus, although the faecal excretion of ash increased three times during scouring the proportion of calcium and phosphorus in the ash was not affected. This large faecal loss of calcium and phosphorus during the alimentary disturbance necessarily resulted in a low balance. The excretion of phosphorus in the urine of Calf №13 increased during the scouring period despite the fact that there was a marked increase in the excretion of phosphorus in the faeces. The reason for this is not known; it was certainly not due to an excessive catabolism of body protein during this time. Excluding the results obtained in the scouring period, the increase in urinary phosphorus with increasing milk intake was statistically significant ($P = 0.01$).

4. Discussion

The effect of nutritional plane on the storage of nitrogen, calcium and phosphorus on growth and on heat production has already been discussed. The two calves, however, differed from one another in several ways. Calf №13 required more milk for maintenance of nitrogen equilibrium than Calf №14 and he also had a higher basal metabolism. These results are inter-related. A high basal metabolism entails a greater catabolism of ingested food or body tissue to meet this demand for energy, and the higher urinary nitrogen, largely made up of urea, ammonia and amino-N reflects the higher rate of deamination of ingested protein which occurred in order to furnish energy for this purpose. Armsby & Fries in 1911 compared the utilisation of feed energy by a purebred steer with that of a scrub steer and concluded that "the energy

requirement of the scrub steer for maintenance, computed to the same liveweight averaged 18.7% higher than that of the pure-bred. Accordingly the latter was able to use a relatively larger proportion of the total energy of his ration for the production of gain". The difference in heat production of the present calves was 13.2% and judging from the photographs of Armsby's animals the present calves did not differ so much in appearance as did his. Both the present calves were essentially dairy type animals of the same breed born on neighbouring farms within one day of one another and were thought to be reasonably comparable. A further indication of the inherent differences between animals was shown by Wood & Yule (1914) who found that the average liveweight increases of individual oxen on the same diets and under the same conditions could vary between 1 lb. and 3 lb. per day. Wood and Hill (1914) later showed that the difference in liveweight increase between such good "doers" and bad "doers" was inversely related to the skin temperature of the animals, and presumably therefore to their heat production. It is surprising that such large differences exist at an early age, and these differences must be of considerable practical importance.

From the results presented it is clear that bodyweight maintenance in the calves involved storage of minerals in bone and deposition of protein. From the equations presented (7, 9 & 10) it can be seen that 2.83 g. nitrogen, 3.13 g. calcium and 1.64 g. phosphorus were stored in this way. The amount of tissue stored in terms of bodyweight may be calculated roughly from the calcium, phosphorus and nitrogen balances. Since the calcium phosphorus ratio in bone is 2.15 (Shohl, 1939) the positive balance of 3.13 g. calcium should have been associated with storage of 1.46 g. phosphorus. If all the nitrogen stored represented a storage of tissue comparable in composition with muscle, then 2.83 g. nitrogen would have been associated with 88 g. muscle substance. This protein would have a ratio of nitrogen:/phosphorus of 14.7 (Shohl, 1939),

giving an estimate of phosphorus retention in muscle of 0.19 g. and an estimate of total phosphorus retention of 1.65 g. against the 1.64 g. actually determined. The total gain of bodyweight would thus be about 90 g., which is presumably balanced by a loss of body fat and possibly by slight dehydration of the tissues. This general picture of maintenance in the young growing animal is in complete accordance with Water's observations (1908, 1910) in which he found that the body form of cattle did not remain infantile when he kept them at constant bodyweight, but that there was an increase in their skeletal proportions and a loss of subcutaneous and depot fat. The results of the present experiment provide a quantitative estimate of these changes in terms of calcium, phosphorus and nitrogen storages.

In nitrogen metabolism experiments, differences between animals in their basal metabolism and associated phenomena must be taken into account when experiments are planned, and also in the interpretation of the results of these experiments. Thus, four animals were included in each of the following experiments and it was arranged that at least two animals should receive the same treatments.

E. PROTEIN FREE SUPPLEMENTS FOR THE CALF

1. Introduction

It was suggested, (page 62) that if a protein-free supplement was added to a basal diet, nitrogen retention would increase due to reduction of the "basal deamination component"

The following are substances which might be used as protein-free supplements:-

(a) Cellulose

The utilisation of cellulose by the calf essentially comprises bacterial breakdown and rumen development. These conditions were specifically excluded from this series of experiments, since rumen fermentation, besides breaking down cellulose, also involves at any rate, some destruction of dietary amino acids, with the synthesis of bacterial protein.

(b) Fat

The supplementation of skim milk with various fats was attempted by Lindsey as early as 1894. Gullickson, Fountaine & Fitch (1942) have reviewed the earlier literature and further work has been carried out by Jacobson & Cannon, (1947), Jacobson, Cannon & Thomas (1949) and Murley, Jacobson, Wise & Allen (1949). The general conclusions to be drawn are; that butterfat gives the best results, but, owing to its expense, it is obviously of little practical value. Less expensive animal fats have have satisfactory results, especially when homogenised into the diet (Wiese, Johnson & Mitchell 1947); but vegetable fats, which are frequently very unsaturated, have proved unsatis: factory since they tend towards unthriftiness, excessive scouring and high mortality. When these vegetable fats are hydrogenated, however, it is claimed that they compare favourably with butterfat when given to the calf, although Jarvis & Waugh (1949) could not confirm these observations and showed that animals given hydrogenated vegetable oils suffered from fatty livers.

In all these tests, however, the percentage of

fat in the diet was generally 3 and, as already mentioned calves 7 - 12 (Expt.C) had difficulty in assimilating diets containing 4.6% of fat. Aschaffenburg et al (1949) found that diets containing 2% of margarine gave good results. It therefore seemed unlikely that the fat percentage could be raised above this level of 4.6% as a supplement without causing severe distress to the animals.

(c) Simple sugars such as lactose and glucose

Rojas, Schweigert & Rupel (1948) showed that the calf is very sensitive to large quantities of lactose in its diet, since when the lactose intake was increased from 250 g. to 500 g. per day, scouring resulted within a few hours. It is well known that lactose in the diet of other animals causes an increase in gastro-intestinal motility (Fischer & Sutton, 1949). Nevertheless, Lindsey & Archibald (1925) claimed that lactose at a 30% level in the diet of calves was well utilised but was too expensive to be of practical importance.

Little attention has been given to the utilisation of glucose by the calf. In this laboratory, preliminary attempts to use glucose as a protein-free supplement for the calf did not meet with any great success. Two calves each received 250 g. glucose per day as a supplement to a semi-synthetic diet of a similar composition to diet №6 (Table 25). The animals were generally lethargic, and showed alimentary distress during the whole of the time that glucose was given; large quantities of faeces being produced. Simple addition of the results given in Table 27 shows that the calf normally excretes about 130 g. of wet faeces / day. When glucose was given the mean daily excretion of wet faeces for the two calves rose to 490 g. / day. It was decided not to continue these experiments on the utilisation of glucose by the calf. This intestinal motility effect of glucose in the calf no doubt explains the low digestibility of dietary constituents which was obtained when a nitrogen-free diet was given (page47).

(d) Starch

According to Savage & McCay (1942); "The digestion of starch by the calf has interested workers for nearly a century but little is known about it today". Some of the earlier attempts to supplement whole milk with starchy products were made by Liebig (Kellner,1915). Cooked wheat flour was fermented with ground malt and potassium bicarbonate, and the resulting mixture sieved before feeding. In this case, the supplement was, of course, a mixture of sugars and starch and, although few quantitative results appear to be available, there seems little doubt that this method of calf rearing was used successfully for many years. Most of the experimental work carried out appears to have been concerned with the use of starch as a substitute for the fat of milk, rather than as a supplement to whole milk. Uncooked starch has not given good results when fed with skim milk (Fingerling, 1908), or with reconstituted dried skim milk (Käppli, 1925). Much trouble was experienced throughout these latter experiments as the result of digestive disorders, and the same trouble was experienced when flake potatoes were given to the calf (Scholz, 1930). The results of Hittcher (1909), claiming to show that starch treated with malt before feeding gave better results, are of little value since out of 37 calves, 15 died - probably from vitamin A deficiency. Good results have, however, been obtained with skim milk and potato starch treated with malt diastase after the starch had been boiled (Hanne, 1907). An experiment carried out by Shaw, Woodward & Norton (1912) on two calves showed that uncooked starch when given as a supplement to whole milk was digested to the extent of only 21% when the calves were 4-7 days of age. At 30 days however, digestion was 96% although it is not known to what extent rumen fermentation had taken place in these older calves.

Due to this scarcity of information on the utilisation of starch by the calf, an experiment was carried out to determine accurately the utilisation of

gelatinised starch by the young calf, especially from the point of view of its protein-sparing action, or effect on nitrogen balance. It was required to know the extent to which fermentation of the starch took place. Subsidiary objects were to determine mineral balances during starch feeding, and also the effect of this nitrogen-free supplement on the distribution of nitrogen in the urine.

2. Plan of experiment to determine the nutritive value of gelatinised starch for the calf

Four calves, numbers 21, 22, 23 and 24 each received a basic diet of 3.0 litres of whole milk and 2 litres of water per day. In addition, the calves were given 0, 50, 100 or 200 g. starch according to the plan shown in Table 50.

TABLE 50

Plan of experiment

Weight of starch received by each calf (g. /day)

Calf №	Period 1	Period 2	Period 3	Period 4
21	0	50	100	200
22	100	200	0	50
23	200	0	50	100
24	50	100	200	0

The collection periods lasted 10 days and 6 days were allowed between each period for the change-over. The calves were 10 days old when the experiment began.

The weighed amount of starch was first gelatinised in 2 litres of boiling water contained in a two-gallon churn with constant stirring. Exactly 3.0 litres of whole milk was then added followed by 10 ml. of a supplementary mineral solution, the composition of which was the same as in the last experiment (page 69). Stirring was continued for about 5 min. For the evening feed, approximately

half the contents of the churn were given, and the remainder the following morning when the churn was well rinsed with 200-300 ml. water to ensure that the calf received the total amount of feed. Twice weekly, each calf received two halibut liver oil capsules (containing 4500 I.U. vitamin A and 450 I.U. vitamin D per capsule), and 50 mg. α-tocopheryl acetate.

A milk sample was taken every day before the diets were prepared for the determination of total nitrogen and total fat. Samples were also taken daily and pooled for subsequent mineral analysis. The following analyses were carried out:-

Milk

Total nitrogen, total fat (Gerber) were determined each day. Calcium, magnesium, phosphorus, sodium and chloride determinations were made on pooled aliquots for the period.

Urine

Total nitrogen (determined daily); creatine, creatinine (determined every two days); urea, ammonia, protein, amino-nitrogen, purines, uric acid and allantoin (determined once per 10 day period). Mineral determinations were carried out as for milk.

Faeces

Dry matter, total nitrogen, total 'fat' (determined every two days); 'fat' fractionation into soaps, free fatty acids and non-saponifiable residue (one per 10 day period), starch (determined once per 10 day period); mineral analyses as for milk.

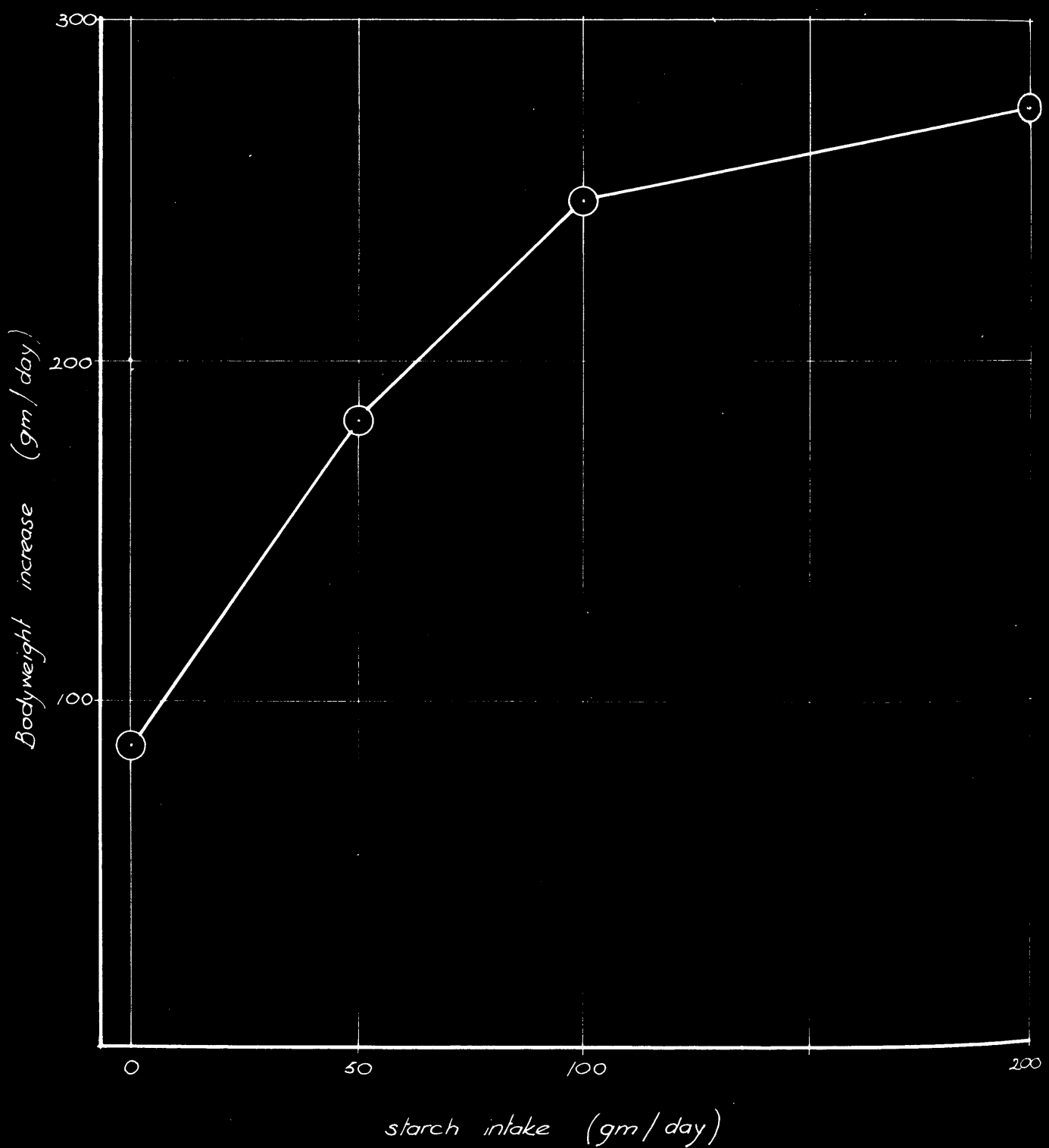
3. Results

(a) General observations

All the calves behaved normally throughout the experiment. No difficulty was experienced by the calves in drinking the milk containing gelatinised starch, although the viscosity of the 200 g. mixture was high. The only difference noted when the calves received starch was in the consistency and volume of the faeces they produced. The faeces of calves 22, 23 and particularly calf 24 became loose

figure 14.

The effect of various quantities of starch on the mean bodyweight increases of the calves.



on changing from the diet containing 200 g. starch to the diet containing no starch. Large quantities of watery faeces were produced by these three calves whereas calf 21 excreted quite normal faeces when receiving no starch. This may be explained by the difference in the viscosity between these two diets - presumably a viscous diet will tend to pass more slowly through the gut of the animal. Calf 21 had not previously been given a diet containing starch.

As the starch intake was increased, the faeces became more and more gelatinous, no doubt due to the excretion of undigested starch. The mean weights of faeces excreted when the calves received 0,50, 100 and 200 g. of starch per day were 823, 327, 336 and 809 g. respectively, which shows that when the calves were receiving 200 g. starch the amount of faeces produced was high and was of the same order as that excreted by the animals suffering from diarrhoea.

A preliminary attempt to feed 300 g. starch per day to a calf caused extreme distention of the gut and pain to the animal. About 3000 g. of highly gelatinous faeces were produced each day and it was concluded that the calf was unable to digest starch when given in such large quantities.

(b) Bodyweight gain

Table 51 summarises the bodyweight gains per day calculated by regression analysis of the individual weights, and the means of these values plotted against starch intake are presented in Figure 14.

TABLE 51

Bodyweight gains of the calves (g. / day)

Calf Nº	Weight of starch fed (g./day)			
	0	50	100	200
	Bodyweight gains (g./day)			
21	111	228	189	301
22	86	226	197	255
23	54	128	291	178
24	97	149	301	367
Mean	87	183	245	275

The variation between calves in their bodyweight gain

TABLE 52

Excretion of fat in g. / day, and the apparent digestibilities of milk fat and nitrogen by the calves when receiving various quantities of starch.

Amount of starch (g.)		0	50	100	200
Faecal fat excretion (g.)	Mean	2.35	2.56	4.09	2.63
Apparent digestibility of fat (%)	Calf №21	97.2	97.7	98.1	98.5
	Calf №22	98.5	98.6	90.3	97.3
	Calf №23	97.6	96.3	97.9	97.3
	Calf №24	98.7	96.9	98.4	98.3
	Mean	98.0	97.4	96.2	97.9
Apparent digestibility of nitrogen (%)	Calf №21	94.0	96.0	93.9	89.0
	Calf №22	91.8	95.1	77.4	83.9
	Calf №23	82.7	86.4	93.7	89.2
	Calf №24	89.1	91.5	90.5	90.3
	Mean	89.4	92.3	88.9	88.1

TABLE 53

Fractionation of faecal fat excreted by the calves into soaps, free fatty acids, non-saponifiable residue and neutral fat (% total fat).

Calf Nº	Starch fed (g./ day)	0	50	100	200
21	Soaps	56.2	36.4	31.8	51.2
	Free fatty acids	20.6	18.2	13.0	13.4
	Non-saponifiable res.	14.5	41.9	47.1	35.4
	Neutral fat	9.6	3.9	7.6	- -
22	Soaps	30.2	58.4	53.3	29.8
	Free fatty acids	25.2	18.2	35.5	21.9
	Non-saponifiable res.	44.5	23.4	11.1	36.5
	Neutral fat	- -	- -	- -	11.8
23	Soaps	27.2	24.4	18.4	56.0
	Free fatty acids	30.4	21.4	44.8	21.1
	Non-saponifiable res.	43.0	53.8	27.6	22.6
	Neutral fat	- -	- -	9.2	- -
24	Soaps	16.4	31.7	36.8	18.9
	Free fatty acids	32.9	52.6	13.5	31.4
	Non-saponifiable res.	50.7	6.9	39.9	49.7
	Neutral fat	- -	8.7	9.8	- -

on the same quantities of starch was large, but these differences were not statistically significant. The relationship between starch intake and bodyweight gain was significant (P = 0.05) although it will be seen that the utilisation of starch in terms of gain in bodyweight decreased when 200 g. of starch was given.

(c) The apparent digestibility of the diets

i. Apparent digestibility of fat

Table 52 shows the mean excretion of faecal fat together with the fat digestibility, from which it will be seen that starch intake had little effect on the digestion of fat. The results of Shaw, Woodward & Norton (1912) and of Fingerling (1908) suggest that the excretion of faecal fat was comparitively high when ungelatinised starch was given to the calf. The mean excretions of fat in the faeces per day were 4.5 g. and 3.9 g. respectively, compared with the mean excretion of 3.0 g. obtained in the present experiment. Of this quantity, however, 36% of the fat was present as soaps (Table 53) and since neither Shaw et al (1912) nor Fingerling (1908) determined fat present as soaps in the faeces it is probable that the figures cited above are about 36% too low. This suggests that gelatinised starch has not the same effect as raw starch on the excretion of fat in the faeces.

Since a latin square layout was used, the experiment permits a test of differences between the individual calves in their digestion of fat, as well as differences between periods. There was a tendency for the excretion of faecal fat to fall as the calves became older. These differences were not, however, significant at P = 0.05.

Soaps, free fatty acids, non-saponifiable residue and neutral fat expressed as a percentage of the mean total fat excretion for the period are shown in Table 53. As the calves became older they excreted a significantly larger proportion of non-saponifiable residue in the faeces. This may be explained by an increased liver turnover of bile

compounds, which would presumably be associated with increased absorption of dietary fat. There were no significant differences in the total excretion of soaps, free fatty acids and neutral fat. This confirms work with human adults (Hill & Bloor, 1922; Annegers, Boutwell & Ivy, 1948) and children (Williams, Shepherd & Endicott, 1939), showing that the type and quantity of food ingested does not significantly affect the composition of faecal fat. This indicates that "faecal fat" may be derived primarily from residues of secretions into the intestine and the lipids of faecal bacteria.

ii. Apparent digestibility of nitrogen

Table 52 shows that the digestibility of dietary nitrogen did not change when starch was given to the calves. The mean apparent digestibility of 89.6 was rather lower than that previously obtained for whole milk (Table 44). It would appear then that the apparent digestibility of nitrogen when the calves were receiving starch and producing firm faeces was not significantly different from the digestibility when scouring occurred. This, in effect, means that the feeding of starch results in a slight decline in the digestibility of the total nitrogen of the diet, since scouring has previously been noted to increase the nitrogen excreted in the faeces.

iii. Apparent digestibility of starch

When a water extract of faeces from those calves receiving starch was tested with iodine, a red colour was produced, indicating that dextrins or other starch breakdown products were present. There appeared to be little true starch in the faeces, except with animals receiving 200 g. starch / day, when a blue colour with iodine was obtained. During the first period, starch was determined by the A.O.A.C. method (1945) which was found to be unsuitable, not only because alcoholic potash did not dissolve the faecal material completely, but mainly because the starch, which had become dextrinised on passage through

TABLE 54

The excretion and apparent digestibility of starch when fed to calves at four different levels.

Amount of starch fed (g./day)	0	50	100	200
Starch in faeces directly determined (g.)	0	2.83	1.87	20.50
Starch in faeces calculated from faeces analysis i.e. 'residual' (g.)	2.12	2.52	2.99	37.28
Apparent digestibility of starch calculated from 'residual' figures (%)		96.1	97.6	81.6

the tract was not measured. A colorimetric method (Nielsen, 1943, 1945) was later used since some of the starch breakdown products are measured in this determination as well as true starch. This method gave a negative result with faeces from calves which were not receiving starch. The mean faecal excretion of starch per day obtained by this method is shown in Table 54. It was thought, however, that a truer estimation of starch and starch breakdown products in the faeces could be made from the residual component of the faeces. This was obtained by analysing the faeces for fat, ash and crude protein (nitrogen x 6.25) and substracting the total from 100. The difference is presumed to be carbohydrate material, which, besides starch and its breakdown products, could have included unabsorbed lactose or bacterial polysaccharides. The mean residual figures are also shown in Table 54 from which it will be seen that their magnitude is of the same order as the values obtained by direct determination of starch. The agreement, however, was not good. Except for the 50 g. level of starch feeding, the residual figures were higher than those obtained by direct analysis. The difference, especially at the highest starch level being due to the lower starch breakdown products which do not give a colour with iodine and therefore are not measured by Nielsen's method. With sheep, Jucker (1948) obtained a higher digestibility coefficient when starch was determined directly on faeces than when the residual component was used for the calculation. The comparatively high residual figures obtained on the faeces of calves receiving no starch was found only when scouring occurred and were probably due to unabsorbed lactose. The residual figure of 0.56 g./ day obtained for Calf 21 when receiving whole milk alone may have been due to bacterial polysaccharide.

Analysis of variance of the residual fraction of the faeces showed that it was significantly affected by the intake of starch, but there was no decrease in

TABLE 55

Nitrogen balance results for calves receiving different amounts of starch (g. / day).

Calf №	Amount of starch fed (g. / day)		0	50	100	200
21	Excretion	Urine	9.50	8.60	7.31	6.10
		Faeces	1.00	0.66	0.99	1.76
	Nitrogen balance		6.10	7.16	7.79	8.20
22	Excretion	Urine	8.18	7.90	6.00	4.99
		Faeces	1.32	0.79	3.74	2.68
	Nitrogen balance		6.59	7.36	6.85	8.76
23	Excretion	Urine	7.55	8.41	6.99	5.79
		Faeces	2.84	2.19	1.01	1.79
	Nitrogen balance		6.03	5.48	8.05	9.02
24	Excretion	Urine	7.50	8.99	6.09	5.39
		Faeces	1.75	1.44	1.56	1.57
	Nitrogen balance		6.81	6.56	8.71	9.13
Mean	Excretion	Urine	8.18	8.48	6.60	5.57
		Faeces	1.73	1.27	1.83	1.95
	Nitrogen balance		6.38	6.64	7.85	8.78

figure 15

The effect of various quantities of starch on the mean nitrogen balances of the calves.

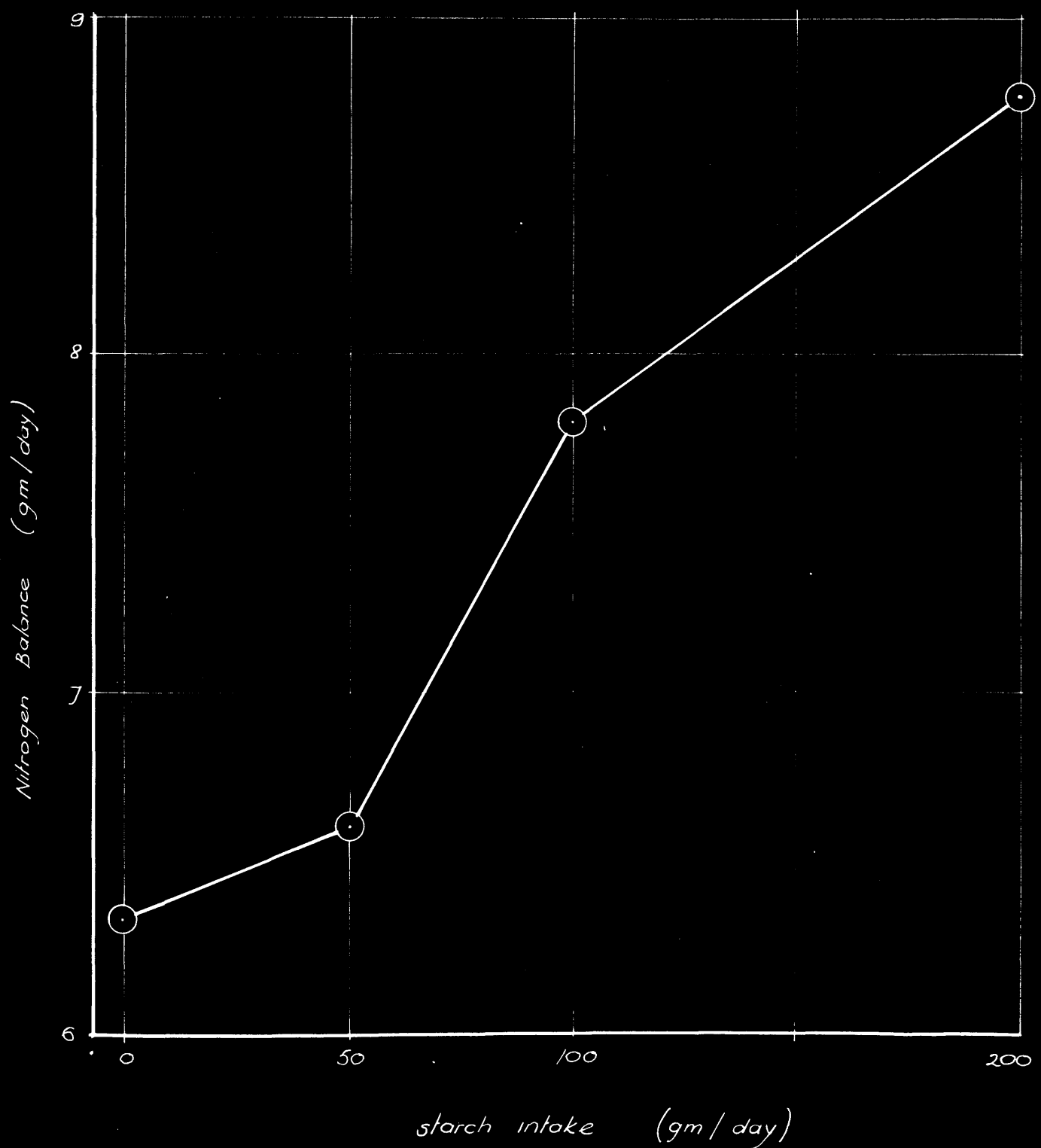
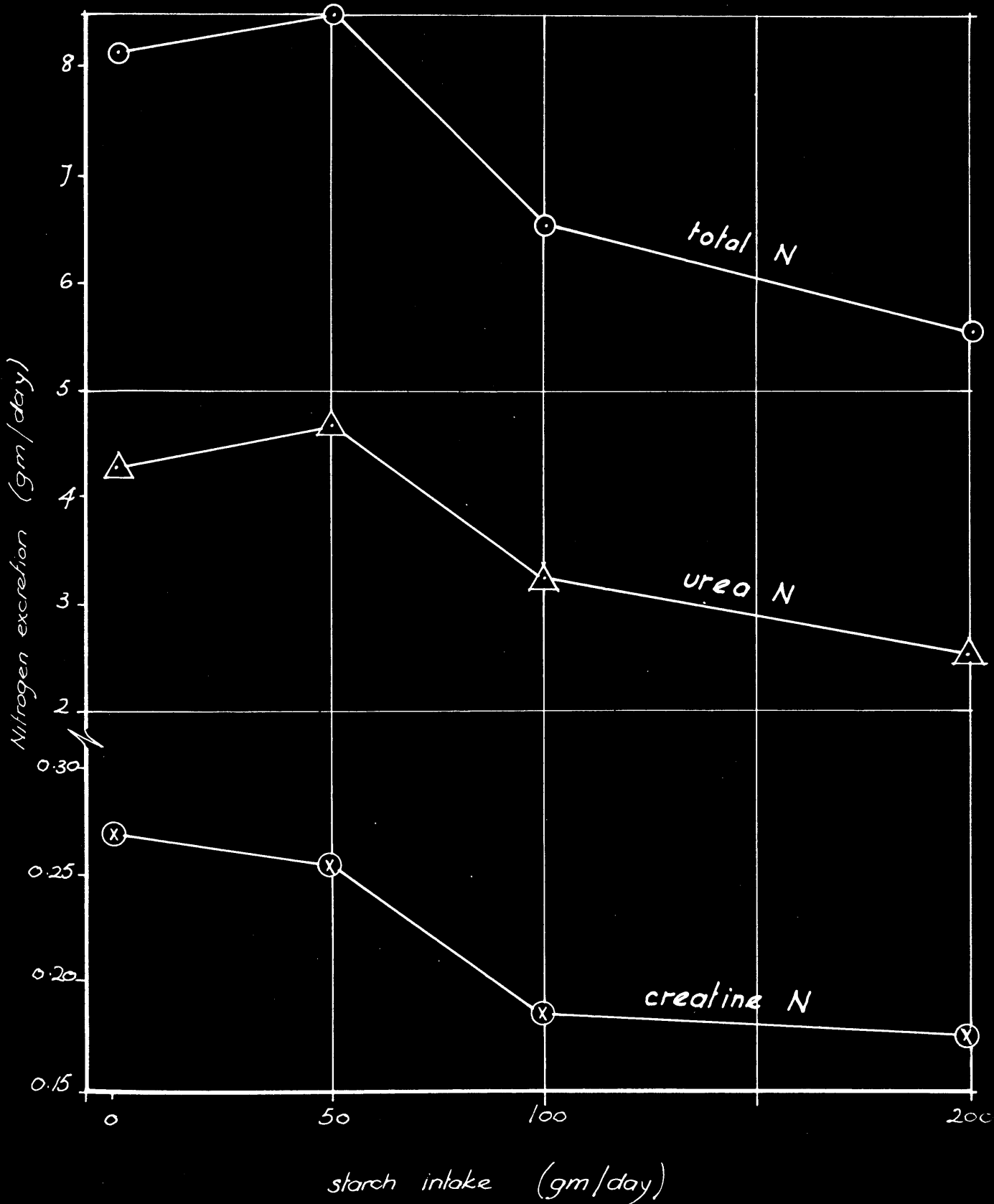


figure 16

The effect of starch on the excretion of total N, urea N. and creatine N, in the urine of the calves.



the residual component with the age of the calves. This result differs from that obtained by Shaw, Woodward & Norton (1912) using uncooked starch. These workers showed that the amount of starch in the faeces decreased from 34 g. per day at 12 days of age to 0.7 g. at 39 days of age. Evidently the calf is able to utilise gelatinised starch much more easily than raw starch when about 12 days old. The mean apparent digestibilities of starch at the three levels of intake are shown in Table 54 and have been calculated using the faecal residual as a measure of excretion, assuming that the excretion of 0.56 g. of the faecal residual component remained constant throughout the experiment. The errors in this assumption are, in any case less than 1%.

(d) Nitrogen balance

Daily nitrogen balances together with urinary and faecal nitrogen excretions are detailed in Table 55. As originally surmised, there was a significant increase in nitrogen balance with increasing starch intake ($P = 0.05$). The mean values are shown plotted in Figure 15. It will be seen from Figure 16 that there was a highly significant decrease in total urinary nitrogen excretion ($P = 0.001$). The faecal nitrogen excretion remained constant throughout. Thus, the increase in nitrogen balance was due entirely to the reduction which occurred in the urinary nitrogen excretion; in other words starch had a protein-sparing effect. This is in agreement with the contention given on page 79).

It will also be seen from Figure 16 that the decrease in urinary nitrogen was due almost entirely to a reduction in the excretion of urea. There was also a significant decrease in the creatine excretion ($P = 0.01$). These changes will be discussed in more detail later (page 111). The excretion of other nitrogenous constituents remained comparatively constant, although there were significant differences between calves in their excretion of purine nitrogen, uric acid nitrogen, creatine nitrogen and creatinine

TABLE 57

Respiratory exchange data of the calves receiving different amounts of starch (arranged according to the experimental period)

Variable	Calf №21				Calf №22				Calf №23				Calf №24				Mean (all calves)			
	Period №				Period №				Period №				Period №				Period №			
	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
Starch (g./day)	0	50	100	200	100	200	0	50	200	0	50	100	50	100	200	0				
Pulse rate/min.	80	84	78	72	102	107	96	79	102	93	88	89	102	96	87	80	97	95	87	80
Respiration rate /min.	19.3	16.8	13.8	14.0	12.1	12.0	13.2	8.9	19.5	13.3	12.3	11.1	11.6	12.1	11.2	9.4	15.6	13.6	12.6	10.9
Minute volume (l.)	3.16	4.03	4.13	5.58	3.56	3.94	4.66	4.14	5.97	3.92	3.91	4.06	3.01	3.22	4.14	3.51	3.93	3.78	4.21	4.32
Tidal air (ml.)	164	240	299	399	294	328	353	465	306	295	318	365	260	276	369	374	256	282	335	401
Oxygen consumption (l./hr.)	9.83	12.90	14.06	15.84	12.92	15.09	19.86	16.32	15.83	13.76	14.18	11.57	11.19	11.04	15.15	13.42	12.44	13.20	15.81	14.29
R.Q.	0.817	0.769	0.775	0.812	0.778	0.793	0.747	0.779	0.807	0.878	0.841	0.920	0.783	0.891	0.747	0.763	0.796	0.833	0.778	0.819
Heat production (cal./hr.)	47.42	61.46	67.15	76.22	61.69	57.29	94.10	77.94	76.21	67.41	68.75	57.25	53.45	54.23	71.79	63.77	59.69	60.10	75.45	68.80
Heat production (cal./kg./ 24 hr.)	28.07	34.54	35.11	37.37	41.58	35.90	58.44	44.64	47.33	41.54	41.25	31.02	38.29	34.07	42.43	35.93	38.82	36.51	44.31	37.24

TABLE 56

Daily excretion of nitrogen in different nitrogenous metabolites in the urine of calves receiving various quantities of starch (g. / day).

Metabolite	Calf №21 Starch received (g./day)				Calf №22 Starch received (g./day)				Calf №23 Starch (g./day)		received		Calf №24 Starch received (g. / day)				Mean Starch received (g. / day)			
	0	50	100	200	0	50	100	200	0	50	100	200	0	50	100	200	0	50	100	200
Urea	5.434	4.948	4.128	2.860	4.367	4.636	2.297	1.849	4.523	4.137	2.892	3.373	2.749	4.893	3.763	2.162	4.268	4.654	3.270	2.561
Ammonia	0.171	0.220	0.227	0.229	0.566	0.463	0.121	0.377	0.352	0.420	0.234	0.432	0.294	0.874	0.213	0.285	0.346	0.494	0.199	0.331
Protein	0.038	0.088	0.145	0.063	0.088	0.214	0.031	0.151	0.123	0.103	0.099	0.089	0.095	0.093	0.128	0.077	0.086	0.124	0.101	0.095
Amino-acids	0.143	0.077	0.055	0.172	0.032	0.141	0.163	0.107	0.144	0.141	0.222	0.196	0.161	0.603	0.102	0.061	0.120	0.241	0.136	0.134
Purines	0.067	0.096	0.131	0.078	0.101	0.095	0.075	0.091	0.088	0.092	0.115	0.121	0.078	0.148	0.073	0.098	0.084	0.108	0.099	0.097
Uric acid	0.034	0.024	0.029	0.047	0.043	0.038	0.036	0.037	0.058	0.063	0.101	0.089	0.033	0.021	0.021	0.044	0.042	0.037	0.047	0.054
Allantoin	0.399	0.479	0.421	0.465	0.473	0.441	0.417	0.533	0.451	0.446	0.480	0.477	0.314	0.352	0.354	0.446	0.409	0.429	0.418	0.480
Creatinine	0.472	0.327	0.430	0.417	0.294	0.292	0.310	0.250	0.367	0.448	0.475	0.461	0.320	0.313	0.246	0.323	0.363	0.345	0.365	0.363
Creatine	0.182	0.151	0.138	0.147	0.381	0.311	0.240	0.205	0.218	0.266	0.201	0.160	0.293	0.290	0.169	0.205	0.269	0.255	0.187	0.179

nitrogen. Tests on the protein fraction showed it to consist entirely of skin debris and hair washed from the bottom of the crate by urine. The nitrogen partition in the urine is given in detail in Table 56.

(e) Respiratory exchange data

It will be seen from Table 57 which gives details of the respiratory exchange data, that the basal metabolic rate of the calves tended to increase with the increasing starch intake. These differences were not, however, statistically significant. Some general changes in metabolism with the age of the calf were found. Pulse rate per minute significantly decreased as the animals became older. This was not due entirely to a general decrease in metabolism of the calves, since the basal metabolism of these animals did not change significantly with the age of the calves. There was also a decrease in respiratory rate with a concommitant increase in tidal air - these two variables being related linearly. An increase in tidal air with the age of the animals would be expected, since the lung size and capacity increases with the increase in body growth. In order to maintain a similar pulmonary ventilation, the respiratory rate is proportionately decreased.

The results for methane and hydrogen which are given in Table 58 showed that there was little, if any fermentation resulting in these end products, taking place on the starch diets.

Table 58

Production of methane and hydrogen by the calves receiving various quantities of starch (ml./hr.)

	Starch (g./day)				
	0	50	100	200	Production by the sheep Brody (1945)
Methane	45.8	28.8	10.2	23.1	1200.0
Hydrogen	7.8	4.9	2.1	0.0	--

TABLE 59

Daily mineral balances of the calves receiving various quantities of starch. (g./day)

Calf №		Starch received (g./day)			
		0	50	100	200
21	Calcium	3.328	3.187	3.246	3.314
	Phosphorus	1.825	1.786	1.976	1.872
	Magnesium	0.300	0.280	0.233	0.292
	Sodium	0.512	0.540	0.627	0.710
	Chloride	1.654	1.614	0.905	1.106
22	Calcium	3.126	3.199	3.021	3.260
	Phosphorus	1.573	1.940	1.749	1.799
	Magnesium	0.316	0.320	0.322	0.309
	Sodium	0.505	0.760	0.647	0.491
	Chloride	0.910	1.008	1.968	1.735
23	Calcium	3.017	3.173	3.270	3.425
	Phosphorus	1.589	1.738	1.951	2.127
	Magnesium	0.273	0.282	0.334	0.306
	Sodium	0.457	0.982	0.154	0.637
	Chloride	1.333	1.329	1.111	1.753
24	Calcium	3.172	3.160	3.308	3.241
	Phosphorus	1.827	1.541	2.016	2.045
	Magnesium	0.297	0.243	0.295	0.280
	Sodium	0.787	1.167	0.676	0.748
	Chloride	1.267	0.747	1.659	1.073
Mean	Calcium	3.161	3.180	3.211	3.310
	Phosphorus	1.653	1.751	1.923	1.961
	Magnesium	0.296	0.281	0.298	0.297
	Sodium	0.565	0.862	0.526	0.646
	Chloride	1.289	1.174	1.411	1.417

TABLE 60

Excretion of total ash, and individual minerals in the faeces of the calves receiving various quantities of starch (mg./day)

Calf Nº		Starch intake (g./day)			
		0	50	100	200
21	Total ash	2110	2600	2200	1770
	Calcium	243	314	208	185
	Phosphorus	431	360	181	199
	Magnesium	45	68	80	75
	Sodium	45	18	17	48
	Chloride	67	48	57	42
22	Total ash	2350	2170	4460	3030
	Calcium	311	290	565	243
	Phosphorus	106	110	336	217
	Magnesium	45	62	82	81
	Sodium	211	15	129	205
	Chloride	99	24	43	9
23	Total ash	5750	3330	1460	2760
	Calcium	419	247	182	169
	Phosphorus	304	113	83	159
	Magnesium	175	84	55	51
	Sodium	1168	177	24	246
	Chloride	993	130	32	80
24	Total ash	3860	2440	1690	2380
	Calcium	321	346	198	235
	Phosphorus	102	196	83	128
	Magnesium	42	46	66	71
	Sodium	540	29	22	71
	Chloride	617	51	49	54
Mean	Total ash	3520	2640	2450	2490
	Calcium	323	299	288	208
	Phosphorus	236	195	171	176
	Magnesium	48	65	71	70
	Sodium	491	60	48	142
	Chloride	463	63	45	46

TABLE 61

Excretion of minerals in the urine of calves (mg./day) receiving various quantities of starch

Calf Nº		Starch received (g. /day)			
		0	50	100	200
21	Calcium	27	11	27	13
	Phosphorus	969	929	1098	1124
	Magnesium	118	77	106	57
	Sodium	1490	1273	1227	1071
	Chloride	3060	2762	3034	2562
22	Calcium	44	22	11	9
	Phosphorus	1576	1145	1140	1059
	Magnesium	57	42	49	35
	Sodium	1155	1054	1271	1135
	Chloride	2911	2678	2770	2680
23	Calcium	77	61	59	4
	Phosphorus	1382	1404	1161	940
	Magnesium	93	52	35	105
	Sodium	206	712	1651	1164
	Chloride	2098	2537	2567	2948
24	Calcium	18	92	7	5
	Phosphorus	1266	1488	976	1082
	Magnesium	85	173	65	68
	Sodium	501	851	1133	1052
	Chloride	1826	3983	2716	2869
Mean	Calcium	42	47	26	8
	Phosphorus	1298	1242	1094	1051
	Magnesium	88	86	64	66
	Sodium	838	973	1321	1106
	Chloride	2474	2990	2772	2765

In fact the highest values for both methane and hydrogen were obtained when no starch was given, suggesting that the presence of starch in the rumen produced such a condition that bacteria which normally attack lactose were unable to thrive. This result will be discussed in more detail later.

(f) Mineral metabolism

Since the feeding of starch was accompanied by an increase in bodyweight, which is essentially bone and muscle growth in the young animal, an increase in the storage of calcium, phosphorus, sodium, and chloride might have been expected. Although there was a tendency for an increase in each case, the differences were not significant. Table 59 shows the results for each calf, and also the means for all the calves.

Since there was no significant difference in the mineral balances due to the feeding of starch, it was not expected that there would be any differences in the route of excretion of the minerals. This was in fact, the case. There was a tendency for the calcium, phosphorus and magnesium to decrease in the urine and also in the faeces when starch was given, but these differences were not significant. The complete data for faecal ash, and the fractionation of this into the constituent minerals is shown in Table 60, and the urinary excretion of minerals in Table 61.

It may be, that the increases in growth which were found when starch was given, were not large enough to cause any significant changes in the balance of minerals. On the other hand, from Tables 55 and 59, it may be calculated that the ratio of nitrogen to calcium stored by the calves was 2.02, 2.09, 2.44, 2.65 when 0, 50, 100 and 200 g. of starch were given. Evidently the increase in nitrogen storage was not associated with a corresponding increase in calcium balance. It is therefore probable that the availability of this mineral limits its storage. The reduction in the excretion of calcium in the urine and faeces of the calves receiving starch may have been an attempt to improve the utilisation of the amount ingested.

This was not sufficient, however, to meet the extra demands corresponding to the increase in nitrogen storage. When the calves were given no starch, 3.16 g. calcium and 6.38 g. nitrogen were stored. When 200 g. of starch were given nitrogen retention increased to 8.78 g. per day. If, as seems logical in a rapidly growing animal, the ratio of calcium retention to nitrogen retention remains constant, a calcium retention of 4.35 g. would be expected. The diet at this time, however, contained only 3.53 g. suggesting that a dietary shortage of calcium can occur when starch is added to whole milk. A similar suggestion may be made regarding the storage of phosphorus, since the ratio of nitrogen to phosphorus stored was 3.86, 3.79, 4.08 and 4.48 for 0,50,100 and 200 g. of starch ingested. Alternatively, it is more likely that the phosphorus storage is limited by the calcium storage. Since the diet contained 3.19 g. phosphorus and the calcium / phosphorus ratio in bone is 2.15 (Shohl,1939) it is seen that the diet contained an excess of phosphorus over calcium for the growth of bone, indicating that calcium is the limiting factor in the utilisation of the constituents of whole milk when a nitrogen free supplement is used. It is, however, an important observation that the feeding of starch does not cause any abnormal excretion of minerals in the faeces.

(g) Post-mortem examination

The calves were slaughtered 3 hr. after feeding and immediately dissected. The digestive tract was then ligatured between the abomasum and small intestine, and between the small intestine and caecum. The contents of each section were emptied and weighed, aliquots being taken for the determination of dry matter percentage and total steam volatile fatty acids.

On dissection, milk was found in the rumen of only one of the calves, Calf №22. The abomasal contents of the calves receiving 0, 50 and 100 g. starch consisted mainly of large milk clots, whereas the rumen contents of Calf №21 which was receiving 200 g. of

TABLE 62

The weight of the intestinal contents of the calves, the quantity of steam volatile fatty acids found in the tracts and a comparison of the steam volatile fatty acids of the calf with those of other animals for various sections of the digestive tract.

	Section of digestive tract	Starch fed (g./ day)			
		0	50	100	200
Intestinal contents (g.wet)	Rumen	350	710	350	100
	Abomasum	460	263	370	400
	Small intestine	333	750	480	650
	Caecum	80	110	180	120
	Total	1223	1833	1830	1270
Total steam volatile fatty acids (ml. N/10 acid)	Rumen	49.8	436.9	45.5	18.7
	Abomasum	25.9	63.5	118.1	45.2
	Small intestine	43.7	151.1	70.7	68.3
	Caecum	48.0	160.5	30.3	73.8
	Total	167.4	812.0	264.6	206.0
Total steam-volatile fatty acids (g.acetic acid/100 g. D.M.)	Rumen	0.60	5.90	0.67	0.64
	Abomasum	0.31	0.13	1.34	0.73
	Small intestine	0.87	1.64	0.97	0.64
	Caecum	2.84	5.58	1.12	3.89

	Sheep *	Ox *	Pig *	Rat *	Calf
	Acetic acid (g./100 g. dry matter in the tract)				
Rumen	7.24	4.82	---	--	1.95
Abomasum	0.37	1.08	1.51	0.23	0.63
Small intestine	0.47	1.07	0.47	0.43	1.03
Caecum	3.51	3.17	3.91	4.64	3.36

* Results of Elsden, Hitchcock, Marshall & Phillipson (1946)

starch were more liquid, with relatively few small clots. No particular differences were noted between the animals in the contents of their small intestines or caeca.

The weight of the digesta in each section of the intestinal tract and the quantity of steam volatile acids present (expressed as ml.N/10 acid and as g. acetic acid per 100g. dry matter) are shown in Table 62. The intestinal contents and also the concentration of volatile acid were greatest in the calf receiving 50 g. of starch per day. It will be noticed, however, that there was no large increase in the volatile acid content of the digesta of those animals receiving the highest quantities of starch when compared with the digesta of the calf which received no starch. The amount of volatile acid present in the digesta depends, of course, upon the rate at which it is produced and the rate at which it disappears. Therefore it is not an infallible measure of the amount of fermentation which takes place, though it does give some indication when comparing animals of the same species. Taking into account the negligible production of methane and hydrogen which took place when starch was given (page 89), the above results lend weight to the suggestion that little bacterial breakdown of starch took place with these calves.

A comparison is also made in Table 62 between the mean volatile acid content of the digesta of the calves in the present experiment, with that of other ruminant and non-ruminant animals. These mean data were calculated from the results of work carried out by Elsden, Hitchcock, Marshall & Phillipson (1946). The variation found in the volatile acid content of the digesta of the calves whether or not they received starch, was no greater than the variation found in the digesta of animals of the same species. It will be seen that the highest content of volatile acid was found in the caecum of the calf, as is the case with other non-ruminating animals. Baker, Nasr, Morrice & Bruce (1950) recently determined the sites and agents

of breakdown of various starches in the gut of different animal species by microscopic methods. They found that in non-ruminants, raw tuber starches can escape the action of digestive juices and are attacked by bacteria in the caecum. Microbial breakdown of starch is usually incomplete, however, and even with ruminating animals starch appears in the faeces. Boiled starch is almost completely digested before reaching the caecum of the rat. From these results it might be suggested therefore, that the young calf is unable to completely digest cooked starch by means of intestinal amalyses, and that the starch which remains undigested in the small intestine is subsequently attacked by micro-organisms in the caecum or large intestine.

4. Discussion

(a) The availability of the metabolisable energy of starch

Assuming that the residual component of the faeces gives a reliable estimation of the starch excreted by the calf, the starch absorbed, or the metabolisable starch, may be calculated. From the regression equation relating digested starch to nitrogen balance, it was found that one calorie of digested starch resulted in an increased nitrogen balance of 3.88 mg.. On the other hand, from the data given on pages 116-7, it will be seen that with animals receiving mixed diets of fat, protein and carbohydrate, one calorie of diet resulted in a bodyweight gain of 0.326 g. A nitrogen balance of 2.62 g. was associated with a bodyweight gain of 100 g. and thus for one calorie of a mixed diet the nitrogen storage was 8.54 mg.. This is considerably greater than the value of 3.88 mg./ calorie obtained with starch. In so far that the nitrogen excretion in the urine of those calves which received starch did not reach the endogenous level, this result suggests that the net availability of the metabolisable energy of starch for the calf was low. A more quantitative estimate cannot be made, however, from the present data. The low availability of starch may be partly due to its high specific dynamic

action during metabolism, but the discrepancy is too large to be due entirely to this factor. It is possible that the calories of starch are only partly utilised to spare protein, the balance being stored as fat. A true interpretation of the results may not be made without the determination of the energy balances of animals given comparable levels of starch.

(b) Gelatinised starch as a food for the calf

No signs of discomfort or distress were experienced by the calves when they received 50 - 200 g. starch per day; in fact scouring only occurred when whole milk was given. It is possible that starch had a tendency to slow down the passage of food through the digestive tract - a very beneficial property where the calf is concerned, since this animal is easily susceptible to changes of feeding routine which result in diarrhoea.

No abnormal digestion of milk fat was found, nor were mineral constituents excreted in abnormal amounts. There may have been a small reduction in the digestibility of the nitrogen, but the general conclusion to be drawn is, that starch had no ill-effects upon the metabolism of the calf, unless such large quantities as 300 g. per day were given.

Limitations to the feeding of gelatinised starch to the calf are therefore as follows. Firstly, the apparent digestibility of starch at high levels of intake is low. Thus the apparent digestibility fell to 82% when 200 g. of starch per day were given. Secondly, the net availability of the metabolisable energy is possibly low. These two factors suggest that it is doubtful whether the practice of starch feeding would ever become economically sound. Thirdly, it is possible that the primary limiting factor with any nitrogen-free supplement for whole milk given to the calf is, that insufficient calcium is supplied. This could presumably be overcome by adding the necessary supplements.

From the results of this and the previous experiment it might appear that considerable quantities of protein are wasted when calves are given whole milk

at recognised levels of feeding. As was inferred when the present experiment was planned (see page 79) limitations of energy supply were thought of first importance and that, as a diet for the calf, whole milk was regarded as containing an excess of nitrogen relative to total energy and as is now presumed, to total calcium. This suggests that one advantage of the high protein content of whole milk may be that it helps to maintain high levels of calcium. This is probably not the case, since only 20% of the calcium of whole milk is associated with the casein fraction, the remainder being colloidal and inorganic calcium phosphate (Ling, 1944). At high intakes of nitrogen, however, even this small percentage may be of considerable importance.

Why Nature has apparently provided the young calf with an unbalanced diet is of great interest. It may be that excess protein would be of value in increasing the heat production in an animal born with little, if any, heat insulating reserves of body-fat. On the other hand, the imbalance of the protein and energy of whole milk may be apparent rather than real. At the levels of intake employed in farm practice, much of the protein is catabolised to meet the intense basal metabolism of the young calf. When the intake is raised, however, less and less energy is required for this purpose and whole milk becomes a more balanced food.

F. THE BIOLOGICAL VALUE OF CASEIN AND GELATIN WHEN GIVEN AS THE SOLE SOURCE OF PROTEIN

1. Introduction

The previous experiments have been concerned with the various factors which affect the storages and losses of body nitrogen in calves. These experiments showed the effects of (a) the level of intake of diets of constant composition; (b) diets containing different percentages of protein; (c) diets containing no protein whatever; (d) nitrogen-free supplements, and (e) starvation. Simultaneously, information was obtained on the effect of digestive disturbances (see pages 26,60). Many of these factors must be taken into consideration when differences in nitrogen balance occur during experiments in which proteins of different amino-acid composition are components of the diet.

There seems little doubt that the adult ruminant is to a large extent independent of a dietary source of essential amino-acids to meet the demands of bodyweight maintenance, growth of tissues, wool and hair, and possibly milk secretion. Both Zuntz (1891) and Hageman (1891) originally propounded the theory that the micro-flora of the rumen is capable of converting non-protein nitrogenous substances into proteins which are digested and absorbed in the lower part of the tract. There was much controversy on this problem during the early part of the twentieth century. Mitchell & Hamilton (1929) concluded from experiments carried out during the first world war that the practical value of protein synthesis in the rumen was doubtful and that much further work was required. Krebs (1937), after writing an extensive review admitted that urea in the food of the ruminant appeared to have some kind of 'protein-sparing effect'. He was reluctant to believe that compounds such as urea and glycine were converted to protein in amounts which were important to the animal. McNaught & Smith (1947) have reviewed the more recent literature on this subject. Synthesis of protein from non-protein nitrogen was shown in metabolism trials with ruminants by Harris &

TABLE 63

"Essential" amino-acid composition of gelatin and casein and the percentage deviation from the corresponding values for whole egg proteins.

Amino-acid	Amino-acid content (%)			Deviation from the corresponding values for whole egg proteins	
	Gelatin	Casein	Whole egg	Gelatin	Casein
Arginine	8.7	4.2	6.4	+ 36	- 34
Histidine	0.9	3.0	2.1	- 57	+ 43
Lysine	5.8	7.9	7.2	- 19	+ 10
Tyrosine	0.7	6.9	4.5	- 84	+ 53
Tryptophan	0.0	1.2	1.5	-100*	- 20
Phenylalanine	2.1	5.6	6.3	- 66	- 11
Cystine	0.1	0.3	2.4	- 96	- 87*
Methionine	0.8	3.5	4.1	- 78	- 15
Cystine + Methionine	0.9	3.8	6.5	- 86	- 42
Threonine	2.0	4.1	4.9	- 59	- 16
Leucine	3.1	9.9	9.2	- 66	+ 8
Isoleucine	1.7	6.5	8.0	- 79	- 19
Valine	2.8	6.7	7.3	- 62	- 8
Glycine	24.0	2.1	2.2	+ 83	0

* First limiting amino-acid

Mitchell (1941 a,b) and Work, Hamre, Henke & Harris (1943). In vitro experiments (Pearson & Smith, 1943 a, b, c) have confirmed these results. Nitrogen balance experiments with sheep indicate that with rations containing a wide variety of proteins the biological value of the dietary nitrogen remains constant (Miller & Morrison, 1942; Johnson, Hamilton, Mitchell & Robinson, 1942). Thus the original hypothesis has been shown to be, in the main part, true. While true of the adult ruminant, this partial independence of a dietary source of amino-acids is unlikely to hold for the calf shortly after birth before the development of its rumen takes place.

An experiment was therefore carried out to test the validity of the hypothesis that the ruminant animal before the development of its rumen occurs is as dependent on its diet for a supply of essential amino-acids as is man, the rat or the dog. The proteins, gelatin and casein were used as the sole source of protein and the results compared with the proteins of dried skim milk for which much data had already been collected.

It has been known since the experiments of Edwards & Balzac in 1832 that gelatin is not a sufficient source of protein and the concept of essential amino-acids may be regarded as having commenced with Escher's (1876) experiments with gelatin supplemented with tyrosine. For the rat, the biological value of gelatin was found to be 23 (Maso & Palmer, 1935) and a preparation of pork cracklings containing 33.5% collagen and 25.7% elastin gave a value of 25, (Mitchell, Beadles & Kruger, 1927). Casein is quite a good source of protein for the rat having a biological value of 69 (Kon, 1928; Beadles, Quisenberry, Nakamura & Mitchell, 1933; Kik, 1938). Table 63 summarises the amino-acid composition of the two proteins and the percentage deficit of amino-acids calculated according to the method of Block & Mitchell (1945-6) using dried whole egg as a standard. This does not imply that whole egg can be used as a standard for measurement of the

TABLE 65

Composition of the three diets (g. / l.)

Ingredient	Dried skim milk diet	Gelatin diet diet №13	Casein diet diet №14
Lard	42.2	42.7	42.7
Glucose	38.1	65.0	65.0
Spray dried-skim milk	47.4	----	----
Casein	----	----	18.9
Gelatin	----	17.7	----
Arachis oil containing 2,000 I.U. vitamin A and 200 I.U. vitamin D/ml.	3.3	3.3	3.3
Yeast extract	----	10ml./calf/day	10ml./calf/ day.
Mineral mixture 1	3.8	10.6	----
Mineral mixture 2	----	----	10.6
DL α-tocopherol acetate	50mg./calf/ day	50mg./calf/ day	50mg./calf/ day
Cholesterol	----	0.1	----
Lecithin	----	0.3	0.3
Glyceryl monostearate	----	0.7	0.7
Riboflavin	----	2mg./calf/ day	2mg./calf/ day
Composition of diet (%)			
Fat	4.6	4.6	4.6
Nitrogen	0.266	0.263	0.263
Calories/l.(calculated)	771	772	772
Percent of calories present as protein	11.9	11.9	11.7

Footnote: Mineral mixture №1 is given in Table 2. Mixture №2 had a lower phosphorus content to allow for the phosphorus present in the casein.

The gelatin was the highest grade commercial gelatin purchased from Messrs. Richard Hodgson & Sons Ltd.. It was very light in colour and was free from elastin and heavy metal impurities. The casein was commercial light white casin purchased from Messrs. Prideaux Milk Foods Ltd.

amino-acid requirements of the calf. The table is merely meant to indicate the large differences between the composition of gelatin and casein and a protein which has been considered to be completely adequate for the rat. From Table 63 it can be seen that casein is deficient in cystine and that gelatine is an extremely unbalanced protein owing to the absence of tryptophan the very high content of glycine and deficiences of tyrosine, cystine, methionine and the leucines.

2. Plan of experiment

Four calves were used, and the plan of the experiment is shown in Table 64.

TABLE 64

Treatment of experimental animals in the gelatin and casein experiment

Calf Nº	Diet given (l./ day)	Adjustment period	Prelim: inary period	First exper: imental period	Recov: ery period	Second experi: mental period
		(6 days)	(12 days)	(12 days)	(8 days)	(12 days)
16	4.8	D.S.M.	D.S.M.	Casein	D.S.M.	Gelatin
17	4.4	D.S.M.	D.S.M.	Casein	D.S.M.	Gelatin
18	4.8	D.S.M.	D.S.M.	Gelatin	D.S.M.	Casein
19	4.4	D.S.M.	D.S.M.	Gelatin	D.S.M.	Casein

D.S.M. = Dried skim milk diet.

It will be noted that each calf received both the gelatin and the casein diet following a period during which dried skim milk was given as the sole source of protein. The composition of the three diets which were given is detailed in Table 65. Glyceryl monostearate and egg lecithin were used in the preparation of the diets as emulsifying agents. Daily supplements of dl - α-tocopherol acetate were given throughout. During the periods in which gelatin and casein were given, 19 ml. of yeast extract and 2mg. riboflavin were given per day. In order to minimise difficulties in the interpretation of the results, the

TABLE 66

Mean gains (-) or losses (-) in bodyweight (g./ day)

Calf Nº	Initial weight (kg.)	Preliminary period D.S.M.	Experimental period 1.	Recovery period D.S.M.	Experimental period 2.
16	35.7	+ 248	+ 257 (casein)	+ 250	- 212 (gelatin)
17	37.2	+ 210	+ 204 (casein)	+ 255	- 239 (gelatin)
18	37.2	+ 377	- 189 (gelatin)	+ 298	+ 200 (casein)
19	33.4	+ 234	- 443 (gelatin)	+ 320	+ 204 (casein)

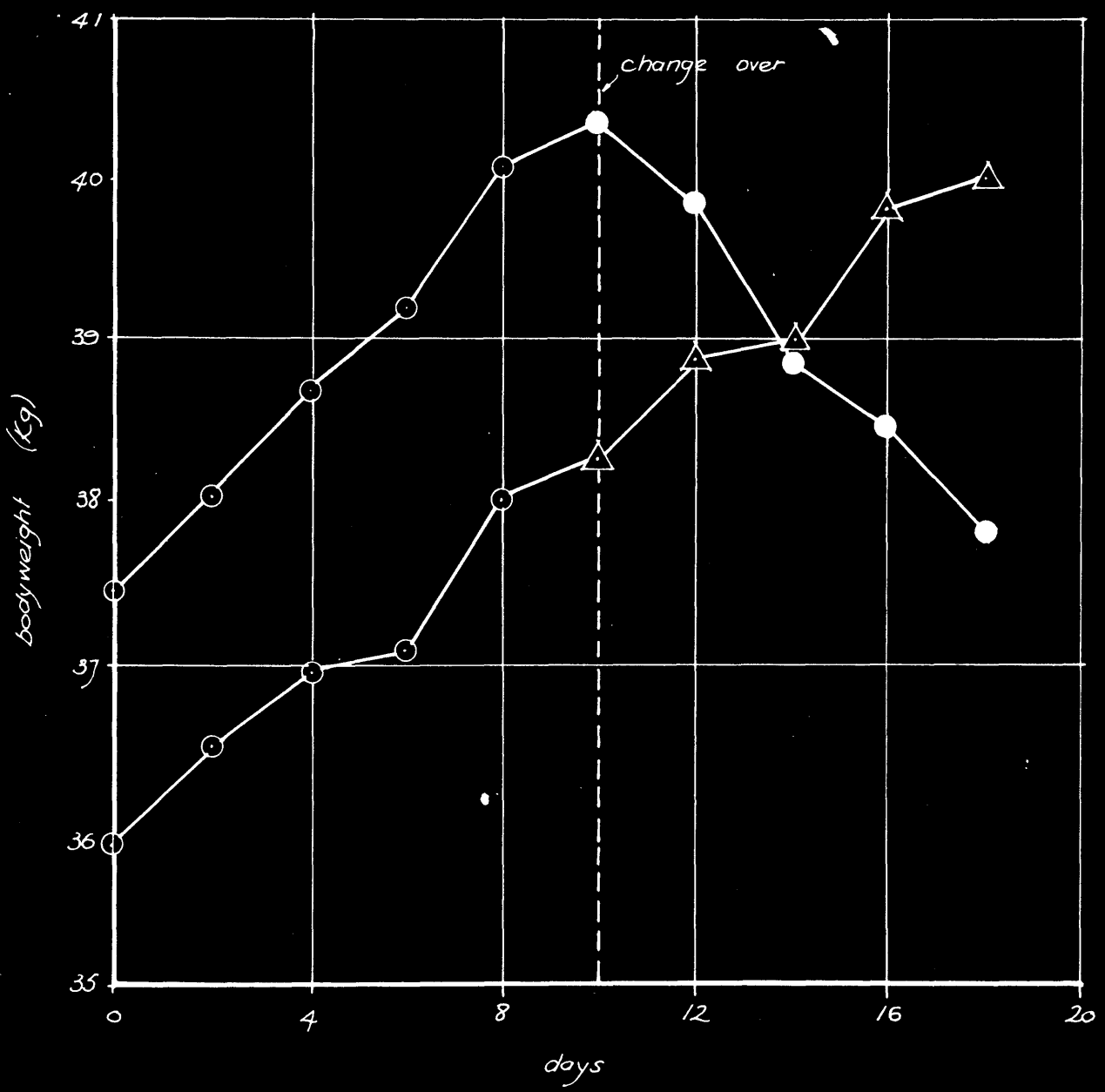
TABLE 67

Mean daily changes in weight (g. / day) classified according to experimental treatment

Level of feeding (l./day)	Protein source		
	Dried skim milk	Casein	Gelatin
4.4 (L)	+ 222	+ 204	- 341
4.8 (H)	+ 313	+ 278	- 201
Mean	+ 268	+ 241	- 271

figure 17

Mean changes in Bodyweight on changing from a Dried Skim Milk diet to Casein and Gelatin diets



calves were given a constant amount of the diet throughout the experiment. Calves №16 and 18 were given 4.8 l. / day and calves №17 and 19, 4.4 l. The gelatin diet set to a solid jelly at low temperatures, but was given to the calves in the liquid form at 37°C as were all the diets.

The following analyses were carried out:-

Diets

Total nitrogen, total fat and dry matter (determined on each sample)

Urine

Total nitrogen (determined each day), nitrogen distribution (determined every two days).

Faeces

Total nitrogen, total fat and dry matter (determined every two days).

3. Results

(a) Bodyweight changes

Figure 17 shows the mean changes in bodyweight on transference from the dried skim milk diet to the casein or gelatin diets. Table 66 summarises these data, by expressing them as gains or losses in weight per day, calculated by linear regression analysis. All gains in weight of the calves when given the dried skim milk diet, or the casein diet were very low indeed. This must have been due to the low level of protein in the diet since in the previous experiments in which a similar diet containing more dried skim milk protein were given in equivalent amounts, far greater gains in bodyweight occurred.

Excluding for the moment the recovery period, the calf which received the higher level of milk per day gained more than its pair mate, or, when the gelatin diet was given, lost less. The mean change in weight resulting from an increase in the allowance of diet from 4.4 to 4.8 litres can be evaluated from Table 67. Statistical analysis of the results given in this table showed that the loss in weight when the gelatin diet was given was very highly significantly

different from the gains which occurred when dried skim milk diet or casein diet was given. The difference in gain between calves given the casein diet and those given the dried skim milk diet was not significant.

During the recovery period the two calves which had been given the gelatin diet and had lost considerable weight gained more than those which had been given the casein diet in the preceeding period. The animals previously given casein gained 252 g.; those which had previously received gelatin gained 307 g. The difference was significant at the odds of 16:1. From the appearance of the calves, it was seen that a slight dehydration occurred during the periods when gelatin was given, and the more rapid gain probably reflects an increase in tissue hydration which took place on transference to the dried skim milk diet.

(b) The apparent digestibility of the diets

The mean apparent digestibility of the dry matter fat, and total nitrogen as well as the calculated "apparent digestibility" coefficients of the dietary energy are given in Table 68.

TABLE 68.

Summary of apparent digestibility coefficients and their statistical significance

Diet	Apparent digestibility coefficients			
	Dry matter	Total fat	Total N	Calories
Dried skim milk	90.7±1.48	84.3±2.63	79.6±1.94	84.4±2.56
Casein	87.5±1.48	77.4±2.63	77.4±1.94	84.6±2.56
Gelatin	85.4±1.48	76.2±2.63	63.5±1.94	82.2±2.56

The standard errors of the means were derived from analysis of variance. The digestibility of all the dietary components was low. This was probably due to the low level of protein in the diet and will be discussed in more detail later (page 109). The apparent digestibility of both the fat and dry matter tended to be reduced when the animals received the

TABLE 69

Heat production of the calves determined 15 hours after feeding calculated from respiratory exchange determinations.

Calf Nº	Diet	Heat production (cal./ 24 hr.)	Heat production (cal./ kg./24 hr)
16	D.S.M. (1)	1704	45.47
	Casein	1818	45.33
	D.S.M. (2)	1881	45.75
	Gelatin	1974	47.34
17	D.S.M. (1)	1713	44.66
	Casein	1830	44.85
	D.S.M. (2)	1431	37.69
	Gelatin	1805	43.98
18	D.S.M. (1)	1771	43.84
	Gelatin	1962	49.91
	D.S.M. (2)	1828	46.69
	Casein	2010	47.75
19	D.S.M. (1)	1590	44.54
	Gelatin	1230	39.66
	D.S.M. (2)	1706	51.08
	Casein	1680	47.67
Mean	D.S.M. (1)	1699	44.63
	D.S.M. (2)	1712	45.05
	Casein	1834	46.40
	Gelatin	1743	45.22

gelatin diet, but the differences were not statistically significant. The apparent digestibility of the total nitrogen of the gelatin diet, was, however, significantly smaller than that of the dried skim milk diet. The true digestibilities of the nitrogen of the three diets were 90.5, 87.3 and 85.3 for the dried skim milk, casein and gelatin diets respectively.

The last column of Table 68 shows the percentage of the calorie intake which did not appear in the faeces. These values are low because the fat digestibility was low. Nevertheless the calculated number of calories apparently digested by any calf was higher than its basal metabolism at the same time (see below). In only one sub-period of the experiment was the apparent uptake of dietary energy from the gut low enough to be exceeded by the directly determined basal metabolism, and that sub-period for calf №19 during the period in which the gelatin diet was given has been omitted in calculating the mean results.

(c) Energy metabolism

The heat production of the calves, calculated from their respiratory exchange were used to calculate the endogenous metabolism using the previously determined factor of 1.9 mg. of endogenous nitrogen per basal calorie. Table 69 summarises the heat production of the calves. Two low values were encountered; one for calf №17 in the recovery period, and the other for calf №19 in the period in which gelatin was given. In both cases there had been a short history of diarrhoea with a concomitant reduction in the amount of energy apparently digested. In view of the instability of the calf's heat production during inanition (pages 33, 74) such an occurrence is understandable. The mean heat production of the calves given 4.8 litres of the diet each day was 46.52 cal./kg./24 hr. and of those given 4.4 litres per day, 44.50 cal./kg./day. The difference between them was statistically significant, confirming previous findings (page 74) that the plane of nutrition affects the heat production of the calf when it is determined 15 - 24 hr. after the last feed.

TABLE 70

Mean nitrogen balance data per day.

Calf Nº	Amount of diet (l. / day)	Diet N	intake (g.)	Faecal N (g.)	Urine N (g.)	N Balance (g.)
16	4.8	D.S.M.	12.75	2.68	4.43	+ 5.64
		Casein	12.63	2.46	6.64	+ 3.53
		'recov: ery'	12.12	3.53	5.97	+ 2.62
		Gelatin	12.54	3.37	12.48	- 3.30
17	4.4	D.S.M.	11.69	2.06	4.14	+ 5.48
		Casein	11.66	3.17	6.12	+ 2.73
		'Recovery'	11.10	4.61	4.72	+ 1.78
		Gelatin	11.13	2.79	9.27	- 0.92
18	4.8	D.S.M.	12.75	2.06	3.67	+ 7.01
		Gelatin	12.85	4.26	10.81	- 2.22
		'Recovery'	12.12	2.37	5.51	+ 4.24
		Casein	12.49	2.61	5.51	+ 4.73
19	4.4	D.S.M.	11.69	3.12	4.51	+ 4.05
		Gelatin	10.98	5.89	9.46	- 4.37
		'Recovery'	11.10	4.01	4.07	+ 3.01
		Casein	11.67	2.98	4.39	+ 4.32
Mean irrespect: ive of feeding level		D.S.M.	12.22	2.48	4.19	+ 5.55
		Casein	12.11	2.81	5.66	+ 3.64
		Gelatin	12.10	4.30	10.50	- 2.70
Standard error of the means				±0.477	± 0.449	± 1.62

In this connection it should be noted that the present mean figures for basal metabolism were higher than those observed when calves were given nitrogen-free diets and subsequently subjected to short fasts, (page 51) this again being due to the effect of the previous calorie intake.

(d) Nitrogen metabolism

The mean data relating to the nitrogen balances of each calf are presented in Table 70. Each value for the preliminary period and main experimental periods represents the mean of five 2-day sub-periods, except for calf №19 where, for reasons already given, one sub-period was omitted. The balances recorded for the recovery period refer to determinations made over 4 days.

Analysis of variance showed that there were no significant differences due to treatment as far as faecal nitrogen excretion was concerned, the high mean value for gelatin being largely due to one high value obtained with calf №19. The effect of diet on urinary nitrogen excretion was very highly significant ($P = 0.001$) as was the effect on nitrogen balance ($P = 0.01$). The effect on urinary nitrogen excretion is shown in Figure 18 in a block diagram, which shows clearly the large excretion of nitrogen which occurred in the urine of those animals receiving the gelatin diet. Even the difference in the excretion of urinary nitrogen between the calves given casein and those given dried skim milk was statistically significant at odds of 17:1.

All balances were of course low, since all the diets were protein-deficient irrespective of the amino-acid composition of the proteins employed. Despite this protein deficiency the animals given dried skim milk or casein gained in weight, though at a lower rate than normal. When the nitrogen balances of the calves were related to their gains in bodyweight the following equation was found:-

$$NB = 0.0128G - 0.898 \quad \ldots\ldots (11)$$

where NB is the nitrogen balance in g. per day and G

TABLE 71

Calculated biological value of dietary protein (%)

Calf Nº	Dried skim milk	Casein	Gelatin
16	89.4	72.0	19.6
17	91.6	74.4	39.7
18	97.3	80.6	29.3
19	90.7	88.1	4.4
Mean	92.3	78.8	29.5

TABLE 72

Biological value of proteins in man, rat and calf

Protein	Calf	Rat	Man	Relative values D.S.M. = 100		
				Calf	Rat	Man
Dried milk	92	84	74	100	100	100
Casein	79	69	58	85	82	78
Gelatin	29	25	--	32	30	--

is the gain in weight per day. This regression was highly significant statistically (P = 0.01), and will be discussed in more detail later (page116).

(e) Biological value of the proteins

Biological values were calculated from the nitrogen balances and basal metabolism of the calves making the assumption that the endogenous metabolism of nitrogen in the calf is proportional to its basal heat loss. Dried skim milk had the highest biological value, casein was intermediate and gelatin was the lowest. The differences between the means were statistically highly significant. The high value obtained for calf №19 when given casein is anomalous. This animal showed the greatest loss of nitrogen when given the gelatin diet, and the subsequent high value obtained when casein was given may reflect the increased retention of nitrogen following severe depletion. While this may be an explanation of the high value it does not provide a reason for omitting it in calculating the mean.

It is of interest to compare these values with those obtained for other animals. Table 71 shows the biological values obtained in the present experiment and Table 72 shows the means compared with values summarised by Block & Mitchell (1945-6). The values obtained with the calves were all higher than those obtained with rats, which were in turn higher than those obtained with men. As shown in the second part of Table 72, the relative values obtained when dried skim milk was taken as equal to 100, were, however, very similar, irrespective of species. It appears therefore, that the nutritive value of proteins which are as diverse in amino-acid content as gelatin and casein, is the same in the calf as in simple stomached animals. The reason why the biological values determined with calves were higher than those determined with other species may be due to a species difference in the utilisation of the constituent amino-acids, but since the ratios of the three proteins to one another in the present experiment and in other

TABLE 73.

Summary of mean daily excretion of urinary metabolites

(mg. N / day)

Nitrogenous metabolite	Dried skim milk diet	Casein diet	Gelatin diet	Standard error of difference between means	
Urea + ammonia	2010.8	2997.8	6326.8	± 378	***
Protein	128.8	143.5	149.9	± 33	N.S.
Amino-N	268.9	282.0	711.5	± 83	***
Creatinine	407.8	378.4	384.0	± 17	N.S.
Creatine	296.0	330.9	499.3	± 55	*
Purine base	93.6	146.0	252.1	± 27	*
Uric acid	69.9	70.4	50.0	± 4.2	*
Allantoin	599.1	518.7	530.6	± 12.5	N.S.

*** Significant when P = 0.001

* Significant when P = 0.05

N.S. = not statistically significant

Block diagrams showing the effect of the proteins of dried skim milk : gelatin, and casein on the excretion of N in the urine of the calves.

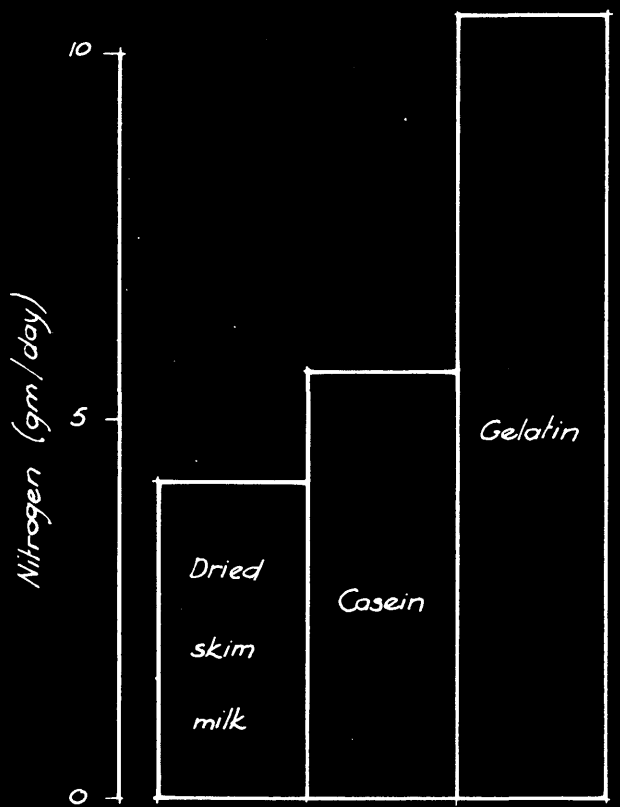


figure 18 TOTAL NITROGEN

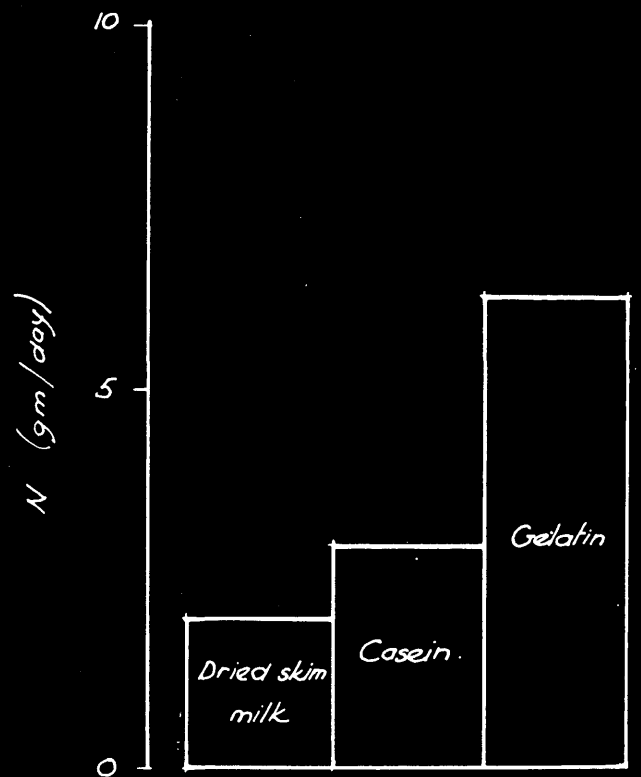


figure 19 UREA & AMMONIA N.

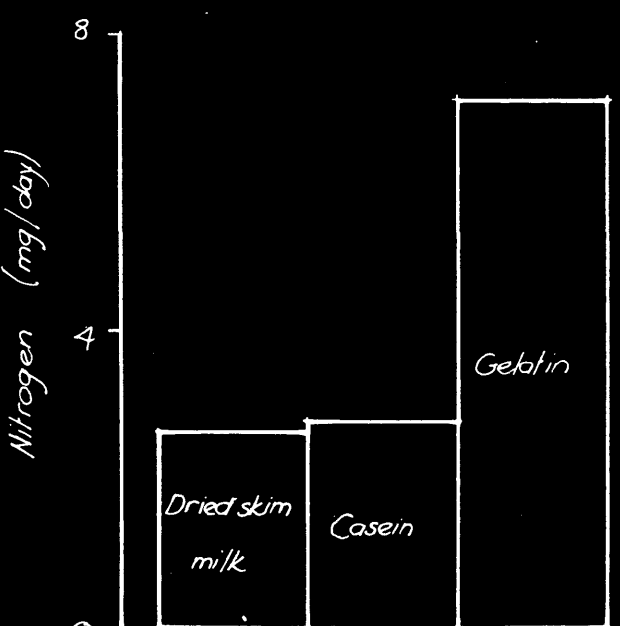


figure 20 AMINO N

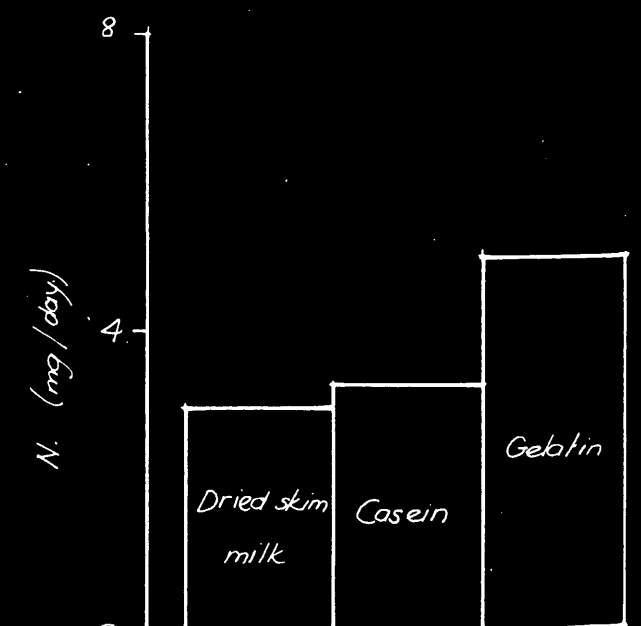


figure 21 CREATINE N.

experiments (Block & Mitchell, 1945-6) are very similar, a more likely explanation is that the level of protein in the present diets led to a comparatively greater degree of protein deficiency than occurred in rat or man. This is explained by the extremely high demand of the rapidly growing tissues of the calf for the essential amino-acids given.

(f) The distribution of urinary nitrogenous metabolites

The mean results obtained on analysis of the urine for nitrogenous metabolites are given in Table 73. These means refer to the last 6 days of each period except for calf №19 when given the gelatin diet and calf №18 when given the casein diet. A single sample of urine in these periods had to be rejected and the mean daily excretion was measured over 4 days. Albumin nitrogen, creatinine nitrogen, allantoin nitrogen and the total purine nitrogen were unaffected by the dietary source of protein. There was a small decline in the excretion of purine nitrogen as uric acid, and this was accompanied by an increase in the excretion of purine bases. These changes, however, involved only small quantities of nitrogen and were not sufficiently large to result in any marked change in the total excretion of purine. The major part of the increase in urine nitrogen excretion was in the urea, ammonia and amino-nitrogen fractions. These three fractions alone accounted for nearly 90% of the increased nitrogen excretion by the calves when given the gelatin diet. Creatine nitrogen also increased when both the gelatin diet and the casein diet were given. The changes are shown in block diagram form in Figures 19, 20, 21 where they may be compared with the total nitrogen excretion shown in Figure 18.

These results are quite compatible with the contention that there was an increase in the de-amination of dietary amino-acids when the calves were given either gelatin or casein as the sole source of protein, and will be discussed in detail later (page 110)

(g) General observations /

TABLE 74

Serum protein concentrations (g. / 100 ml.) in calves receiving low protein diets and in a normal calf given colostrum.

Fraction of serum protein		Calf №					
Na_2SO_4 conctn. (g./l.)	Fraction	16 Gelatin diet	17 Gelatin diet	18 Casein diet	19 Casein diet	Mean	Normal calf (A1) given adequate colostrum
0 - 159	" γ globulin"	1.84	1.09	1.15	1.05	1.26	2.14
159 - 270	" $\alpha + \beta$ globulin"	1.84	1.81	1.65	1.59	1.72	1.85
above 270	albumen	1.97	2.29	1.67	1.94	1.97	2.64
Total		5.67	5.19	4.57	4.62	4.95	6.61

(g) General observations

In general the health of the calves remained good throughout the experiments, but there were a number of instances in which abnormalities occurred and, as the animals were given protein deficient diets their behaviour was not really normal. All calves tended to become a little weak, as judged by a lower incidence of playing in their metabolism cages and their general lethargy. Hair loss was considerable throughout, increasing in severity when the casein diet was given. Mild alimentary disturbances were invariably present as has previously been discussed. When the calves were given the gelatin diet small refusals of the diet occurred after 8-10 days and there was a tendency for the calves to become dehydrated. On these occasions extra water was added to their feed and in only one case (calf №19) was it necessary to resort to the injection of a normal salt solution to restore tissue hydration. When the gelatin diet was given a pigmentation of the tongue developed in this calf, and the whole oral cavity became a dark magenta colour. Nicotinic acid deficiency was suspected in this tryptophan deficient calf but no additional nicotinic acid was given in view of the metabolic relationships between tryptophan and nicotinic acid. Similar oral lesions have been reported in calves given riboflavin deficient diets (Wiese, Johnson, Mitchell & Nevens 1947). There was, however, no response to additional riboflavin (2mg./ day) and the condition slowly improved as the experimental period progressed. The exclusion of nicotinic acid from the diets containing large quantities of casein does not produce any abnormalities at all in young calves (Johnson, Wiese, Mitchell & Nevens, 1947). A similar condition, though not so severe was noted in calf №16 when transferred to gelatin.

At slaughter nothing abnormal was noted in the carcases of the calves. Sera were collected at this time and the plasma proteins determined by sodium sulphate fractionation. Table 74 summarises the

results.

Results obtained for a normal calf aged 6 days given cow's whole milk are included for comparison. The serum protein concentration was low in all the experimental calves the reduction being in both the globulin and albumin fractions. In adult cattle the total serum protein concentration is approximately 7-8 g. / 100 ml., this higher figure being accounted for mainly by a greater concentration of globulins. The low globulin content of the blood of the present calves may be partially accounted for by the fact that they were not fed colostrum, but the low albumin content is clearly related to the inadequate level of protein nutrition.

4. Discussion

Since the young calf can store nitrogen and grow when given a protein known to be of good nutritive value in rat nutrition and yet will lose body nitrogen and weight when given a protein which is inadequate, it is obvious that it is dependent on its diet for at least a part of its amino-acid supply. Since there was a close proximity between biological values obtained with the rat, man and calf for a good protein- dried skim milk, a fair protein, - casein and a very poor protein - gelatin, it would seem that the calf is just as dependent as the rat or man, on a dietary source of amino-acids. These results seem to be the first unequivocal demonstration that large farm animals do require dietary sources of amino-acids for nitrogen metabolism processes of the body. The results apply, however, only to the calf reared under conditions in which the development of an active rumen could not possibly occur, and this, considered together with the fact that the adult ruminant does not react in a similar manner provides additional confirmation of the Zuntz (1891) and Hageman (1891) hypothesis of rumen function.

This result has a number of implications; firstly, the protein quality of calf meals and gruels

which are used for calf rearing. The amino-acid composition of these mixtures is seldom, if ever, taken into consideration. Gruels usually contain a large quantity of linseed meal which has a very low content of lysine. Bean and pea meals are also widely advocated for the young calf and are very low in tyrosine - in fact they have a biological value of only 38 and 48 for the rat (Block & Mitchell, 1946). Thus, better quality proteins should be used for the young calf and the proteins of low biological value would be better utilised if given to the cow.

Secondly, the present results supplement others showing that the requirement of the young calf for dietary essentials differs from the requirement in the adult. It is known that microbial synthesis of the following vitamins takes place in the rumen of mature animals:- K, aneurin, riboflavin, biotin, folic acid, nicotinic acid, pantothenic acid and vitamin B 6 (Kon & Porter, 1947). Normally, these vitamins are synthesised in sufficient quantities to meet the requirements of the animal. In the calf with no synthetic flora, a deficiency of riboflavin (Wiese, Johnson, Mitchell & Nevens, 1947; Warner & Sutton, 1948; Brisson & Sutton, 1951), biotin (Wiese, Johnson & Nevens, 1946), aneurin (Johnson, Hamilton, Nevens & Boley, 1948), and pyridoxine (Johnson, Pinkos & Burke, 1950) has been demonstrated. An attempt to produce nicotinic acid deficiency in the calf was not successful (Johnson, Wiese, Mitchell, & Nevens, 1947). Similar studies concerning the requirements of the calf for other vitamins will undoubtedly follow, but there is sufficient evidence available to show that the calf must receive a dietary source of many of the vitamins and amino-acids which the mature animal is able to synthesise. During its early life, and even when rumen function has commenced but has not reached its maximal synthetic activity, the calf may therefore be liable to shortages of these essentials.

Thirdly, the fact that losses in weight occurred when gelatin was given suggests that tryptophan - the first limiting amino-acid of gelatin, is required for maintenance of nitrogen equilibrium and bodyweight. The determination of the specific requirement for this amino-acid and for other essential amino-acids by the young calf may now follow.

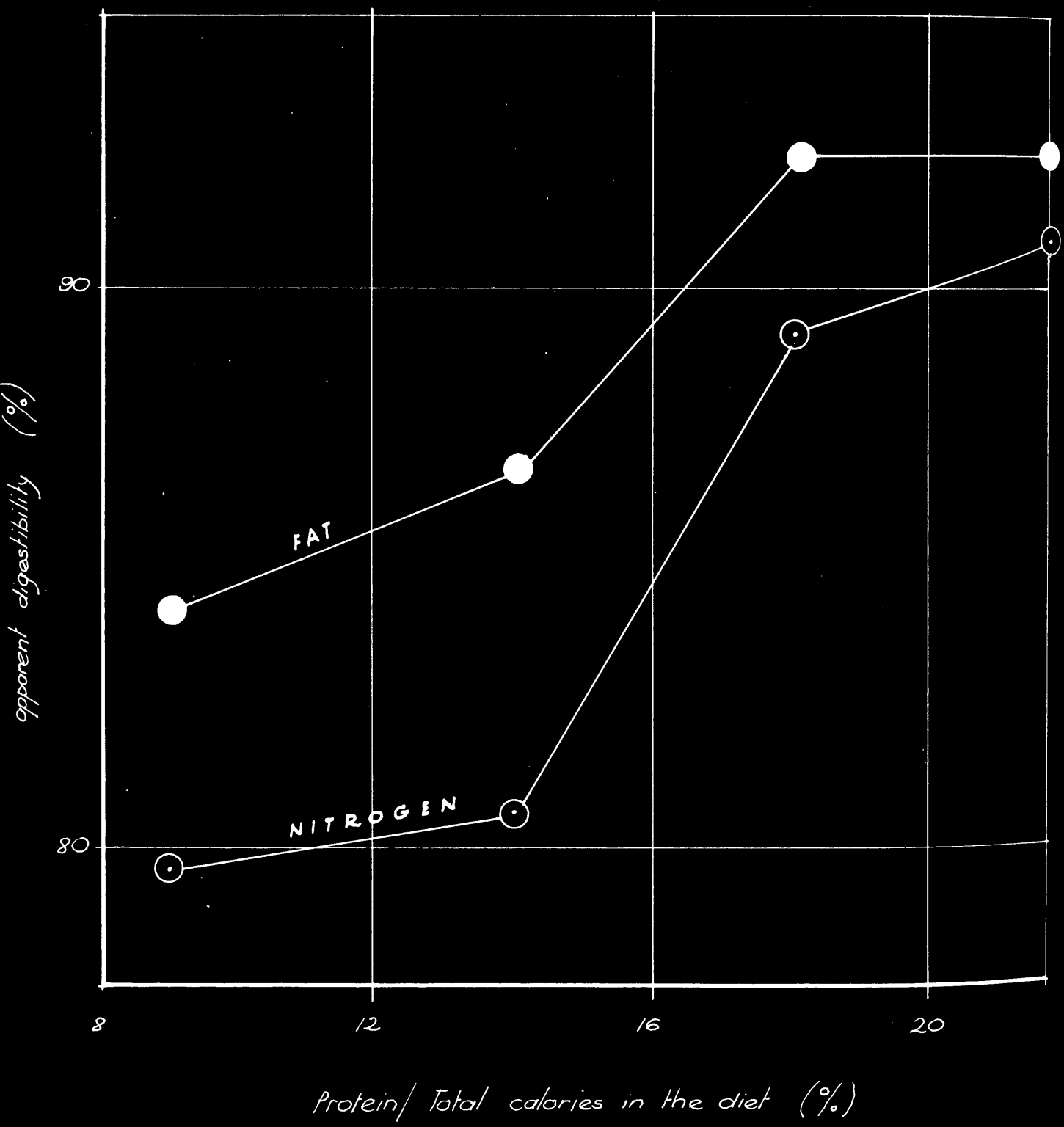
TABLE 75

The mean apparent digestibilities of the constituents of dried skim milk in semi-synthetic diets, and of whole milk compared with those obtained by other workers.

Reference	Dry matter	Fat	Nitrogen
Semi-synthetic diets (all protein) and energy percentages - see Tables 8, 27,36,37 & 68)	95.1	92.9	90.3
Skimmed milk with fat added and emulsified (Fingerling 1908)	98.0	93.0	95.0
Whole milk (see Table 44)	96.5	93.8	95.7
Whole milk (Tomme & Taranenko, 1939)	----	95.1	94.5
Whole milk Schneider (1937)	98.0	100	96.0

figure 22

. The effect of the percentage of protein in the diet, on the apparent digestibilities of fat and nitrogen



(G) SOME GENERAL ASPECTS OF METABOLISM OF THE YOUNG CALF

Many aspects of protein metabolism have been discussed in the sections dealing with individual experiments. Further aspects of metabolism may, however, be investigated by combining the individual results of each experiment.

1. The apparent digestibility of synthetic diets and whole milk

The mean apparent digestibilities of the dry matter, fat and nitrogen in the synthetic diets containing dried skim milk (Tables 8,27,36,37 & 68) and those of whole milk (Table 44) are summarised in Table 75, which also shows some of the results obtained by other workers. The apparent digestibility of the dry matter and fat of the synthetic diets was within the normal range when compared with the apparent digestibility of whole milk constituents. The results obtained in the present series of experiments for the apparent digestibility of the fat in the semi-synthetic diets and also in whole milk are much lower than the earlier German results summarised by Schneider (1947). This was no doubt due to the fact that earlier workers determined faecal fat by simple extraction, so that the soaps which constitute about 50% of the fat in faeces were not determined. The apparent digestibility of the nitrogen of the semi-synthetic diets appears to have been slightly lower than that of whole milk. This might perhaps be due to an effect of heat on the milk proteins during drying, or during preparation of the diets. (Nevens & Shaw, 1933; Cook, Morgan, Weast & Parker, 1951). A more likely explanation is that the lower protein content is alone responsible for the lowered apparent digestibility of the nitrogen. That this is so may be seen from Figure 22 which shows that as the percentage of protein in the diet was reduced, a marked decline in the apparent digestibility of the protein occurred. Part of this decline was possibly due to the presence of metabolic faecal nitrogen. Figure 22 also shows that a lower percentage of protein

in the diet resulted in a lower apparent digestibility of the dietary fat. One suggestion is that the protein with its associated phospholipid is necessary for the efficient absorption of fat, or another, that the percentage of protein in the diet affects the type of clot produced in the abomasum of the calf, which in turn might be related to the efficiency of fat digestion.

2. Excretion of nitrogenous metabolites in the urine

Distribution of nitrogen in the urine of the calf evidently has not been determined before. It is therefore of interest to compare the results obtained in these experiments with those obtained with other animals, and also to compare the changes in distribution which occur under different conditions. Table 76 summarises the nitrogen distributions which were obtained during the present series of experiments. For comparative purposes the figures are calculated as mg. nitrogen / kg. bodyweight.

Since the so-called endogenous excretion of metabolites (Folin, 1905) does not alter essentially during various treatments of the calf, it is present in each determination of the metabolites excreted during the various experiments. During starvation, the body relies entirely on its own substance for energy and thus the difference between the endogenous excretion of metabolites and the excretion of metabolites during starvation gives a good indication of the end-products of protein metabolism arising from the breakdown of body constituents to provide energy for vital processes. In neither case is the result disturbed by a dietary source of nitrogenous compounds. In most animals, urea is regarded as the end-product of the catabolic processes of protein metabolism that contribute to energy production. Allantoin and the other purines, as well as creatinine, may be regarded as the excretory products of compounds which play specialised roles in essential metabolic processes of the body. It will be seen that, with the calf, as with other animals, the differences in nitrogen excretion were almost entirely in the urea and creatine

TABLE 76

Comparison of nitrogen excreted as different metabolites in the urine of calves subjected to various experimental treatments

Metabolite	Excretion (mg.N/kg. bodyweight/24 hrs.) during:-					
	N-free feeding (expt. B)	Starvation (expt. A)	Whole milk (expt. D)	Whole milk + 200 g. starch (expt E)	Low protein D.S.M.(expt. F.	Low protein gelatin (expt. F
Total	81.9	245.6	256.5	134.0	112.0	263.9
Urea + ammonia	47.7	196.7	192.1	70.6	53.8	159.0
Amino-acids	---	---	6.5	3.2	7.2	17.9
Creatine	2.9	7.5	6.3	4.4	7.9	12.5
Creatinine	10.1	9.1	10.3	8.8	10.9	9.6
Allantoin	---	14.7	12.8	11.8	16.0	13.3
Purine base	---	4.5	3.4	2.4	2.5	6.3
Uric acid	1.3	1.4	1.0	1.4	1.9	1.3
Total purine	---	20.6	17.2	15.6	20.4	20.9
Protein	---	---	4.1	2.4	3.4	3.8

fractions, as previously inferred (see Figures 2 - 7, 18 - 21). Of the total increase in nitrogen excretion above the endogenous level during starvation, 91.1% was due to the excretion of urea and ammonia, and 2.8% to the excretion of creatine. Thus, changes in these same fractions during other experiments may be explained by their production during catabolism of nitrogenous compounds to supply energy to the tissues.

The comparatively high excretion of urea and ammonia N when whole milk was given is a reflection on the high protein content of whole milk, and the reduction of the urea fraction when starch was given as a nitrogen free supplement (Table 56) is a certain indication that dietary protein was averted from the process of deamination to one of storage.

When the calves received a low-protein diet, the amino-acids in the diet became the limiting factor. The amino acids in excess of those required would be deaminated because they could not be utilised by the body for maintenance or growth. Thus, the urea concentration in the urine of the calves on the diet containing low percentage of dried skim milk approached the endogenous level (Table 76) and yet, when the calves received the gelatin diet containing the same percentage of protein they excreted a quantity of urea and ammonia nitrogen closely approaching that excreted during starvation.

Amino-nitrogen determinations were not made throughout the experiments, but it will be seen from Table 76 and Figure 20 that there was a large increase in the excretion of amino-N when the poor protein gelatin was given. This may indicate a concentration of unrequired amino-acids in the blood higher than the renal threshold for these compounds.

It is certain from Table 76 that creatine is a normal constituent of calf urine. On one occasion only, during the period when a nitrogen-free diet was given did this constituent disappear completely from the urine. This excretion of creatine by the calf

is in agreement with results obtained with other animals since during tissue growth, including pregnancy, creatine is a normal constituent of urine (Rose, 1911-2). The present experiments indicate that creatine excretion is in some way connected with the deamination of amino-acids and the assimilation of their carbon containing compounds. As already discussed, there was an increase in the excretion of creatine during starvation much higher than the endogenous excretion. There was also a significant lowering of creatine excretion when starch was given (Figure 16). The loss of creatine during starvation was understandable since the breakdown of muscle would also entail a loss of creatine from the muscle cell. From the reduction in creatine excretion which occurred when the nitrogen balance increased on feeding starch, two suggestions follow. Firstly, assuming that the production of creatine in the body is constant, an increased growth of muscular tissue may mean an increased retention of creatine in the tissues. Secondly, that the production of creatine is not constant, but is reduced due to the assimilation of creatine precursors, possibly arginine or methyl groups, into muscle tissue. Arginine is known to be a precursor of creatine, and thus the second suggestion is supported by the fact that the excretion of creatine was high during the time that the gelatin diet was given (Table 76), since the arginine content of gelatin is comparatively high (see Table 63).

The excretion of creatinine /kg. bodyweight by the calf remained constant during all the experiments, despite large changes in nitrogen metabolism. The mean excretion was 9.9 mg. nitrogen / kg. bodyweight, which is in good agreement with results obtained for other species e.g. man with a ratio of 7.5 - 10 mg. N / kg. bodyweight (Peters & Van Slyke, 1931). The concept of the endogenous metabolism of Folin (1905) is therefore confirmed. As the creatinine nitrogen of the urine remained constant irrespective of the source of nitrogen, and since poor utilisation of

TABLE 77

Analysis of variance of linear regression and logarithmic regression of biological value of dietary protein on % total nitrogen in the urine present as creatinine.

Component of biological value variation	Degrees of freedom	Estimated variance	Variance ratio (e^{2z})
Total	26		
Linear regression	1	7,544.96	59.7
Deviations	25	126.48	
Logarithmic regression	1	9,582.77	215.0
Deviations	25	44.57	

dietary protein entailed an increase in total urinary nitrogen excretion, then the ratio of creatinine nitrogen to total nitrogen in the urine should reflect quite closely the biological value of the ingested protein (Murlin, Szymansky & Nasset, 1938). When a protein with a biological value of 100 is given this percentage of creatine nitrogen should be 11.8, that is the same as the percentage of the nitrogen present as creatinine when the animal is given no protein whatever. Analysing statistically the data obtained in the present series of experiments, it was found that the linear and logarithmic regression between the percentage of total nitrogen present in the urine as creatinine and the biological value of the ingested protein was significant. Table 77 shows the analysis of variance. The relationship tends to be curvilinear, probably due to the fact that the biological value of a protein is not entirely a function of endogenous nitrogen and total urinary nitrogen. As previously given,

$$BV = \frac{NB + MN + EN}{ADN + MN} \times 100 \quad \ldots\ldots \quad (3b)$$

where BV = biological value, NB = nitrogen balance, MN = metabolic faecal nitrogen, EN = endogenous nitrogen, and ADN = apparently digested nitrogen.

$$\text{Thus } BV = \frac{ADN - UN + MN + EN}{ADN + MN} \times 100 \quad \ldots \quad (12a)$$

where UN = urinary nitrogen.

For simplification, substituting TDN for ADN + MN, where TDN is the truly digestible nitrogen,

$$BV = \frac{EN - UN - TDN}{TDN} \times 100 \quad \ldots\ldots \quad (12b)$$

showing that the biological value of a protein is a function of TDN as well as EN and UN.

By simple algebra,

$$\frac{EN}{UN} = 1 + \frac{TDN\ (BV - 1)}{UN} \times 100 \quad \ldots \quad (13)$$

Thus the ratio creatinine nitrogen / total urinary nitrogen, using creatinine nitrogen as an index of the endogenous nitrogen excretion, is affected by the apparently digested nitrogen and to a lesser extent by the metabolic faecal nitrogen as well as by the

TABLE 78

Percentage distribution of total purines in the urine of different species (Dukes, 1947)

Species	Allantoin N	Purine N	Uric acid N
Calf	72.4	20.1	7.4
Cow	92.1	0.7	7.3
Sheep	64.0	20.0	16.0
Goat	81.0	12.0	7.0
Horse	88.0	0.5	12.0
Pig	92.3	5.8	1.8
Dog	97.1	1.3	1.9
Man	2.0	8.0	90.0

biological value of the absorbed nitrogen.

It will be noticed from Table 76 that purine nitrogen excretion also remained fairly constant throughout the experiments, and although the data relating to endogenous purine excretion are not complete, there seems little doubt that the purine nitrogen excretion was almost as constant as was the excretion of creatinine. The excretion of purine compounds also remains constant in other species during various dietary stresses and physical exercise (Garry, 1926-7) but the variation in the distribution of purine nitrogen in the urine of various species is considerable. Table 78 shows the percentage distribution of purines in the urine of a number of animals taken from a summary by Dukes (1947). It will be noticed that the calf excretes a slightly higher percentage of its total purine as purine bases than does the mature animal. There is a corresponding reduction in the excretion of allantoin by the calf, when compared with the excretion by all other species except man, the excretion of uric acid as a percentage of the total purine excretion remaining constant. It is not known, however, whether the different species of animal summarised by Dukes received diets which were free from purines. If they did not, wrong conclusions may be drawn. For instance, the conclusions made by Morris & Ray (1939) and Hutchinson & Morris (1936b) may be criticised in this respect. These workers concluded that in the ruminant there is a marked reduction of nuclear cell metabolism during fasting. These conclusions were based on the reduction of the excretion of purine nitrogen in the urine when the animals were fasted, following the ingestion of diets which contained normal foods such as hay. These diets were not purine free, and the reduction which was noted merely reflects the cessation of an external purine source. This emphasises still more the species differences shown in the table since purine bases, if present in the diet would presumably be excreted in the urine as such. The

TABLE 79

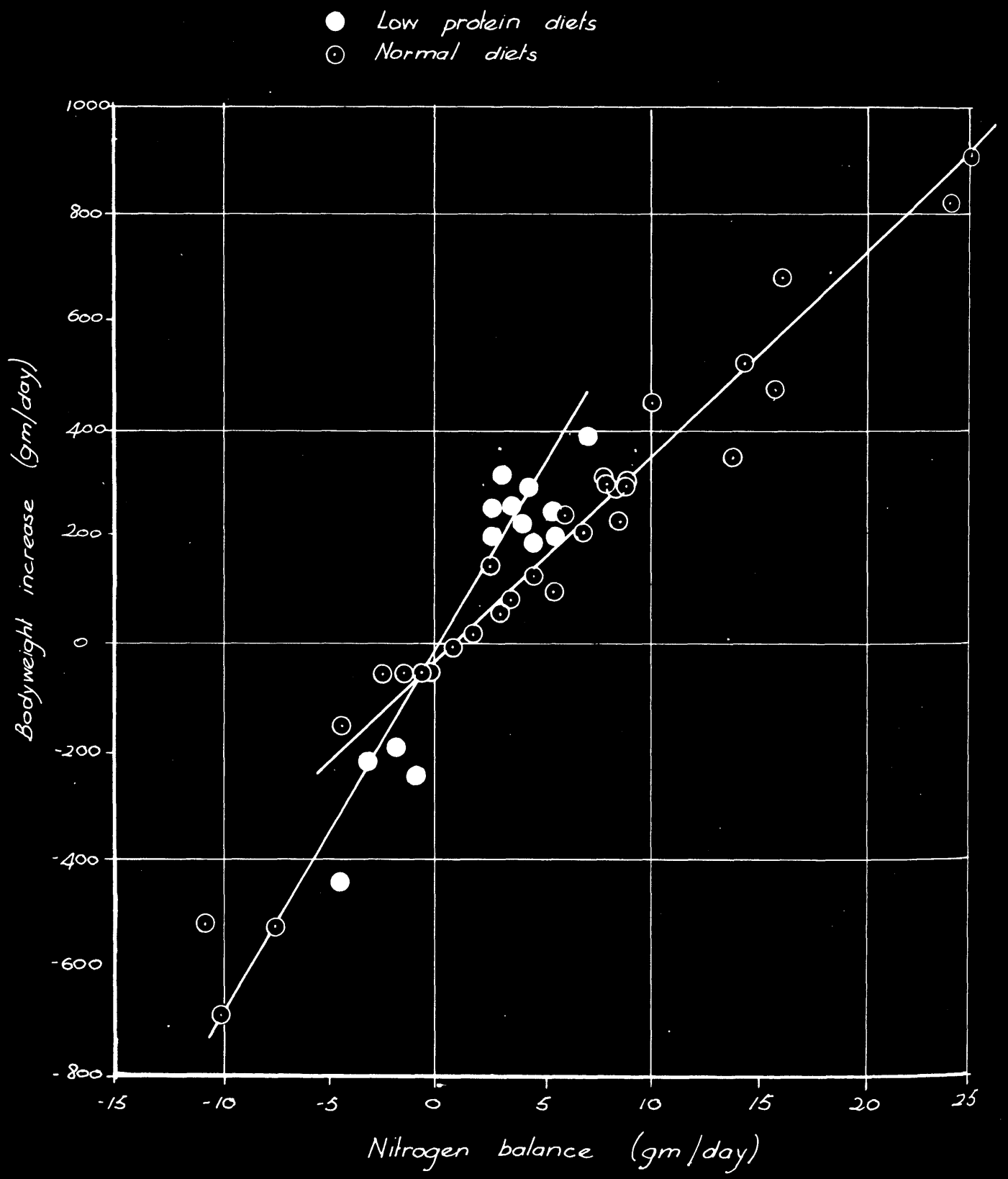
Distribution of nitrogen in different nitrogenous metabolites in the urine of starving calves, goats, sheep and cows expressed as percentages of total urinary nitrogen.

Metabolite	Calf	Goat ✱	Sheep ✱	Cow ✱
Urea	72.49	64.42	57.56	30.39
Ammonia	7.57	3.64	3.87	31.77
Total urea + ammonia	80.06	68.06	61.43	62.16
Creatinine	3.70	6.12	7.88	7.35
Creatine	3.06	3.63	4.86	4.77
Uric acid	0.57	1.08	1.44	0.58
Purine base	1.83	6.16	4.66	2.14
Allantoin	5.95	11.12	18.41	14.24
Total purine	8.35	18.48	24.51	16.96

✱ Results of Morris & Ray (1939) recalculated and expressed in a form suitable for comparing with present results for calves.

figure 23

Relationships between nitrogen balance and Bodyweight increase of the calves receiving various quantities of protein in the diets.



higher excretion of purine base nitrogen by the calf suggests therefore a lower activity of the purine enzyme systems. From the pathway of purine oxidation in the body (Baldwin, 1948) it is possible that the xanthine oxidase system has a lower activity in the calf than in other species.

Pathway of purine oxidation (Baldwin, 1948)

Xanthine $\xrightarrow[\text{oxidase}]{\text{xanthine}}$ uric acid $\xrightarrow[\text{oxidase}]{\text{urico-}}$ allantoin

The fraction designated as 'protein' was shown to consist largely of skin and hair debris washed from the bottom of the crate by urine. In the younger animal, however, this fraction would include small amounts of heat coagulable protein such as reported by Smith & Little (1924) in the urine of newly born calves. Separation of these fractions was not carried out as a routine determination.

The nitrogenous constituents of the urine of different species are most suitably compared during starvation since the results are not then complicated by an exogenous source of nitrogen. Table 79 compares the nitrogen distribution of the urine during starvation of the calf with that found on starvation of the adult ruminant. Expressed as a percentage of the total nitrogen excreted the starvation metabolism of the calf differed mainly from that of the cow in that the creatinine nitrogen and also the purine nitrogen was only half of that found in the same species at maturity, or in mature animals of similar bodyweight. The excretion of urea and ammonia nitrogen was a considerably greater percentage of the total nitrogen in the calf than in these species, again emphasising the intense metabolism of the calf.

3. Storage of nitrogen during growth

The information relating nitrogen balance to bodyweight gain from all the determinations in this series of experiments is shown in Figure 23. It will be noticed that there are two relationships, one obtained when low nitrogen diets were given (12%

calories as protein - see page 102) and the other obtained with diets less deficient in protein (14% and over). Both were statistically highly significant. The equations for the two regressions were:-

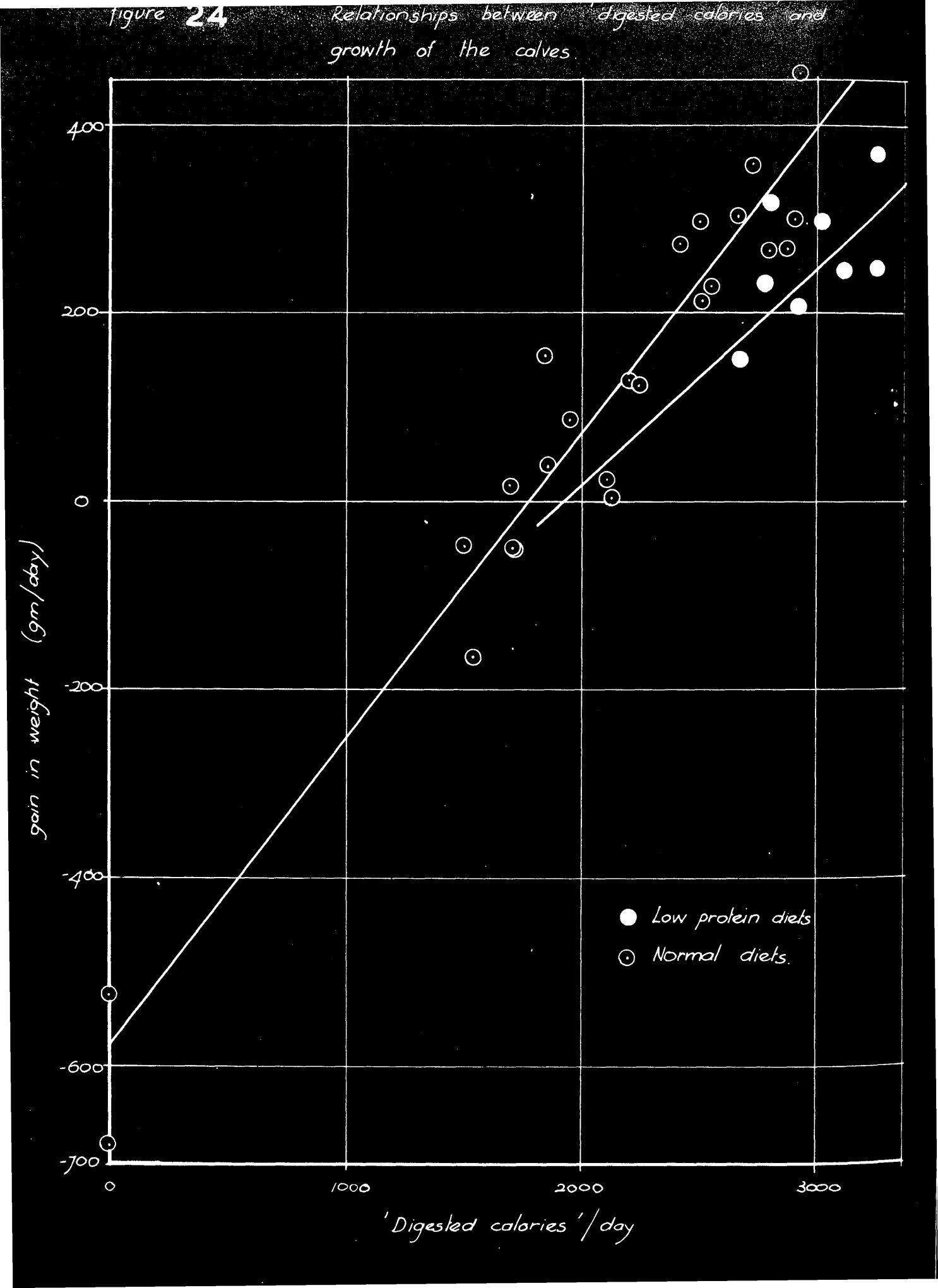
Low protein diets $NB = 0.0139\ G + 0.158$ ... (14a)

Other diets $NB = 0.0262\ G + 0.703$ ... (14b)

where NB is the nitrogen balance in g. / day and G the gain in bodyweight in g. / day. The results in the region of negative nitrogen balance were statistically significantly different from the regression obtained for normal diets, but not significantly different from the regression for low protein diets. Equation (14a) was therefore calculated from positive nitrogen balance results obtained with low protein diets together with negative nitrogen balance results obtained on all diets. The intercepts of 0.2 and 0.7 for the low- and adequate-protein diets respectively imply that when there is no gain in weight, storage of nitrogen still takes place, that is bodyweight maintenance does not entail cessation of growth. This confirms the results which were obtained when varying quantities of whole milk were given to the calf (see page 77).

The equations also show that for low-protein diets every 100 g. bodyweight gain is associated with the storage of 1.39 g. nitrogen (or.8.7 g. protein). During normal growth, the regression coefficient of 0.0262 shows that for every 100 g. bodyweight gain the calf stores 2.62 g. nitrogen (or 16.5 g. protein). This may be interpreted to mean that the major part of bodyweight gain in the young calf is a gain of flesh, since 16.5 g. protein would be expected to be present in about 80 - 85 g. muscle. The remainder must represent fat, bone and water. The difference between these two estimates (2.62 ± 0.122 less 1.39 ± 0.199) was highly significant indicating that the animals given the low-protein diet stored considerably less nitrogen per 100 g. body gain than did those given less protein-deficient rations. A larger part of the bodyweight gain of those animals receiving the low protein diet must therefore have been body-fat.

Figure 24 Relationships between 'digested calories' and growth of the calves.



4. Storage of calories during bodygrowth

The energy stored during body growth can only be determined calorimetrically or by slaughter analysis, but a first approximation can be made by statistical analysis of results obtained with diets containing low and adequate protein, and relating the digested energy intake to the gain in bodyweight. The regression of calorie intake (DC) on bodyweight gain (G) was:-

For low protein diets, $DC = 4.38\ G - 1921$ ... (15a)

and for other diets, $DC = 3.07\ G - 1771$ ... (15b)

where DC represents the total number of calories ingested daily less the calories excreted in the faeces. The regressions were both highly significant statistically ($P = 0.01$ and 0.001 respectively), although, as may be seen from Figure 24 the variation about the regressions was high. This variability is understandable since the errors involved in determining gain in weight and caloric intake are high; no account is taken of the loss of energy in the urine for the diets high in protein, or of the variation from animal to animal in energy requirements for maintenance and growth. The equations are only generalisations, but it is probable that they represent the average relationships which exist.

The regression constants in these two equations represent the number of digested calories required per g. gain in bodyweight (4.38 and 3.07 respectively). The difference between these two estimates was highly significant. This result agrees with the data showing that the nitrogen stored for every 100 g. gain in bodyweight was lower in those animals given low-protein diets. Presumably a larger part of the gain of these animals was fat having a greater calorific value per gram. It is not possible to calculate the calorific value of the retained material, for the fat, water and ash values associated with the gains is not known.

The intercepts of these two equations represent the theoretical maintenance requirement of the calves

TABLE 80

Net protein requirements, uncorrected for losses in digestion or in metabolism, and digestible energy requirements of young calves weighing 30 kg. together with an estimate of the percentage of the total calories which must be present as protein in order to avoid deamination of dietary protein to meet energy requirements.

Gain in weight (g./day)	Nitrogen retention (g./day)	Endogenous nitrogen (g./day) ≠	Total protein required by the tissues (g./day)	Digestible energy required (cal./day) ⁂	Percentage of the digestible energy needed as protein to avoid deamination
0	0.70	2.40	19.4	1572	6.9
100	3.32	2.40	35.7	1879	10.7
200	5.94	2.40	52.1	2186	13.5
400	11.18	2.40	84.9	2800	17.1
800	21.66	2.40	150.4	4028	21.1
1000	26.90	2.40	183.1	4642	22.2

≠ 80 mg./ kg. body-weight

⁂ 52.4 Cal./kg. bodyweight + 307 Cal./100 g. gain in weight

in terms of digested calories per day (1921 and 1771 cal. respectively.) The difference between these two estimates was not significant. The mean estimate of 1846 calories (58.1 calories per kg.) is in excess of the determined basal metabolism of 40 - 45 cal./kg./day by an amount of energy which may be accounted for by an activity increment of about 30%. This is extremely reasonable for an animal confined in a metabolism crate (Blaxter, 1948).

5. The net protein requirements of the calf

It was shown (see Table 33) that the endogenous nitrogen metabolism of the calf was 80 mg. / kg. bodyweight. This value represents the animals minimum requirement of nitrogen for maintenance of nitrogen equilibrium. Together with the results given in equations (14b) and (15b) (pages 116 and 117), this value permits an estimate of the requirements of protein and digestible energy of the calf. The protein requirements are net requirements since they only represent the protein which is stored or which will meet the endogenous losses in the urine. With proteins of 100% biological value and 100% digestibility they represent the minimal requirements of dietary protein that ensure no deamination of protein for energy purposes.

The results of the calculation are shown in Table 80. The last column of the table shows that the percentage of protein calories in the diet must increase with increasing rate of gain. The figures apply to proteins with a biological value of 100 only, and would be higher if the biological value of the protein was less than 100. If an animal were given a diet containing 20% of its digested energy as a high quality protein, maximal biological values would not be attained unless the total intake was sufficient to result in a gain of at least 700 g./day. If the intake was smaller, the urinary excretion of nitrogen would relatively increase, since the protein given would be greatly in excess of the requirements

TABLE 81

Minimum digestible crude protein requirement (lb. / day) for a 100 lb. Ayrshire calf gaining in weight from 0.86 lb. to 2.2 lb. per day, compared with the requirements advocated by Armsby and by Morrison.

	Gain in weight per day			
	0.86 lb.* (390 g.)	1.32 lb. (600 g.)	1.76 lb. (800 g.)	2.201b. (1000 g.)
Requirements calculated from present results (lb. protein/day) Biological value of the protein 90.	0.21	0.30	0.38	0.47
Requirements calculated from present results (lb. protein / day) Biological value of the protein 50	0.39	0.55	0.70	0.85
Armsby feeding standards. (lb. protein / day)	0.48			
Morrison feeding standards (lb. protein / day)	0.3 - 0.4			

* Ragsdale standard

at that particular rate of gain in weight.

Table 81 shows these results calculated in terms of lb. digestible crude protein / day for Ayrshire calves weighing 100 lb. and gaining in weight from 0.86 lb. per day to 2.2 lb. / day. A liveweight increase of 0.86 lb. per day is the Ragsdale standard rate for growth of a calf weighing 100 lb. (Ragsdale, 1934). In the calculation, the following equation was used:-

$$R = 6.25 \left\{ \left[\frac{1}{BV} (E + G + M.D) \right] - M.D \right\} \quad \ldots \quad (16)$$

as given by Blaxter & Mitchell (1948), where R = requirement for apparently digestible protein, B.V. = biological value of the protein, E = the minimum endogenous loss of nitrogen, G = the daily increment in tissue nitrogen during growth, M = the metabolic faecal nitrogen excretion per 100 g. of dry matter ingested and D is the dry matter intake.

The apparent digestibility of the protein was taken as 96% (see page 109) and if whole milk proteins were used, a biological value of about 90 should be obtained. In order to compare the results with feeding standards given by other workers the results are also calculated in terms of digestible crude protein with a biological value of 50. These requirements again represent minimal requirements without any margin for safety. When compared with allowances of crude protein advocated by Armsby (1917) and Morrison (1948), it will be seen that the protein standards of Morrison are hardly sufficient to maintain the growth of 0.86 lb. per day and certainly no higher rate of liveweight increase, unless whole milk is the sole food. The Armsby feeding standards allow a safety margin of 23% for the Ragsdale growth standard, and are no doubt ample for the growth rate of 1 lb. per day - the rate of growth regarded as ideal by most agricultural advisors. They are sufficient for larger gains, however, only if protein with a high biological value is given.

It may be concluded that the standard rates

of gain of a calf are far from maximal, and that the feeding standards employed are therefore lower than the optimum and are in fact wasteful of protein since, as the growth rate of a calf becomes greater, the endogenous losses become a smaller proportion of the whole. The calf does not have a 'minimum' protein requirement, but only a minimal requirement for a certain rate of growth.

RESUMÉ

The introduction indicated that little is known regarding the protein and energy requirement of the young calf for maintenance and growth, and that the calf should be just as dependent on a dietary source of amino-acids as is the rat or man.

The detailed nitrogen metabolism of the young calf had not previously been studied. In order to establish the basic metabolism of this animal, the losses during starvation and the endogenous nitrogen excretion were determined. The calf has a basal metabolism at least twice that of the mature animal, or that of animals of other species with a similar body size. It was concluded that an estimate of endogenous nitrogen excretion may be made from a determination of the basal energy metabolism.

It was shown that maximal biological values are attained in the calf only when a high intake of artificial diet containing a low percentage of protein is given. With whole milk, maximal biological values were not attained at high intakes, due to the comparatively high content of protein. A nitrogen free supplement (gelatinised starch) therefore increased nitrogen storage.

Gelatin, casein and the proteins of dried skim milk have different biological values for the young calf. These differences are of the same order as those observed in the rat or man. This is the first unequivocal demonstration of a large farm animal requiring a dietary source of amino-acids.

The final section of the thesis related body gains to nitrogen storage and to digested calories from which the protein requirement of the calf was calculated. It was shown that the calf has not a definite minimum protein requirement, but only a minimum requirement for a given rate of growth.

A more detailed summary of the results is given separately.

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REFERENCES

Albanese, A.A., & Irby, V. (1944)
J. biol. Chem. 153, 583

Allison, J.B. (1948)
Ann. Rev. Biochem. 17 , 275

Allison, J.B. (1951)
Fed. Proc. 10 , 676

Allison, J.B., & Anderson, J.A. (1945)
J. Nutrit. 29 , 413

Allison, J.B., Anderson, J.A., & Seeley, R.D. (1946)
Ann. N.Y. Acad. Sci. 47 , 245

Amann, O.V. (1933)
Arch. int. Physiol. 37 , 121

Angels, J., & Cannon, C.Y. (1936)
Amer. Soc. Animal Prod. 223, Proc.29

Annegers, J.H., Boutwell, J.H., & Ivy, A.C. (1948)
Gastroenterologia 10 , 486

Archibald, J.G., Kucinski, K.S., Brook, R.O., & Greeman,S.L. (1938)
J. Dairy Sci. 21 , 59

Armsby, H.P. (1917)
"The nutrition of farm animals"
MacMillan Co: New York.

Armsby, H.P., & Fries, J.A. (1911)
Bull, U.S. Dept. Agric. no.128

Armsby, H.P., & Moulton, C.R. (1925)
"The Animal as a Converter of Matter and Energy."
New York: Chemical Catalogue Co. Inc.

Aschaffenburg, R. Bartlett, S; Kon, S.K.; Terry, P., Thomson, S.Y., Walker, D.M., Briggs, C.; Cotchin, E., & Lovell, R. (1949)
Brit.J. Nutrit. 3 , 187

Association of Official Agricultural Chemists (1945)
"Official and Tentative Methods of Analysis".
Washington: Sixth Edition

Atkeson, F.W. (1931)
Idaho Agr.Exp. Sta. Bull. 179

Baker, F; Nasr, H., Morrice, F. & Bruce, J. (1950)
J. Path. Bact. 62 617

Baldwin, E. (1948)
Dynamic Aspects of Biochemistry.
Cambridge: University Press.

Bate, W., Dwight, E., & Cannon, C.Y. (1946)
J. Dairy Sci. 29 , 41
Beadles, J.R., Quisenberry, J.H., Nakamura, F.I., & Mitchell, H.H. (1933)
J. agric. Res. 47 , 947
Benedict, F.G. (1907)
Publ. Carneg. Instn. no.77
Benedict, F.G. (1915)
Publ. Carneg. Instn. no.203
Benedict, F.G., & Fox, E.L. (1934)
Amer. J. Physiol. 108 , 285
Benedict, F.G., & Ritzman, E.G. (1927)
Publ. Carneg. Instn. no.377
Benedict, F.G., & Ritzman, E.G. (1931)
Arch. Tierernähr. Tierz. 5 , 1
Blackwood, J.H., Morris, S., & Wright, N.C. (1936)
J. Dairy Res. 7 , 228
Blaxter, K.L. (1948)
J. agric. Sci. 38 , 207
Blaxter, K.L., & Howells, A. (1951)
Brit. J. Nutrit 5 , 25
Blaxter, K.L., & Mitchell, H.H. (1948)
J. Animal. Sci. 7 351
Blaxter, K.L., Watts, P.S., & Wood, W.A. (1951)
Brit. J. Nutrit. (In press)
Blaxter, K.L., & Wood, W.A. (1951)
Brit. J. Nutrit. (In press)
Block, R.J., & Mitchell, H.H. (1945-6)
Nutr. Abst. & Rev. 16 , 249
Boussingault, J.B. (1857)
"Rural Economy" trans. by G. Law
C.M. Saxton Co.; New York
Braman, W.W. (1924)
J. biol. Chem. 60 , 79
Bricker, M., Mitchell, H.H., & Kinsman, G.M. (1945)
J. Nutrit. 30 , 269
Brisson, G.J., & Sutton, T.S. (1951)
J. Dairy Sci. 34 , 28
Brody, S. (1930)
Univ. Mo. agric. Exp. Sta. Res. Bull. 143
Brody, S. (1932)
Res. Bull. Mo. agric. Exp. Sta. no.166

Brody, S. (1945)
"Bioenergetics and Growth"
Reinhold Pub. Co.: New York.

Burroughs, E.W., Burroughs, H.S., & Mitchell, H.H.(1940)
J. Nutrit. 19 , 271

Buschmann, A. (1907)
Landw. Vers. Sta. 101 , 1

Cathcart, E.P., & Green, H.H. (1913)
Biochem. J. 7 , 1

Carpenter, T.M. (1927)
Amer. J. Physiol. 81 , 519

Cathcart, E.P. (1907)
Biochem. Z. 6 , 109

Cook, B.B., Morgan, A.F., Weast, E.O., & Parker, J.(1951)
J. Nutrit. 44 , 51

Davidson, J.N. (1945)
Biochem. J. 39, lix.

Davidson, J.N., & Waymouth, C. (1944)
Biochem. J. 38 , 379

Deuel, H.J. Jr., & Gulick, M. (1932)
J. biol. Chem. 96 , 25

Dewar, A.D., & Newton, W.H. (1948-9)
Brit. J. Nutrit. 2 , 123

Dukes, H.H. (1947)
The Physiology of Domestic Animals.
New York: Comstock Publishing Co. Inc.
Sixth Edition

Duncan, C.W., Huffman, C.F., & Robinson, C.S. (1935)
J. biol. Chem. 108 , 35

Edwards, W.F., & Balzac (1832)
Ann. Sci. nat. 26 , 318

Elsden, S.R., Hitchcock, M.W.S., Marshall, R.A., & Phillipson, A.T. (1946)
J.expt. Biol. 22 , 191

Escher, T. (1876)
Vischr. naturf. Ges. Zürich, 21 , 36

Fairbanks, B.W., & Mitchell, H.H. (1935)
J. Agric. Res. 51 , 1107

Ferguson, W.S. & Neave, O. (1943)
J. agric. Sci. 33 , 95

Fingerling, G. (1908)
Landw. Versuchsw. 68 , 141

Fingerling, G. (1912)
Wender. Vers. Sta. 76 , 1
Fischer, J.E., & Sutton, T.S. (1949)
J. Dairy Sci. 32 , 139
Folin, O. (1905)
Amer. J. Physiol. 13 , 117
Gamble, J.L., Ross, G.S., & Tisdall, F.F. (1923)
J. biol. Chem. 57 , 633
Garry, R.C. (1926-7)
J. Physiol. 62 , 364
Gullickson, T.W.; Fountaine, F.C., & Fitch, J.B. (1942)
J. Dairy Sci. 25 , 117
Hagemann, O. (1891)
Landw. Jb. 20 , 264
Hamilton, T.S. (1937)
Thesis; University of Illinois.
Hamilton, T.S. (1938)
Quoted by Mitchell, H.H. (1938) in "The Biological Values of the Proteins and a Criticism of the Methods of its Determination."
Rome: Reale Accademia d'Italia
Hanne, R. (1907)
Milchw. Ztg. 36 , 267
Harris, L.E., & Mitchell, H.H. (1941 a)
J. Nutrit. 22 , 167
Harris, L.E., & Mitchell, H.H. (1941 b)
J. Nutrit. 22 , 183
Hart, E.B., Humphrey, G.C., & Morrison, F.B. (1912)
J. biol. Chem. 13 , 133
Hawk, P.B., Oser, B.L., & Summerson, W.H. (1947)
Practical Physiological Chemistry.
Blakiston Co. : Philadelphia, Toronto
Hector, A., & Rowat, R. (1948)
Scot.J. Agric. 28 , 43
Henry, K.M., Houston, J., Kon, S.K., & Osborne, L.W. (1939)
J. Dairy Res. 10 , 272
Henry, K.M., Kon, S.K., Lea, C.H., & White, J.C.D.(1948)
J. Dairy Res. 15 , 292
Hill, E., & Bloor, W.R. (1922)
J. biol. Chem. 53 , 171
Hittcher, R. (1909)
Landw. Jb. 38 871

Honcamp, F., Koudela, S., & Muller, E. (1923)
Biochem. Z. 143 , 111
Hutchinson, J.C.D., & Morris, S. (1936 a)
Biochem. J. 30 , 1682
Hutchinson, J.C.D., & Morris, S. (1936 b)
Biochem. J. 30 , 1695
Jacobson, N.L., & Canon, C.Y. (1947)
J. Dairy Sci. 30 , 587
Jacobson, N.L., Cannon, C.Y., & Thomas, B.H. (1949)
J. Dairy Sci. 32 , 429
Jamieson, S. & Allcroft, R. (1950)
Brit. J. Nutrit. 4 , 16
Jarvis, R.N., & Waugh, R.K. (1949)
J. Dairy Sci. 32 , 665
Johnson, B.C., Hamilton, T.S., Mitchell, H.H., & Robinson, W.B. (1942)
J. Animal Sci. 1 , 236
Johnson, B.C., Hamilton, T.S., Nevens, W.B., & Boley, L.E. (1948)
J. Nutrit. 35 , 137
Johnson, C.B., Pinkos, J.A., & Burke, K.A. (1950)
J. Nutrit. 40 , 309
Johnson, B.C., Wiese, A.C., Mitchell, H.H. & Nevens, W.B. (1947)
J. biol. Chem. 167 , 729
Jordon, L. (1933)
Vet. J. 89 , 202
Jucker, H. (1948)
Inst. f. Haustierernährung in der E.T.H. Zurich
Käppli, J. (1925)
Landw. Jb. Schweiz S.6
Kellner, O. (1915)
The Scientific Feeding of Animals.
MacMillan Co. New York
King, E.J. (1946)
Micro-analysis in medical Biochemistry.
London : Churchill
Kik, M.C. (1938)
Ark. agric. Exp. Sta. Bull. 352
Kon, S.K. (1928)
Biochem. J. 22 , 261
Kon, S.K. & Porter, J.W.G. (1947-8)
Nutr. Abst. Rev. 17 , 31

Kosterlitz, H.W. (1944)
Nature, Lond., 154 , 207
Krebs, K. (1937)
Tierernährung 9 , 394
Larson, H.W. (1932)
J. biol. Chem. 94 , 727
von Liebig, J. (1843)
"Animal Chemistry, or Organic Chemistry in its application to physiology and pathology".
Taylor and Walton; London.
Lindsey, J.B. (1894)
Mass. agric. Expt. Sta. 12th Ann. Rep. 125
Lindsey, J.B., & Archibald, J.G. (1925)
Mass. agric. Exp. Sta. Bul. 223
Ling, E.R. (1944)
A Textbook of Dairy Chemistry. Vol I. Theoretical.
London: Chapman & Hall Ltd.
Lofgreen, G.P., Loosli, J.K., & Maynard, L.A. (1951)
J. Animal Sci. 10 , 171
Lovell, R., & Hill, A.B. (1940)
J. Dairy Res. 11 , 225
Lusk, G. (1931)
"The Science of Nutrition".
Philadelphia : W.B. Saunders Co.
Magendie, F. (1816)
Ann. chim. (Phys.) 3 , 66
Majoor, C.L.H. (1946)
Yale J. Biol and Med. 18 , 419
Majoor, C.L.H. (1947)
J. biol. Chem. 169 , 583
Marston, H.R. (1948)
Aust. J. Sci. Res. 1, ser B, p.93
Maso, I.D. , & Palmer, L.S. (1935)
J. Nutrit. 9 , 489
McCollum, E.B., & Steenbock, H. (1912)
Wis. agric. Exp.Sta. Res. Bul. 21
McCrae, T.F., El Sadr, M.M., & Sellers, K.C. (1942)
Biochem. J. 36 , 460
McLellan, W.S., & Hannon, R.R. (1932)
J. biol. Chem. 95 , 327
McNaught, M.L., & Smith, J.A.B. (1947)
Nutr. Abs. Rev. 17 , 18

Miller, J.I. (1937)
J. Agric. Res. 55 , 467
Miller, J.I., & Morrison, F.B. (1942)
J. Animal. Sci., 1 , 353
Minett, F.C. (1946)
Indian J. Vet. Sci. 16 , 123
Mitchell, H.H. (1923-4)
J. biol. Chem. 58 , 873
Mitchell, H.H. (1926)
Bull. nat. Res. Coun. No.55 Washington D.C.
Mitchell, H.H. (1929)
Bull. nat. Res. Coun. No.67 Washington D.C.
Mitchell, H.H. (1944)
Indust. Eng. Chem. Anal. Ed., 16 , 696
Mitchell, H.H. (1948)
In "Proteins and Amino Acids in Nutrition" p. 46 (M. Sahyun, editor)
New York: Reinhold Publishing corporation.
Mitchell, H.H., & Beadles, J.R. (1926-7)
J. biol. Chem. 71 , 429
Mitchell, H.H., Beadles, J.R., & Kruger, J.H. (1927)
J. biol. Chem. 73 , 763
Mitchell, H.H. & Hamilton, T.S. (1929)
"The Biochemistry of Amino-acids".
New York: Chemical Catalogue Co.
Morris, S., & Ray, S.C. (1939)
Biochem. J. 33 , 1217
Morrison, F.B. (1948)
"Feeds and Feeding"
New York; Morrison Publication Co.
Mukherjee, R. (1946)
"Studies of protein Metabolism in Relation to Cattle Nutrition".
Thesis, University of Dacca.
Mulder, G.J. (1839)
J. prakt. Chem. 16 , 129
Murley, W.R., Jacobson, N.L., Wise, G.H., & Allen, R.S. (1949)
J. Dairy Sci. 32 , 609
Murlin, J.R., Edwards, L.E., Hawley, E.E., & Clark, L.C. (1946)
J. Nutrit. 31 , 533
Murlin, J.R., Szymanski, T.A., & Nasset, F.C. (1948)
J. Nutrit. 36 , 171

Nevens, W.B., & Shaw, D.D. (1933)
J. Nutrit. 6 , 139
N.Z. Dept. of Agric. (1948)
Annual report.
Nielson, J.P. (1943)
Indust. Eng. Chem. Anal. Ed. 15 , 176
Nielson, J.P. (1945)
Indust. Eng. Chem. Anal. Ed. 17 , 131
Norton, C.L., Eaton, H.D., Loosli, J.K., & Spielman, A.A. (1946)
J. Dairy Sci. 29 , 231
Osborne, T.B., Mendel, L.B. & Ferry, E.L. (1919)
J. biol. Chem. 37 , 223
Palmer, W.W., Means, J.H., & Gamble, J.L. (1914)
J. biol. Chem. 19 , 239
Pearson, R.M., & Smith, J.A.B. (1943 a)
Biochem. J. 37 , 142
Pearson, R.M., & Smith, J.A.B. (1943 b)
Biochem. J. 37 , 148
Pearson, R.M., & Smith, J.A.B. (1943 c)
Biochem. J. 37 , 153
Peters, J.P., & Van Slyke, D.D. (1931)
Quantitative Clinical Chemistry
Vol. 1, Interpretations, 1st. ed.
London: Bailliere Tindall and Cox.
Peters, J.P., & Van Slyke, D.D. (1932)
Quantitative Clinical Chemistry, Vol.II, Methods.
London: Bailliere, Tindall & Cox.
Phillips, P.H., Lundquist, M.S., & Boyer, P.D. (1941)
J. Dairy Sci. 24 , 977
Prout, W. (1855)
"Chemistry, Meteorology, and the function of digestion."
H.G. Bohn; London.
Ragsdale, A.C. (1934)
Bull. Mo.Agr.expt.Sta. no. 336
Ritzman, E.G., & Benedict, F.G. (1938)
Publ. Carneg. Instn. no.494
Ritzman, E.G., Washburn, L.E., & Benedict, F.G. (1936)
Tech. Bull. N.H. agric. Expt.Sta. no.66
Rojas, J., Schweigert, B.S., & Rupel, I.W. (1948)
J. Dairy Sci. 31 , 81

Rose, W.C. (1911-2)
J. biol. Chem. 10 , 265
Savage, E.S., & McCay (1942)
J. Dairy Sci. 25 , 595
Schneider, B.H. (1934)
Biochem. J. 28 , 360
Schneider, B.H. (1935)
J. Biol. Chem. 109 , 249
Schneider, B.H. (1947)
"Feeds of the World, their Digestibility and Composition"
Univ. West Vir. agric.
Exp. Sta.
Schneider, W.C., & Klug, H.L. (1946)
Cancer Res. 6 , 691
Schoenheimer, R., & Rittenberg, D. (1940)
Physiol. Rev. 20 , 218
Scholz, R. (1930)
Tierernährung 1 , 502
Shaw, R.H., Woodward, T.E., & Norton, R.P. (1912)
J. Agric. Res. 12 , 575
Shohl, A.T. (1939)
"Mineral Metabolism"
New York: Reinhold Pub. Co.
Sideris, C.P. (1937)
Indust. Eng. Chem. Anal. Ed. 9 , 145
Sideris, C.P. (1942)
Indust. Eng. Chem. Anal. Ed. 14 , 821
Sjollema, B., & van der Zande, J.E. (1923)
J. metab. Res. 4 , 525
Smith, T., & Little, R.B. (1924)
J. expt. Biol. 39 , 303
Smuts, D.B. (1935)
J. Nutrit. 9 , 403
Sorg-Matter, H. (1928)
Arch. Int. Physiol 30 , 126
Soskin, S., & Levine, R. (1946)
Carbohydrate Metabolism.
Chicago: University Press.
Soxhlet, F. (1878)
"Untersuchungen über den Stoffwechel des Saugkalbes".
Vienna: ist. Rep. Agric. Exp. Sta.

Stamp, J.T., & Blaxter, K.L. (1951)
Unpublished observations
Steenbock, H., Nelson, V.E., & Hart, E.B. (1915)
Res. Bull. Wis. agric. Exp. Sta. no.36
Sumner, E.E. (1938)
J. Nutrit. 16 , 129
Swaminathan, M. (1937 a)
Indian. J.med. Res. 25 , 57
Swaminathan, M. (1937 b)
Indian J. med. Res. 25 , 381
Terroine, E.F., & Sorg - Matter, H. (1927)
Arch. Internat. Physiol. 29 , 121
Tidwell, H.C., & Holt, L.E. (1936)
J. biol. Chem. 112 , 605
Tomme, F., & Taranenko, G.A. (1939)
Proc. Lenin. Acad. Agric. Sci. U.S.S.R. 10 , 36
Treichler, R. (1939)
"The relationship between basal metabolism and endogenous nitrogen metabolism."
Thesis, University of Illinois.
Treichler, R., & Mitchell, H.H. (1941)
J. Nutrit. 22 , 333
Vawter, L.R., & Records, E. (1947)
J. Amer. vet. med. Ass. 110 , 152
Voit, C. (1872)
Cited by McCollum, E.V., Orent-Keiles, E., & Day, H.G.
"The Newer Knowledge of Nutrition"
MacMillan Co. (1939)
Voit, E. (1901)
Z. biol.. 41 , 188
Ward, A.H. (1945)
Proc. N.Z. Soc. Animal Prod. 10
Warner, R.G., & Sutton, T.S. (1948)
J. Dairy Sci. 31 , 976
Waters, H.J. (1908)
Proc. Soc. Prom. agric. Sci. 29th. meeting 71
Waters, H.J. (1910)
Kan. State Board agric. Rep. no. 113 , 59
Weaver, E., Horwood, R.E., & Smiley, E.S. (1949)
Bull. Mich. Quart. 32 , 42

Wesson, L.G. (1930-31)
J. Nutrit. 3 , 503
Wiese, A.C., Johnson, B.C., & Mitchell, H.H. (1947)
J. Dairy Sci. 30 , 87
Wiese, A.C., Johnson, B.C., & Nevens, W.B. (1946)
Proc. Soc. expt. Biol. & Med. 63 , 521
Wiese, A.C., Johnson, B.C., Mitchell, H.H., & Nevens, W.B. (1947)
J. Nutrit. 33 , 263
Williams, H.H., Shepherd, M.L., & Endicott, E.E. (1939)
J. biol. Chem.128 , Proc. cxi - cxii.
Wilson, H.E.C. (1925)
Biochem. J. 19 , 322
Wood, T.B., & Hill, A.V. (1914)
J. agric. Sci. 6 , 252
Wood, T.B., & Yule, U. (1914)
J. agric. Sci. 6 , 233
Work, S.H., Hamre, C.J., Henke, L.A., & Harris, L.E. (1943)
J. Animal. Sci. 2 , 166
Young, E.G., & Conway, C.F. (1942)
J. biol. Chem. 142 , 839
Zuntz, N. (1891)
Pflüg. Arch. 49 , 483