SOME ASPECTS OF THE DEVELOPMENT OF RUMEN FUNCTION IN DAIRY CALVES REARED ON PASTURE.

A Thesis Presented at Massey College in Partial Fulfilment of the Requirements for the degree of Master of Agricultural Science in the Victoria University of Wellington.

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by

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INTRODUCTION.

Economy in feeding the ruminant is based on taking full advantage of rumen function. At birth however the rumen is not functional and the very young animal is dependent on a diet which it can digest without aid from microbial fermentation. The need for a readily assimilated diet is normally fulfilled by the mother's milk. In the case of naturally reared animals this milk may continue to provide a significant proportion of the nutrients in the diet of the young animal even when rumen function has been established and it is capable of obtaining its nutrient requirements from grazing alone.

Recognition of the fact that milk is an ideal food for young animals is reflected in the extended periods of milk feeding which in the past have been characteristic of the rations fed to artificially reared dairy stock. However there may be several disadvantages in such a practice, not the least of which being the amounts of milk and labour involved.

In the search for more economical methods of calf rearing, the possibility of weaning at an earlier age has been investigated. Calves have been weaned as early as 3 weeks of age (Preston 1960), and it is known that calves which are provided with high quality pasture may be weaned at about 8 weeks of age without ill effect.

The age at which weaning may be accomplished will depend on the age at which rumen function develops or is capable of developing. Efforts have been made to elucidate the physiological changes which occur in the rumen during the transition from non ruminant to ruminant and considerable advances have been made in this field. However little information is available on the normal pattern of development of rumen function in dairy calves. Such information as is available suggests that, in line with the experience from early weaning, rumen function can become established at an early age. Nevertheless further investigation of rumen function development seemed desirable, particularly in relation to calves reared on pasture.

Following a preliminary trial to investigate and become familiar with techniques, an experiment was conducted in which some aspects of rumen function development in pasture fed calves were studied. The procedures used in these studies and the results obtained are reported, together with a brief review of the literature and a discussion of the results.

REVIEW OF LITERATURE.

I. Comparative Anatomy and Physiology of the Stomach* of the Newborn and Adult Bovine.

The capacity of the adult rumen varies greatly with the age and size of the animal, but usually accounts for some 80 per cent of the total stomach volume (Annison and Lewis, 1959). Sisson and Grossman (1954) state that, in the newborn ruminant, the rumen and reticulum together are about half as large as the abomasum. For newborn calves, Warner et al (1956) demonstrated that the reticulo-rumen volume was three-quarters of the volume of the abomasum and Flatt et al (1959) showed the rumen alone to be half the size of the abomasum, but these results were obtained with a water filling technique which may have stretched the rumen and reticulum more than the abomasum.

The lining of the adult forestomach consists of a stratified squamous epithelium (Phillipson, 1961) which in the rumen is extensively papiliated; in the reticulum has a honey combed-like structure; and in the omasum is in the form of longitudinal folds which occupy the major portion of its cavity (Sisson and Grossman, 1954). In contrast, the newborn calf has a forestomach that is rudimentary; the papillae of the rumen wall are soft and short, the spaces between the leaves of the omasum are not fully patent and the honeycombed-like structure of the wall of the reticulum is particularly undeveloped (Blaxter, 1954).

Within the adult rumen an environment which supports a large and diversified microbial population is normally maintained (Annison and Lewis 1959). This microbial population is largely responsible for the chemical reactions occurring within the rumen. Among the major functions of the rumen are the digestion of cellulose, the synthesis of protein from non protein nitrogen and the synthesis of B vitamins (Phillipson, 1960). Concomitant with cellulose digestion is the production and absorption of volatile fatty acids (Annison and Lewis 1959).

* In this thesis, "stomach" includes all four compartments (rumen, reticulum, omasum, and abomasum) since they all develop from the embryonic stomach (Blaxter, 1954); "forestomach" refers to the three compartments rumen, reticulum and omasum.

These functions have been shown to be non existent or evident only to a small degree in the very young calf (See Section IV.).

Furthermore the act of rumination, which in the adult is well developed and occupies a large portion of the animals' time (Hancock 1953), is absent in the newborn animal.

Summary.

Whereas the forestomach of the adult bovine is relatively large, well developed and capable of digesting cellulose, producing and absorbing volatile fatty acids and synthesizing protein and B vitamins, the forestomach of the newborn calf is rudimentary both anatomically and physiologically.

II. A Comparison of the Techinques Used in Obtaining Data for the Study of Developing Rumen Function and Anatomy.

(a) Slaughter of the animal.

Slaughter enables the investigator to measure the weight of the stomach compartments and their tissue components. Also papillary growth may be accurately measured and other histological observations made. Such measurements are not possible with other techniques.

The major disadvantage of the slaughter technique is that measurements are obtainable at only one stage in the animal's life. In order to compare rumen development at different stages, a large number of calves is needed if good estimates of mean development and variability are to be obtained at each stage.

(b) Fistulation.

In contrast to slaughter of animals, fistulation makes possible the repeated observations of some criteria on the same animal at different stages. In particular samples of rumen contents for analysis may be taken from known sites; in vivo cellulose digestion may be studied; and in vivo volume measurements may be obtained. It also provides a means of administering known quantities of treatment materials that would not normally be consumed by the animal.

While it is generally conceded that a properly closed fistula in mature animals is not deleterious (e.g. Drori & Loosli, 1959) it is by no means certain that such is the case in calves. Flatt *et al* (1959) found that fistulation resulted in lower dry food consumption and retarded growth rate of their calves. The excellent gains and feed conversion efficiencies of their fistulated milk fed calves indicate that the fistula *per se* was not detrimental where *ad lib* dry feed consumption was not a factor.

While it is possible that the utilization of dry feed was impaired by the imperfect rumen cannula, Flatt *et al* (1959) claim that the data they present does not justify such a conclusion. However their data concerning fistulated and unfistulated calves (four calves each) fed hay and grain show that the fistulated calves ate less dry food, grew at a slower rate, and were less efficient converters of food (as indicated by TDN/lb gain) than the unfistulated calves, indicating that dry food utilization was impaired. Although similar body weight gains and dry feed dry matter intakes were maintained for fistulated and

unfistulated hay fed calves, only one calf per treatment was involved and it is not made clear whether or not different milk intakes were responsible for the similar body weight gains. That such a possibility exists is indicated by their earlier statement that "calves received varying amounts of milk ------- to maintain the desired rates of gain in body weight".

The Cornell workers (Flatt et al, 1959) concluded that fistulation "did not prevent the deposition of stomach tissue, papillary growth, cellulose digestion, and production of VFA, suggesting that fermentation was not seriously impaired." Since no data are presented to enable a comparison of fistulated and unfistulated calves to be made (except for one comparison of papillary index which was lower for fistulated calves fed hay and grain) it is not clear to what extent fermentation was impaired.

(c) Stomach tube sampling.

Use of the stomach tube enables samples of rumen contents to be obtained from intact calves. Although this permits the repeated sampling from the same calf, this method suffers from the disadvantage that the investigator is never sure from which part of the reticulo rumen the sample is taken. Because of the heterogeneity of rumen contents (Bryant, 1961), variation due to position of sampling is not eliminated.

Summary.

Slaughter is the only method of obtaining anatomical data apart from capacity but as measures are obtained at only one stage in the calf's life it is unsuitable for observations on other criteria which may exhibit large daily variation. Fistulation and stomach tube sampling, because they enable repeated sampling overcome this difficulty but provide no anatomical data (except for in vivo capacity in the case of fistulation). However effects of fistulation in calves have not been adequately investigated and the site of sampling with the stomach tube is unknown.

III Some Criteria which have been used in the study of developing rumen function in calves.

(a) The digestion of cellulose.

Evidence of cellulolytic activity in the rumen has been sought by in vivo and in vitro techniques. Flatt *et al* (1959) estimated cellulose digestion by measuring the loss of weight of loops of cotton thread suspended in the rumen via a rumen fistula for 24 hours at a time. These workers found the method

> "quite useful for the qualitative determination of cellulose digestion and for purposes of comparison,"

although no critical evaluation of the method appears to have been made.

A similar method was used in mature animals by Balch and Johnson (1950). They showed that the standard deviation of 10 individual values from a mean loss of 19.1% was ± 1.6% when the loops were suspended together in the ventral sac of the rumen of one of their cows. Considerable differences were found in loss of weight between positions when loops were suspended in dorsal sac, ventral sac or "mid-rumen". These workers also found a highly significant high correlation ($r = +.940$ d.f. = 7) between time required for a 50% loss of dry matter of the cotton loop and dry matter content of the surrounding digesta, which may explain part of the variation encountered between positions in the rumen.

This method of estimating cellulose digestion commends itself because of its apparent simplicity and reliability. However its use in the study of developing rumen function needs care because of the variability which may arise due to the position of the loops in the rumen and the possible effect of dry matter of the surrounding digesta.

Two *in vitro* methods of estimating cellulose digestion were used by Lengemann & Allen (1955; 1959). The first consisted of suspending a weighted cotton thread in rumen fluid and measuring the time taken for the thread to break. However Balch & Johnson (unpublished, cited by Balch & Johnson 1950) found that when measured by attachment of weights, the breaking stress of cotton

Thread varies considerably at different places on the one thread. The second method used by Lengemann & Allen was the in vitro gas production of a sample of rumen liquor using Solka floc as substrate. The reliability of this method has not been tested but the data presented by these workers indicates that results may be highly variable.

It is apparent that further investigation is needed before any of these methods of estimating cellulose digestion can be used with certainty. In particular, the relationship between the estimated cellulose digestibility as obtained by these methods and the true cellulose digestibility is not known. On the basis of present knowledge however, it would appear that the method used by Balch and Johnson and Flatt et al is most promising as a means of comparison.

(b) Production of volatile fatty acids and pH of the rumen contents.

It is now well established that volatile fatty acids (VFA) are produced in the rumen of adult animals as a result of degradation and fermentation of carbohydrate and protein by microorganisms (see Annison & Lewis 1959). Consequently, the presence of VFA in the rumen of the calf may be taken as evidence of microbial fermentation.

The estimation of total VFA is commonly achieved by titrating the distillate from steam distillation of a sample of rumen contents. Because organic acids besides fatty acids are steam volatile, although to a lesser extent, the distillation procedure is adjusted in order that their influence on the titre is minimized. Some steam distillation procedures were reviewed and experimentally tested by Bryant (1961) who concluded that

> "the high levels of VFA present in the ruminal contents can, for routine analysis be estimated sufficiently accurately by the steam distillation procedures outlined. More accurate but time consuming procedures similar to those used for blood are obviated by the high levels of VFA and low levels of interfering substances found in the rumen."

From the work of Bryant and others (see Bryant's review) it is apparent that the concentration of total VFA will differ according to -

(i) the site of sampling

and (ii) the time of sampling; day to day and diurnal variation has been established.

Furthermore, the type of diet has been shown to influence the pattern of VFA production (Balch & Rowland 1957). These variations must be accounted for if a meaningful estimation of VFA is to be made. The use of fistulated animals is the best way in which this may be done (Bryant 1961), as serial sampling may be performed at a known site.

The use of concentration of VFA has one serious limitation in that the actual concentration at any time will be the net result of production, absorption and dilution. However until evidence is obtained to the contrary, it is probably safe and indeed necessary to assume that where the concentration of VFA in rumen of the calf is within the adult range over a period of time, then rumen function has been established. This appears to be the implied assumption of investigators in the field of developing rumen function (e.g. McCarthy & Kesler 1956; Godfrey 1961b; Hibbs et al 1956).

There are several difficulties involved in the use of pH as a criterion of rumen function.

Firstly there is the problem of obtaining a reliable estimate of the pH as it occurs in situ. Turner & Hodgetts (1955a), investigating buffer systems in the sheep's rumen showed that loss of CO_2 on exposure to air resulted in a rise in pH. Aspiration of ingesta from the rumen with a stomach tube may result in a high estimate of pH because of contamination with saliva (Briggs et al 1957), air (Turner & Hodgetts 1955a), or both. Briggs et al reported a method of obtaining rumen samples which, it was reported, gave pH readings identical with those obtained with electrodes in situ. Variation in pH with position and time of sampling has also been demonstrated (Bryant 1961).

Even when a reliable estimate of the pH of ruminal contents has been obtained there remains the problem of interpretation of this data.

Differences in buffering capacity of ruminal fluid have been shown to be associated with differences in interval after feeding, nature of the diet and consumption of drinking water, and were correlated with differences in total and relative concentrations of bicarbonate phosphate and VFA (Turner & Hodgetts 1955b). Cason et al

(1954) claimed that the pH was closely related to ash content of the ingesta. Other factors which may influence pH of ruminal contents are ammonia concentration, lactic acid concentration and salivary secretion (Briggs et al 1957).

(c) Other Criteria.

Several groups of investigators who have studied rumen development in calves have used anatomical criteria as a basis for their study. Those criteria which may be classed as anatomical include abattoir stomach volume (Warner et al 1956), in vivo reticulo-rumen volume (Flatt et al 1956), fresh weights of stomach tissues (Brownlee 1956; Godfrey 1961a), fat-free dry matter of stomach tissues (Warner et al 1956) and mucosal development and papillary growth (Warner et al 1956; Brownlee 1956; Flatt et al 1959; Harrison et al 1960). Differences in anatomical development of the rumen and reticulo-rumen due to age (Warner et al 1956; Godfrey 1961a) and diet (Warner et al 1956; Brownlee 1956) are evident but such differences probably reflect differences in rumen fermentation (See Section IV).

Pounden & Hibbs (1948) proposed the use of type organisms as indicators of the presence or absence of a characteristic microbial population. However proof neither was sought nor obtained that the organisms mentioned were the most important ones in the digestion of feeds present. Although the data of Lengemann and Allen (1959) indicated that there may be some relationship between the establishment of type organisms and VFA production, the establishment of a microbial population characteristic of the adult may not necessarily be essential for rumen function. Russof (1951) who used a classification similar to that proposed by Pounden & Hibbs (1948) contended that:

> "----------any attempt to gain an adequate indication of the true nature of what is happening in the rumen requires more than a microscopic examination of its contents. The heterogeneity of morphologically identical microbial forms makes any such examination of questionable value."

A further criterion which has been used in the study of developing rumen function is that of production of certain B-vitamins (Lengemann & Allen 1959). However insufficient information was given to be able

to assess the reliability of this method. Since the production of B-vitamins is an important activity in the rumen, further investigation into its use as a criterion of functional development seems desirable.

Summary.

Methods are available which give a good indication of whether or not rumen function has been at least partially established. Amongst these, the estimation of cellulose digestion, VFA and pH (and possibly B-vitamin production) give a better idea of when rumen function is established than do anatomical measures and observations on the microbial population.

Loss of weight of cotton thread appears to be the most reliable method of estimating cellulose digestion but is only a semi quantitative method. Variation due to site and time of sampling must be allowed for or eliminated in the measurement of VFA and pH and changes in pH due to sampling need to be guarded against.

IV The Development of Rumen Function in the Calf.

The study of the changes which occur in the ruminant stomach between birth and maturity has followed two main lines of investigation. One has been the investigation of anatomical changes and the other has been concerned with functional development. Although anatomical development is probably important from the standpoint of absorption and utilization of the endproducts of rumen microbial fermentation, the efficient utilization of dry feed, particularly roughages, will be dependant primarily on the attainment of an active rumen fermentation.

Insofar as the rumen is concerned, it would seem that anatomical development is to some extent dependant on functional development, since Cornell experiments report that salts of VFA (Flatt et al 1958), particularly butyrate and propionate (Sander et al 1959), are responsible for rumen mucosal development, provided that they are present in the rumen in sufficient concentration (Flatt et al 1959).

Differences in rumen fermentation in the adult rumen have been mainly attributed to differences in diet (see reviews by Annison & Lewis 1959; Barnett & Reid 1961; & Lewis 1961). Consequently it might be expected that diet could influence the development of rumen function.

It has been demonstrated that calves fed milk alone show little or no development of rumen function (Flatt et al 1959; Godfrey 1961b; Lengemann & Allen 1959) or mucosal tissue (Warner et al 1956; Wing & Ammerman 1960). This does not seem surprising since it is known tha milk and other liquid diets may by-pass the rumen by way of the oesophageal groove (e.g. Hegland et al 1957). However some milk may pass into the rumen (Smith 1960), which might account for the VFA found by Flatt et al (1959) and Lengemann and Allen (1959). The quantities of VFA produced when milk alone is fed are probably too small to cause any appreciable development of rumen mucosa.

Apart from the effect of milk alone, evidence that differences in diet produce differences in rumen functional development is meagre,

although some evidence is available which suggests that the type of fermentation produced may differ. The VFA data of Flatt et al (1959), although perhaps inadequate (see below), suggest that higher levels of VFA are produced earlier on a grain diet than on a hay or hay/grain diet. Although cellulose digestion was less than for the other diets this might not be important insofar as a grain diet is concerned. Differences in anatomical development due to diet (e.g. Warner et al 1956; Brownlee 1956) and differences in the type of organism established on different diets (Pounden & Hibbs 1948b) seem to indicate that diet may affect the type of fermentation produced, as in adult cattle.

Several investigators have demonstrated that there is an increase in the concentration of VFA in rumen contents and an increase in the ability to digest cellulose with age. Flatt et al (1959) report data on VFA concentration and cellulose digestion of fistulated calves on a variety of diets. These indicate that an increase in VFA and cellulose digestion occurs with age when dry feed is given and that dietary differences may occur. However these experiments involved few calves, average determinations only were given, cellulose digestion and VFA determinations were measured fortnightly and considerable variation within groups between sampling times was obtained. Although they concluded that anatomical characteristics were most sensitive to dietary changes it is considered that their evaluation of other criteria was based on insufficient evidence for comparison.

McCarthy and Kesler (1956), who fed calves on hay ad lib plus milk or milk replacer to 6 weeks of age, obtained rumen samples by stomach tube once weekly. They demonstrated maximum concentrations of VFA at 7-9 weeks of age after which the levels fell gradually. However the greater part of the increase in VFA concentration had occurred by 3 weeks in two trials and by 5 weeks in the other. The concentration of VFA at these stages was equivalent to that obtained for all trials from the 12th to 15th week inclusive. These workers also found that the percentage cellulose digestion, as measured by an in vitro technique, increased rapidly during the first 4-6 weeks after which it increased relatively slowly and showed considerable fluctuation.

Conrad et al (1954) found a 50% increase in VFA between 4 and 9 weeks of age although the concentration at 4 weeks was already 60 meq/litre. The proportion of hay to grain fed did not significantly affect the level of VFA.

Hibbs et al (1956) obtained similar results for 3 groups of calves fed differing ratios of hay and grain and inoculated with cud material from adult cattle. They did not report observations on rumen contents of a further group of calves fed milk alone. The group average pH of rumen juice was shown to increase between 4 and 12 weeks although the group range at these ages overlapped. It was stated that there was a highly significant difference in VFA and pH between 4 and 12 weeks and also a highly significant difference in pH between the 4:1 hay/grain fed group and the other groups (3:2 and 2:3 hay/grain) at 4, 6 and 9 weeks but no significant difference at 12 weeks. The high hay group had the higher pH Rumen bacteria and protozoa ratings gradually increased over the experimental period.

Notwithstanding the significant differences obtained by these workers, the significance of the results in terms of developing rumen function must be considered in the light of the following comments.

(i) Rumen samples were obtained by stomach tube at 4, 6, 9 and 12 weeks of age. Thus differences attributed to age and diet could conceivably be due to differences in site of sampling and an imperfect sampling procedure.

(ii) It is apparent that a considerable degree of rumen fermentation had been attained in all groups by 4 weeks of age.

Lengemann and Allen (1959) showed that adult levels of acetic, butyric and propionic acids were attained by 6, 4 and 7 weeks of age respectively for calves with access to dry feed from birth. Rumen samples obtained by stomach tube indicated that calves which had the opportunity consumed appreciable amounts of solid feeds as early as 2 weeks of age. Milk fed calves, when offered solid feed (hay and grain) at 8 weeks of age, avidly consumed it and a marked increase in VFA was found by 9 weeks of age. This emphasizes the need to make observations on rumen contents from as early an age as possible.

It was noted that a liberal milk feeding program, as opposed to a limited milk feeding program, seemed to delay the acquisition of some adult characteristics.

Slaughter date of Godfrey (1961b) indicated that a similar rise in VFA occurred for pasture fed calves. Figures for VFA presented by him showed that the concentration plateaued at 6 weeks of age. The pH of the rumen juice was 5.15 at one week of age, rising to a maximum of 6.95 at 15 weeks.

In a further experiment, Godfrey (loc. cit.) used 3 groups of calves designated A. B. and C. Group A was denied access to pasture until 8 weeks of age; groups B. and C. were allowed to graze from birth but group B was permitted access to pasture for one third of the time spent grazing by group C. Rumen samples were obtained twice weekly by stomach tube. In addition samples were taken periodically at 3 hourly intervals over 24 hours to determine diurnal variation. The level of VFA for all groups at 2 weeks was about 25-30 mM/litre. For groups B and C the level rose to about 65 mM/litre at 4 and 5 weeks, then fell below 60 mM/litre to 8 weeks rising again to 90-100 mM/litre from 9 weeks onwards. The VFA level for group A rose from about 30 mM/litre at 8 weeks to 85 mM/litre at 9 weeks. The level rose further to 100 mM/litre at 10 weeks and then fell to about 65 mM/litre at 12 weeks. Irrespective of age or treatment the diurnal variation was the same, being lowest at 8 a.m., rising to a peak at 8-11 p.m. and falling again to the low level at 8 a.m.

Summary.

It appears that the development of rumen function depends on the calf receiving solid feeds, since a liquid diet may by-pass the rumen. Provided that the calf has access to solid feed from birth, considerable rumen fermentation can occur by 3 or 4 weeks of age. It seems that rumen function similar in many respects to that found in adult animals can be attained by the time the calf is 6 to 8 weeks old. Even when denied solid feed until this time, the rapidity with which the fermentation end products reach high

levels following the ingestion of solid feed suggests that 2 month old calves can quickly adjust to the change in diet.

However although there is good general agreement between most reports on the development of rumen function, most studies have employed stomach tube sampling and with the exception of the diurnal observations of Godfrey (1961b) samples have been obtained no more frequently than once or twice a week. Consequently, the possible variation due to site of sampling has not generally been accounted for, and there appears to be no information on daily changes in fermentation. It is apparent that there is a paucity of information on the development of rumen function in pasture-fed calves.

V. Grazing and Rumination by Calves on Pasture.

It is desirable to know when and to what extent calves eat pasture as this will influence rumen functional development. Observations of rumination are of interest since rumination is characteristic of adult rumen function.

Roy et al (1955) reported that calves on pasture attempted to graze from about the seventh day onwards and at 3 weeks of age spent 1.3 hours grazing in the period from 6 a.m. to 6 p.m. The corresponding rumination time was 1.23 hours. Observations over 24 hours showed that at 7 weeks of age grazing and rumination times were 5.59 hours and 6.83 hours respectively. The average times over 7-21 weeks were 7.85 hours grazing and 7.61 hours ruminating.

These workers discovered that very little grazing took place during the hours of darkness and definite peaks of grazing intensity were observed during the day at 6 a.m., 10 a.m., 1 p.m. and 4-8 p.m. Rumination took place mainly during the hours between sunset and sunrise with small intermittent peaks in the daytime during intervals between grazing.

Godfrey (1961b) reported an increase in grazing time from 2 hr 50 min. at 3 weeks of age to 7 hr 20 min. at 6-7 weeks of age. In general the calves grazed only during the daylight hours with a marked resting period shortly before midday.

Summary.

The information available indicates that calves commence to graze pasture at an early age. They apparently do most of their grazing by day and ruminate mostly at night.

MATERIALS AND METHODS.

For clarity, the experimental work will be reported as two separate experiments. The first was in the nature of a trial run in order to gain familiarity with the methods to be used, to see what difficulties might be involved and to decide on what measures were necessary and practicable.

Experiment I.

Two Friesian bull calves were purchased from a local farmer at the end of March 1961. They had received colostrum for 2 days and had access to pasture. Throughout the trial which lasted 7 weeks, they were fed whole milk obtained from the College herd (monthly average fat test: April, 5.14%; May, 5.07%; June, 4.34%, July, 4.47%). One gallon of this milk was given daily in two feeds (at 0830 hours and 1730 hours) from a nipple pail. Calves were allowed access to pasture between the a.m. and p.m. feeds but were enclosed on hay bedding at night. From one month of age, both animals received one gallon of milk in the evening only and had continuous access to pasture.

Following fistulation (see below) samples of rumen contents were obtained every second day prior to the evening feed for volatile fatty acid (VFA) analysis. On three occasions, samples were obtained at 3 hourly intervals over a 24 hour period to obtain data on diurnal variation.

Experiment II.

Two Friesian bull calves collected at the end of May 1961 and a Friesian and an Ayreshire bull calf obtained 3 weeks later were treated similarly to those in Experiment I but with the following differences and modifications.

(i) They were allowed continuous access to pasture.

(ii) At 2 weeks of age they were gradually changed to a diet of reconstituted butter milk. By 3 weeks of age they were receiving one gallon of fluid per day containing 1.75lb of buttermilk powder. At 4 weeks of age they were changed to once a day feeding as in Experiment I.

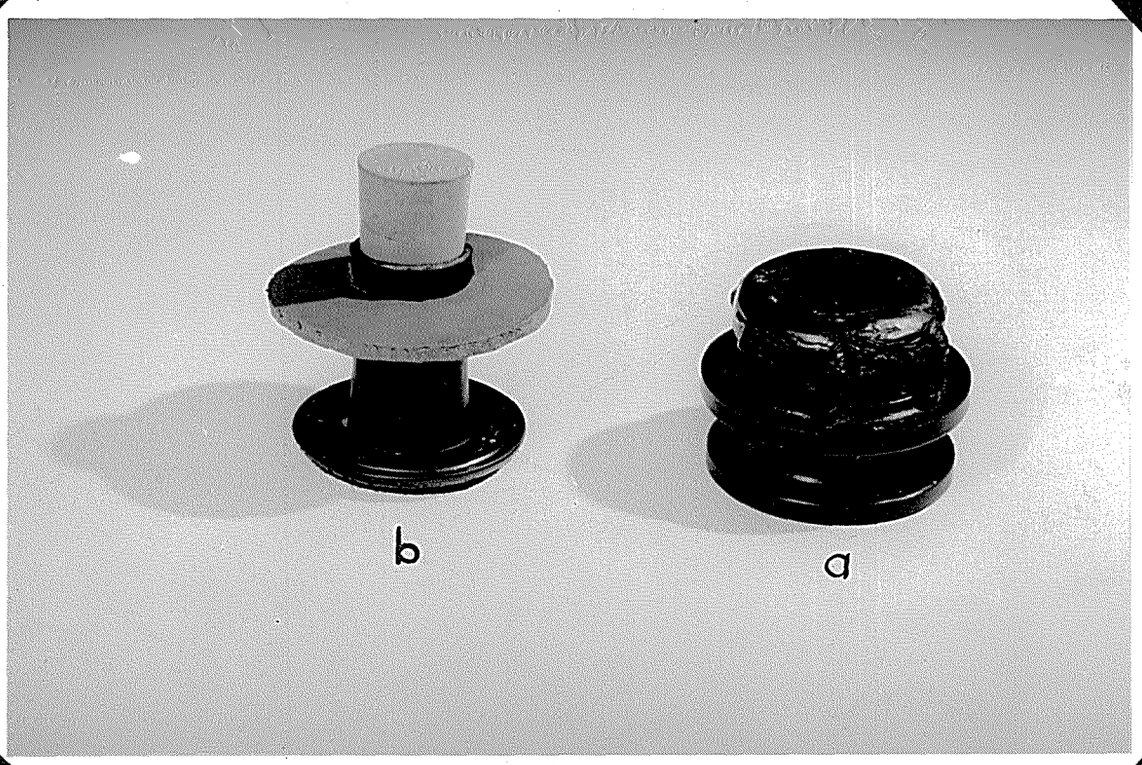


FIGURE 1.

(a) The Vulcathene cannula.

(b) The milking machine inflation adapted as a replacement cannula.

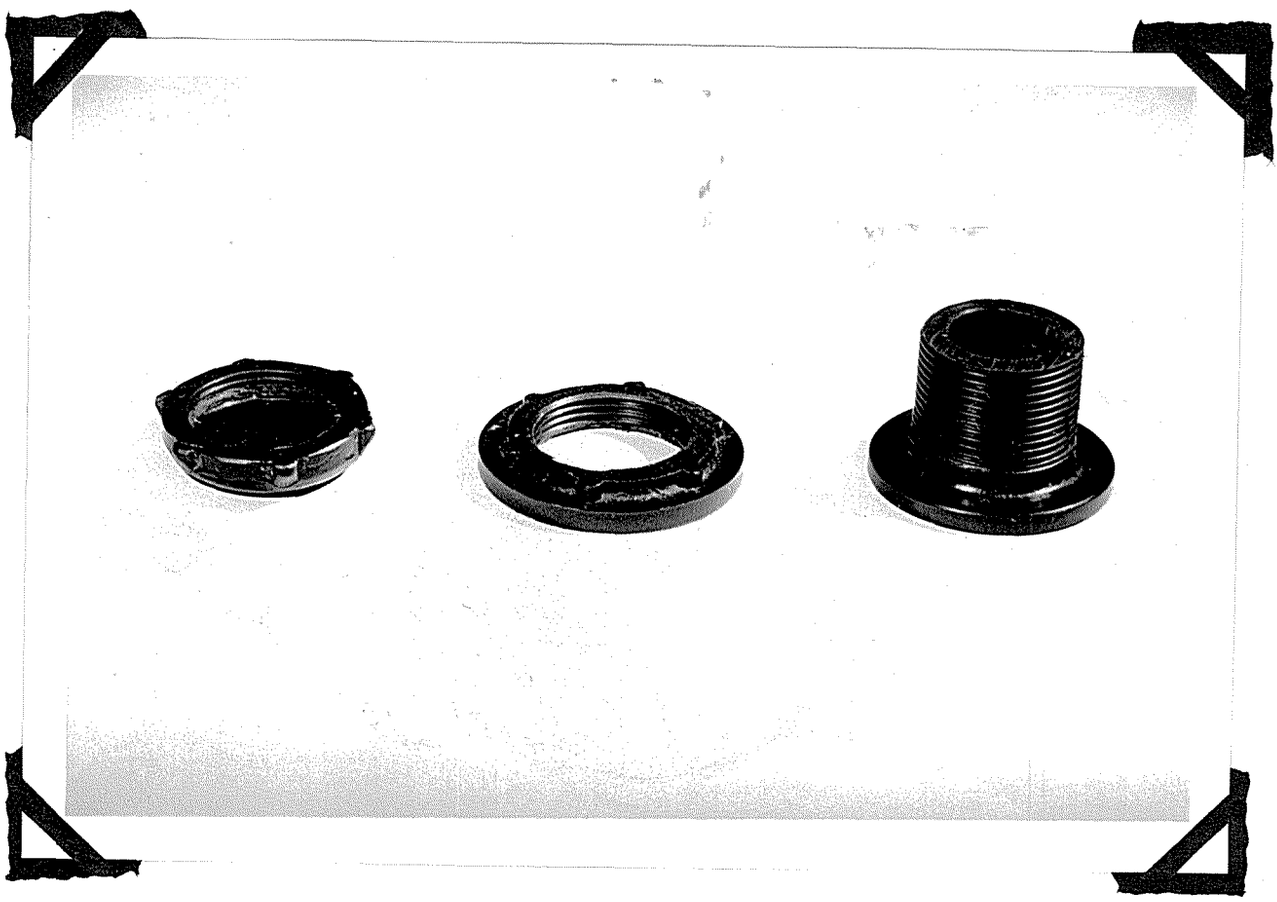


FIGURE 2. The Vulcathene cannula dismantled to show component parts.

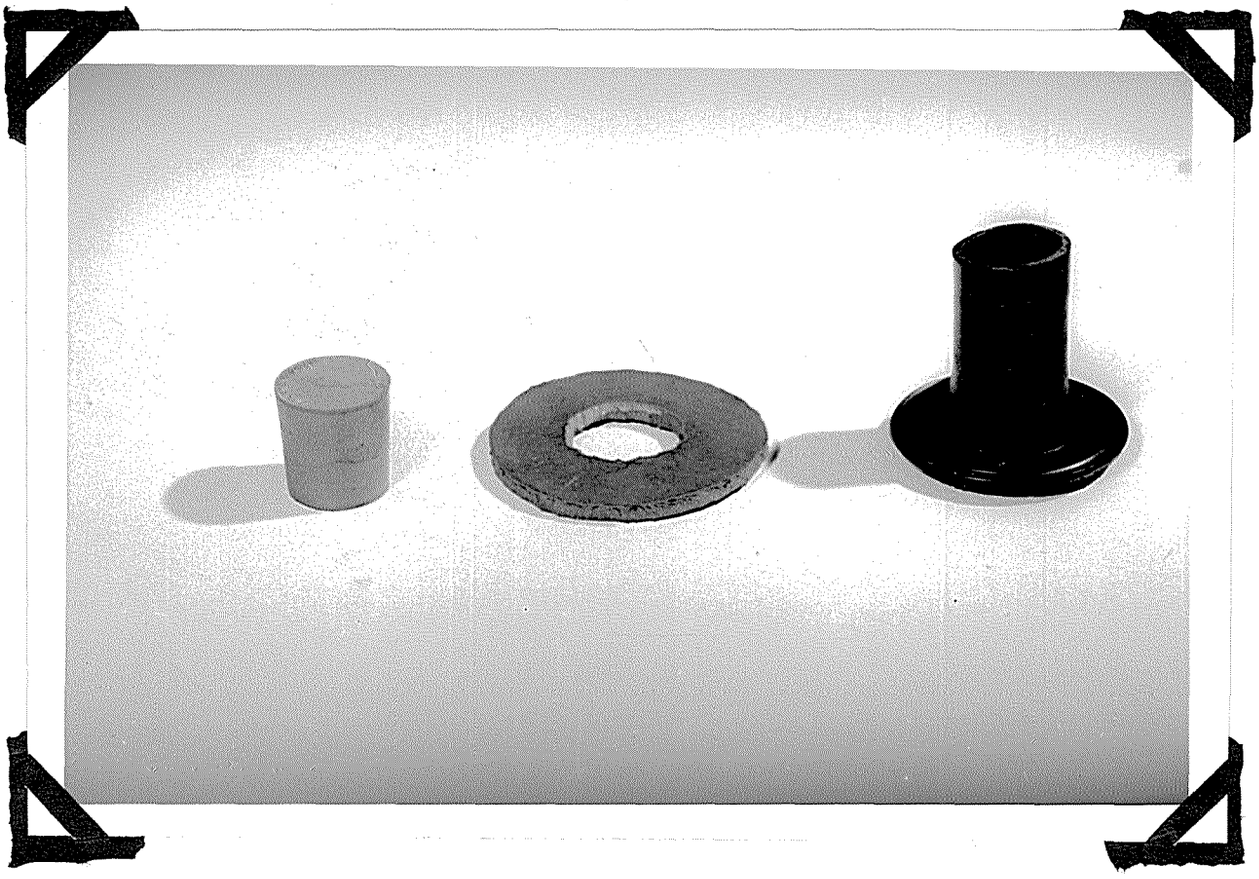


FIGURE 3. The replacement cannula dismantled to show component parts.

(iii) Samples of rumen fluid were obtained by stomach tube prior to fistulation.

(iv) Following fistulation, samples of rumen contents were collected once daily and on two occasions 3 hourly for diurnal variation.

(v) The pH of the rumen fluid was measured as well as VFA concentration.

(vi) An estimate of cellulose digestion was obtained once weekly.

(vii) Behaviour of the calves was recorded at 5 minute intervals over a 24 hour period once weekly.

Fistulation.

A closed rumen fistula was prepared in each calf by a method similar to the stab wound technique reported by Dougherty (1955). The cannula originally inserted was a converted plumber's waste drain fitting made of "Vulcathene". Portion of the threaded tube was cut off and the outer screwed flange was cut to leave the flange proper and a threaded ring. This ring was sealed over with polythene sheet to provide a screw cap. The completed cannula is illustrated in figure 1a and in its dismantled form in figure 2.

When this cannula became dislodged (see results section) it could not be reinserted due to the non flexible nature of the Vulcathene. Consequently a cut down milking machine inflation with an outer flange of rubber sheeting and a rubber stopper was substituted (see figure 1b and figure 3).

The modification to Dougherty's (1955) technique was as follows. Instead of a stab wound being made beside the original incision, a circle of skin equal in diameter to that of the cannula tube was removed. This incision was continued above and below the circle of skin removed in order to accommodate the internal flange of the cannula. These continuations of the incision were later stitched to hold the cannula in place. The outer flange of the cannula, when screwed into place held the skin against the outside of the rumen wall to promote adhesion of the two surfaces.

The Sampling of Rumen Contents.

Samples of rumen contents were obtained by oral suction through a rubber tube inserted into the rumen via the fistula. A trap was

fitted to collect the sample and to prevent rumen contents being sucked into the mouth. The end of the tube was pushed about 5 inches into the ingesta, slightly forward of and below the fistula in an attempt to sample from the same position each time. In the early stages there was insufficient ingesta to do this and the tube had to be inserted further. However since the ingesta at this stage was quite fluid and well mixed, variation due to position of sampling was probably not very great.

Prior to fistulation the tube was passed by way of the calf's mouth down the oesophagus into the reticulo-rumen. The first part of each sample collected in this was discarded because of contamination with saliva.

Following collection the sample was squeezed through a double layer of muslin. The resultant liquor was transferred to a 25 ml. bottle which after being filled to the brim was closed with a tight fitting screw cap.

Analytical procedures.

As soon as possible after collection the sample was transferred to the laboratory where pH was determined in duplicate with a meter. The time interval from collection to determination of pH was never more than 5 minutes. After measuring the pH the sample was stored in a refrigerator at $5^{\circ}C$. Preliminary tests showed that this method of storage gave identical volatile fatty acid figures over an 8 day period. Since in all cases analysis for VFA was performed within 4 days of collection of the sample no further precautions were taken.

The determination of total steam volatile fatty acids was carried out in the following manner.
A 5 ml aliquot of the rumen liquor sample was placed in a Markham (1948) still and 1 ml of 10N $H_2 SO_4$ saturated with $MgSO_4$ added. Steam distillation was carried out until two 50 ml portions of distillate had collected, the second portion being used as a blank correction for organic acids which are slightly steam volatile. Prior to titration with standardized NaOH solution, carbon dioxide

free air was passed through the distillate with a filter stick for two minutes. This aeration was continued during titration and was intended to reduce interference by carbon dioxide. In the initial stages a 0.02N NaOH solution was used for titrations because of the low concentration of VFA, but as this concentration increased over about 25 mM/litre, a 0.05N solution of NaOH was used. These solutions were standardized against potassium hydrogen phthallate. Phenolphthalein was used as the indicator of the titration end point. Preliminary tests of this procedure gave good repeatability of results and a 98.7% (average) recovery of a known mixture of salts of VFA alone or added to a sample of rumen liquor.

For each calf in Experiment II the regression of pH on VFA concentration was calculated (Snedecor 1956).

Estimation of Cellulose Digestion.

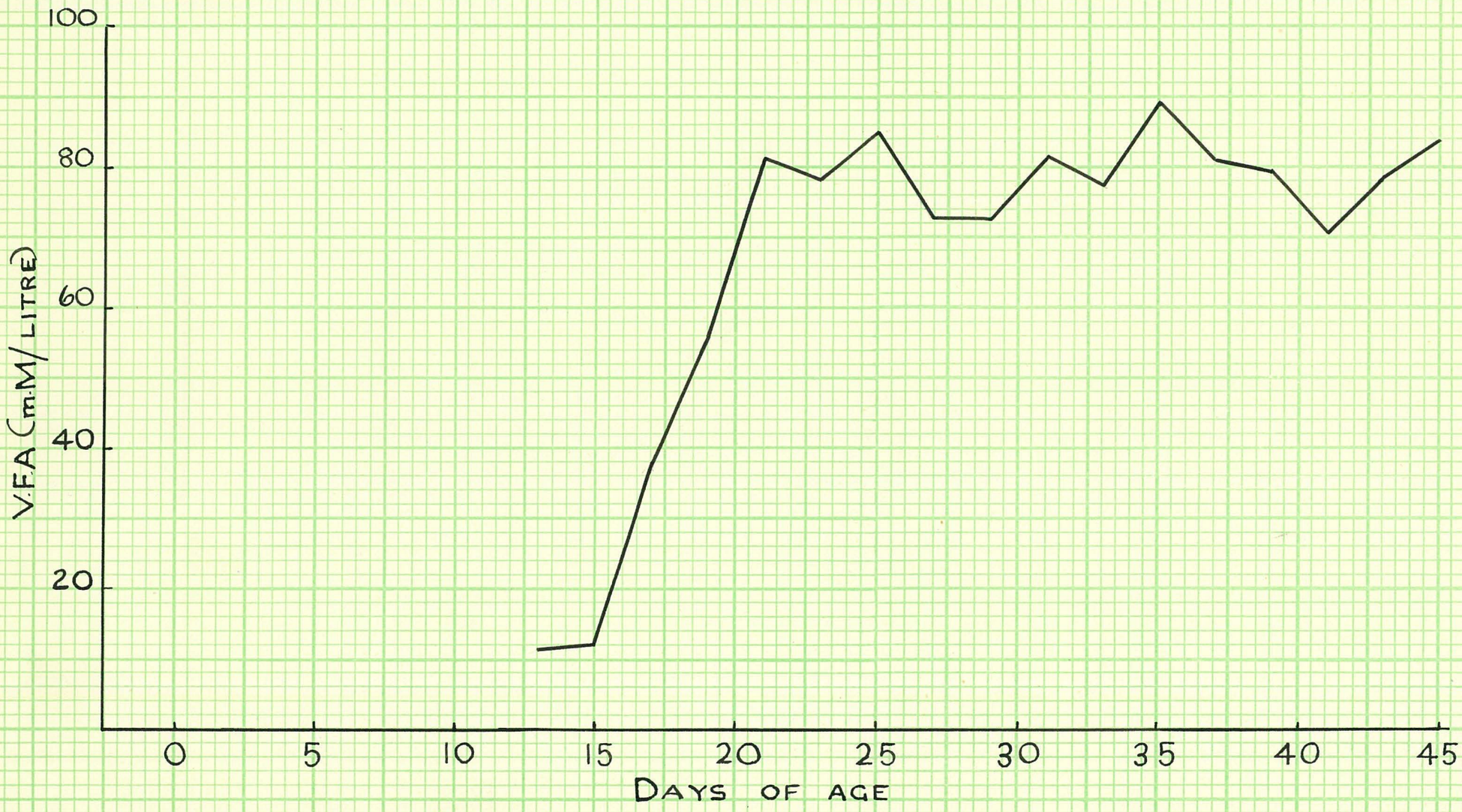
Cellulose digestion was estimated by measuring loss of weight of cotton threads suspended in the rumen for 24 hours as described by Flatt et al (1959). Three loops of thread were used for each determination and a control set of three loops used to obtain a correction for loss of weight due to processing.

FIGURE 4 CALF 1

DAILY V.F.A. CONCENTRATION

FIGURE 5 CALF 2

DAILY V.F.A. CONCENTRATION



R E S U L T S.

EXPERIMENT I.

The two Friesian calves used in this preliminary trial were 4 days old when collected and appeared to be in good health, no evidence of scouring or other abnormality being detected. They were fistulated at 7 and 11 days of age respectively and sampling commenced two days later. Fistulation appeared to have no lasting adverse effect on either calf and healing was rapid. Some leakage of rumen contents around the cannula occurred and in the first calf this resulted in some softening and peeling of the skin below the site of operation soon after fistulation. This soon cleared up despite continued leakage of rumen contents.

After about six weeks in each calf the skin which was holding the cannula in place became stretched sufficiently for the cannula to become dislodged. Since the Vulcathene was not flexible, the cannula could not be replaced and a cut down milking machine inflation was substituted. Although some loss of rumen contents occurred when the cannula fell out, this did not happen on a sampling day and is not recorded in the appendices.

The results of the analyses of the samples of rumen contents for volatile fatty acid (VFA) concentration are presented in graphical form in figures 4 and 5 (opposite). The figures used in the construction of these graphs are found in appendix I and II. These results clearly demonstrate that there is a marked difference in the VFA concentration in rumen liquor between two and four weeks of age.

For calf No.1 the VFA concentration was less than 10 mM/litre of rumen liquor between 9 and 17 days of age. By the time the calf was 27 days old the level of VFA had risen to a value of 78.5 mM/litre and thereafter fluctuated between 72.4 and 91.6 mM/litre. A similar pattern was obtained for calf No.2 although less measurements were obtained in the lower range. For this animal, VFA concentrations in the rumen liquor of 11.5 and 12.1 mM/litre at 13 and 15 days respectively were found, and a rapid rise occurred to 80.9 mM/litre

T A B L E I.

AGE OF COLLECTION AND FISTULATION OF CALVES IN EXPERIMENT II.

Calf No.	Breed	Age collected (days)	Age fistulated (days)
3	Friesian	4	7
4	Friesian	3	6
5	Ayreshire	4	6
6	Friesian	3	5

T A B L E II.

BODY WEIGHT GAINS OF CALVES IN EXPERIMENT II.

Calf No.	Age (days)	Body weight (lbs)
3	5	93
	12	96
	20	107
	27	113
	34	121
	41	136
4	4	91
	11	93
	19	97
	26	103
	33	113
	40	122
5	5	66
	12	69
	19	81
	26	93
6	3	66
	10	69
	17	79
	25	90

at 21 days of age. Thereafter the level fluctuated between 70.3 and 89.2 mM/litre.

Before this rise in VFA concentration had occurred, the rumen contents of both calves were very watery, with little evidence that grass had been eaten apart from one or two pieces of grass in the samples. At this stage the colour of the rumen fluid was a bit more brown than straw coloured. During the period when the rise in VFA concentration occurred, increasing quantities of grass were observed in the rumen, the colour of the rumen contents became greener and its consistency became less and less fluid. By the time high levels of VFA were found, the colour and consistency of the rumen contents resembled those of mature animals fed on pasture.

A considerable diurnal variation was found in VFA concentration of rumen liquor. The general pattern was for VFA concentration to increase during the day, reaching a peak value at about 1700 or 2000 hours and then falling to a minimum level at about 0500 or 0800 hours. This pattern was similar in all except one case, although the actual levels of VFA obtained varied with age of the calf. The exception to the above pattern was for calf 1 at 23 days old when a minimum VFA value occurred at 2030 hours, rising gradually thereafter. The results of the diurnal variation observations are presented in appendix Ia and IIa.

EXPERIMENT II.

The calves used in this experiment were collected and fistulated at the ages shown in table I. Their body weights, which were measured once weekly before the morning feed, are shown in table II, where it is seen that fair growth was made. Calves appeared to be in general good health although some scouring was encountered, particularly in the week when the diet was changed from whole milk to buttermilk.

More difficulty was experienced with the cannulas than in experiment I. The Vulcathene cannula became dislodged in calf 4 at twelve days but was surgically replaced and extra reinforcing stitches

FIGURE 8 CALF 5

DAILY V.F.A. CONCENTRATION AND pH OF RUMEN LIQUOR

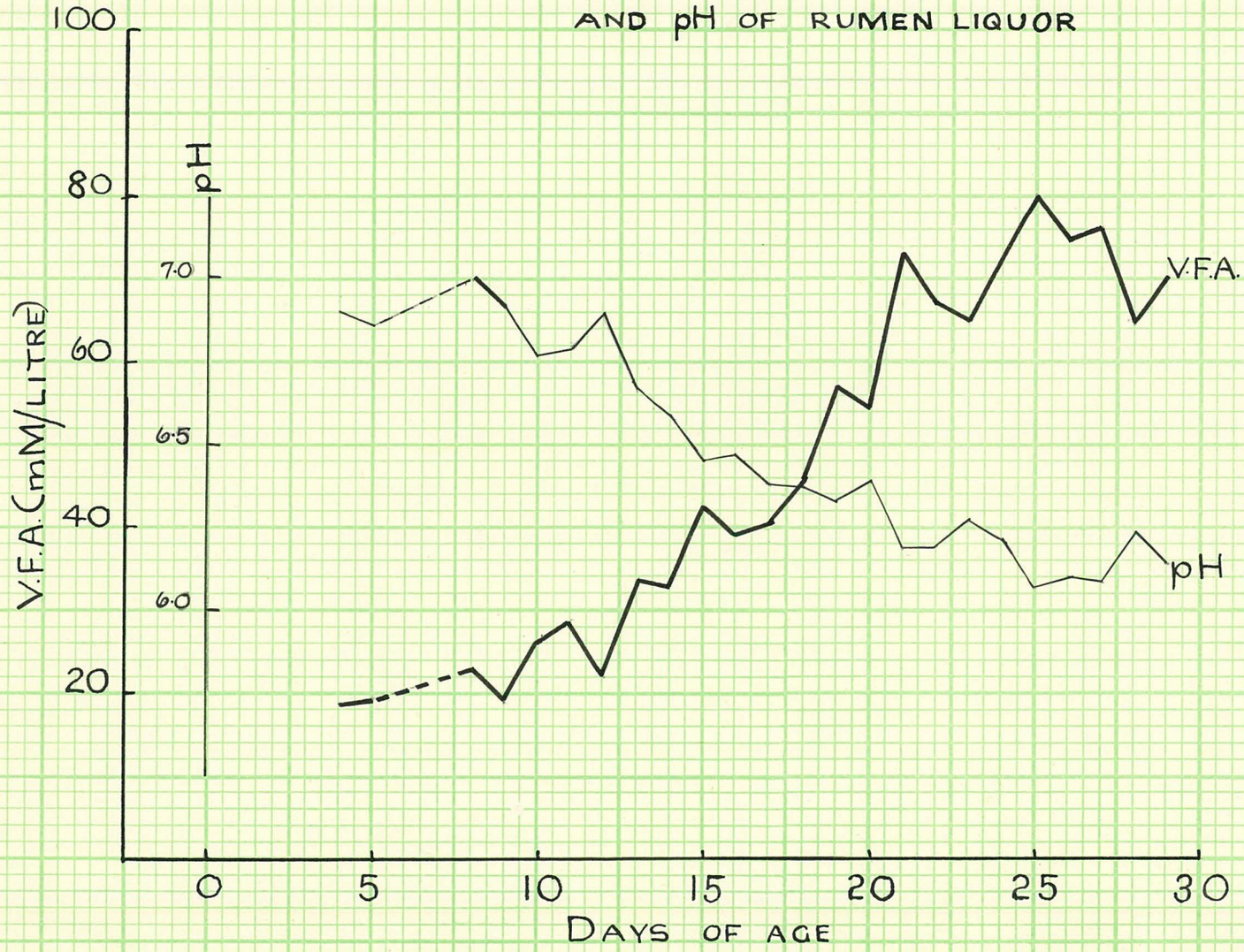


FIGURE 7a CALF 4

REGRESSION OF pH ON V.F.A. CONCENTRATION

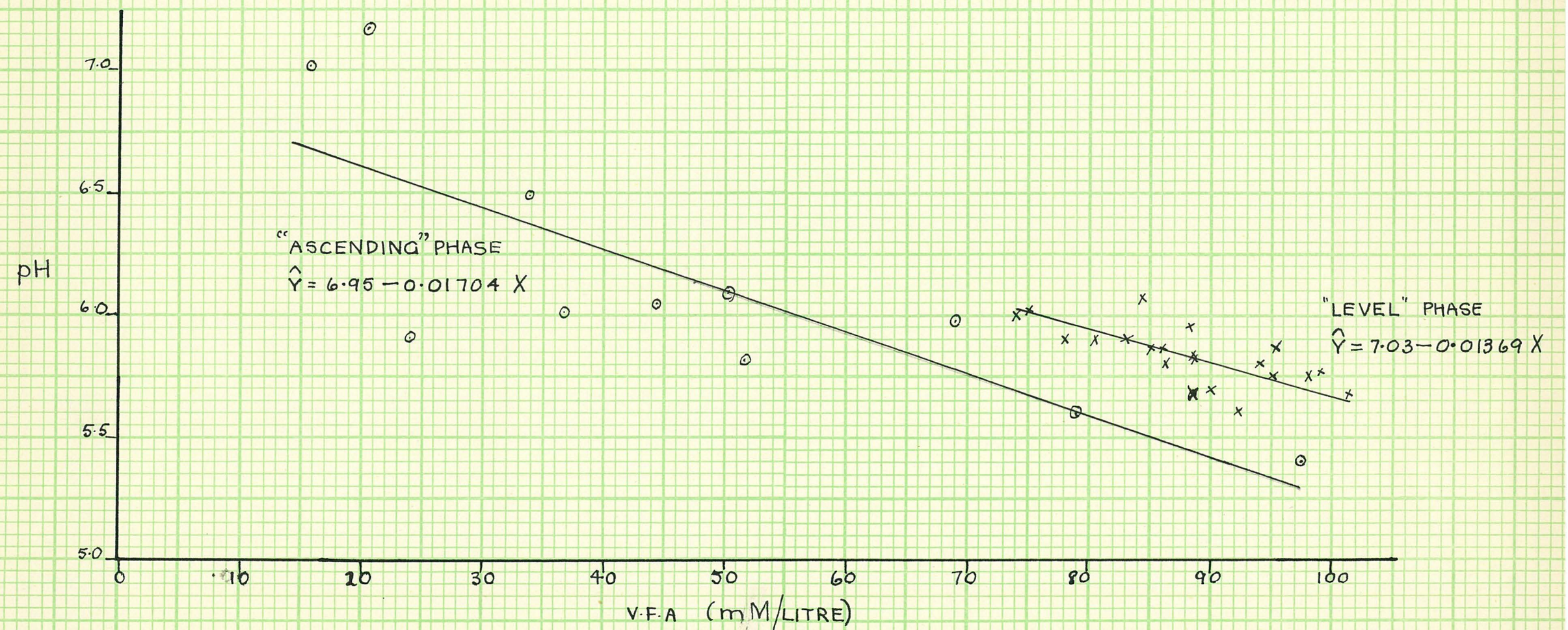


FIGURE 7 CALF 4

DAILY V.F.A. CONCENTRATION AND pH OF RUMEN LIQUOR.

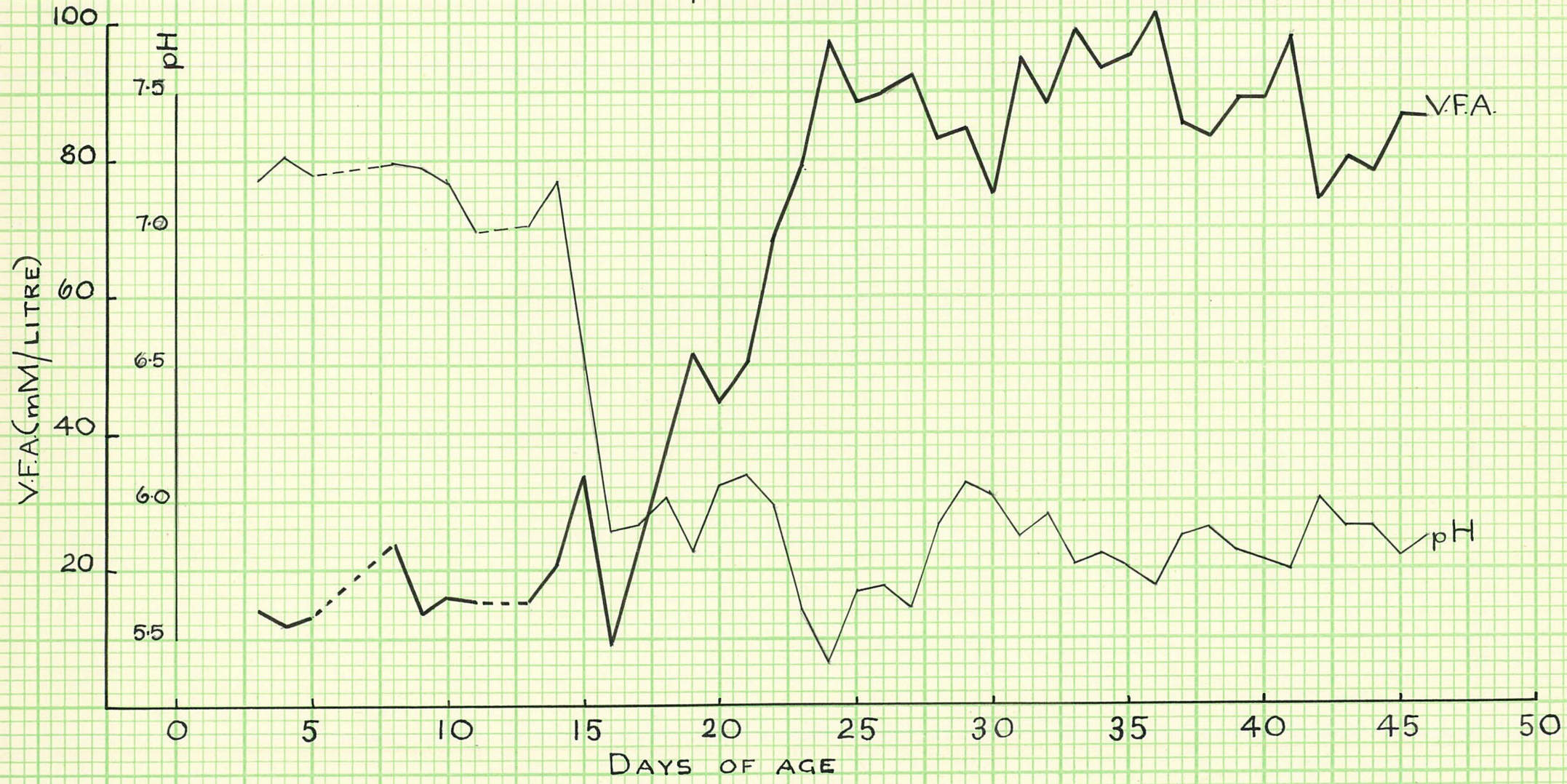


FIGURE 6a

CALF 3

REGRESSION OF pH ON V.F.A. CONCENTRATION.

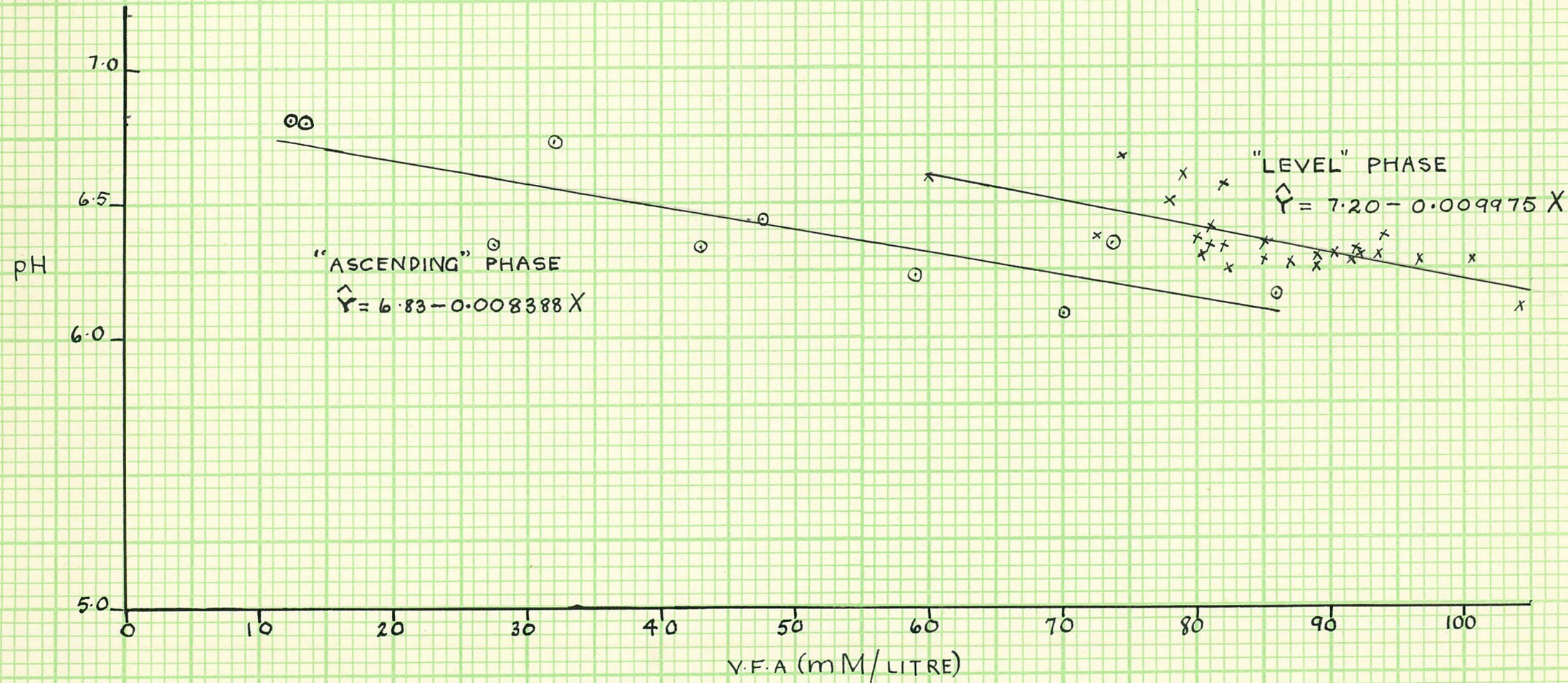


FIGURE 6 CALF 3

DAILY V.F.A. CONCENTRATION AND pH OF RUMEN LIQUOR.

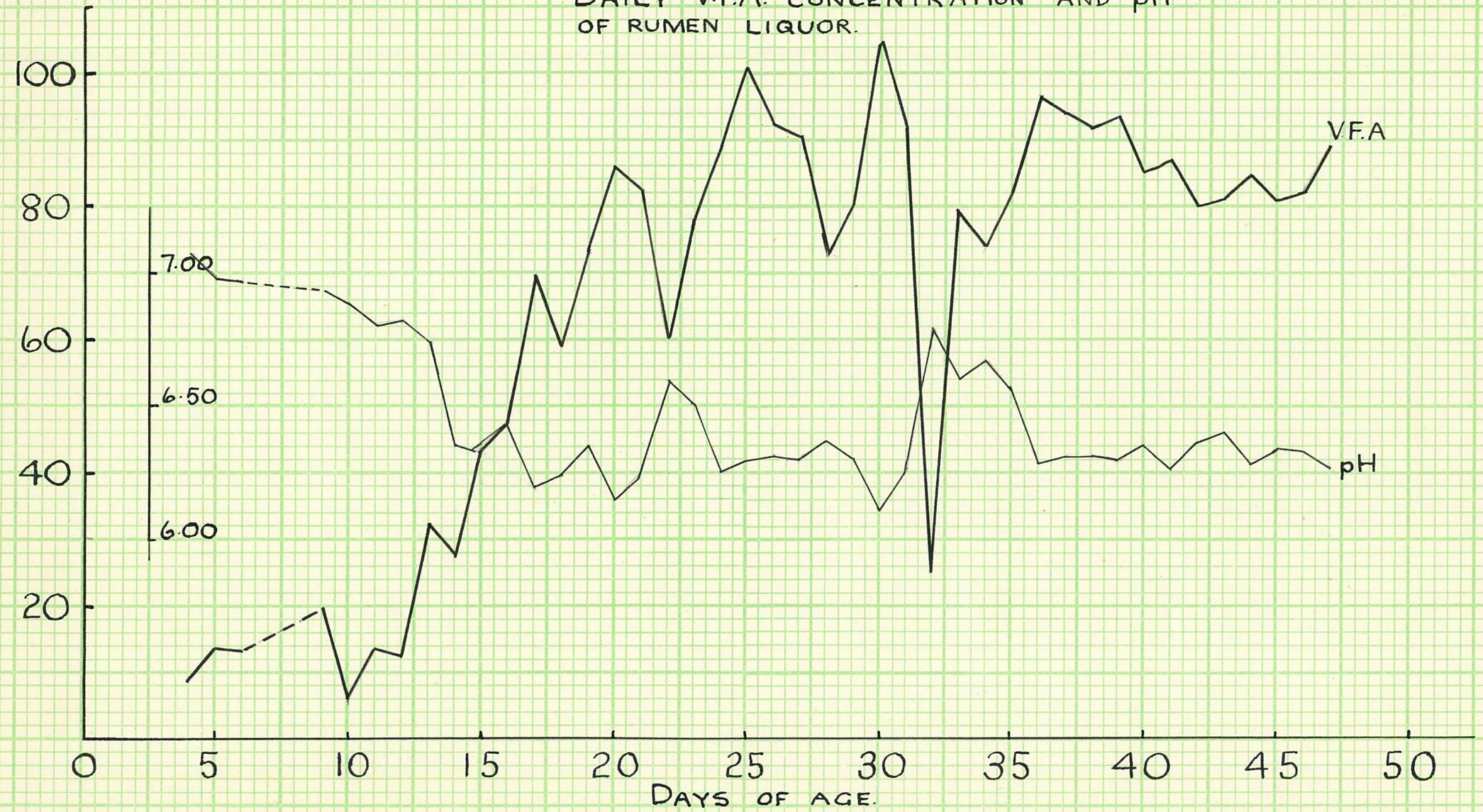


FIGURE 9a

CALF 6

REGRESSION OF pH ON V.F.A. CONCENTRATION

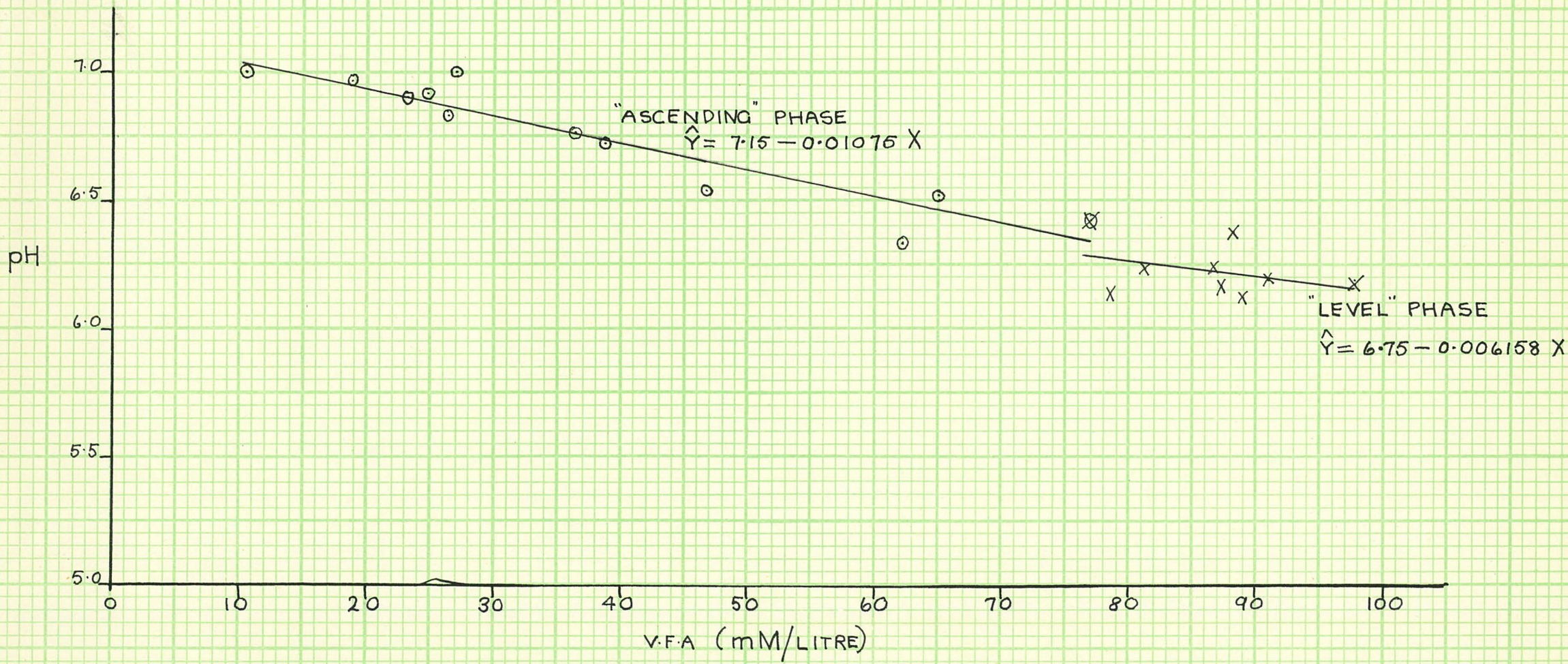


FIGURE 9 CALF 6

DAILY V.F.A. CONCENTRATION AND pH OF RUMEN LIQUOR.

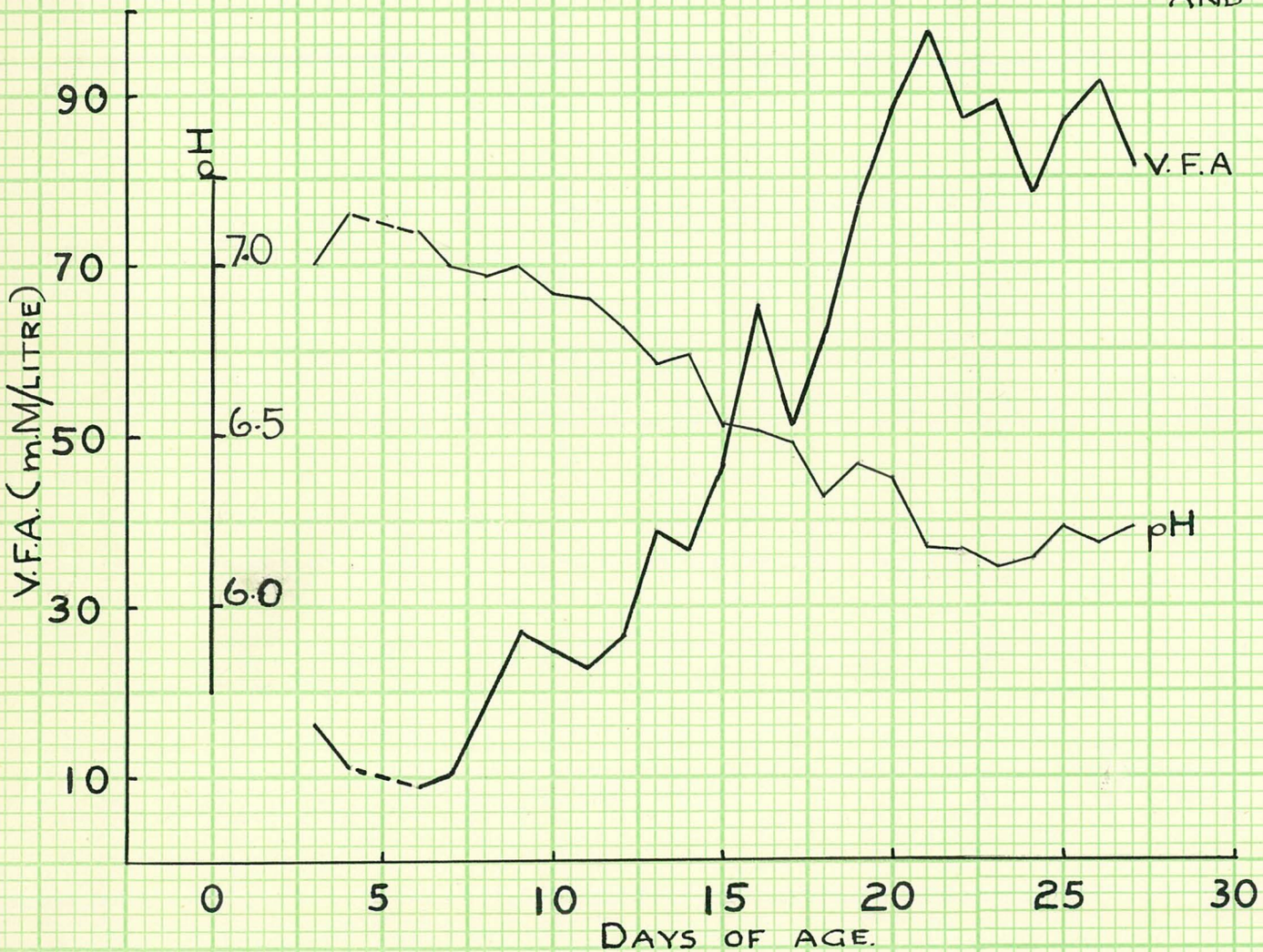
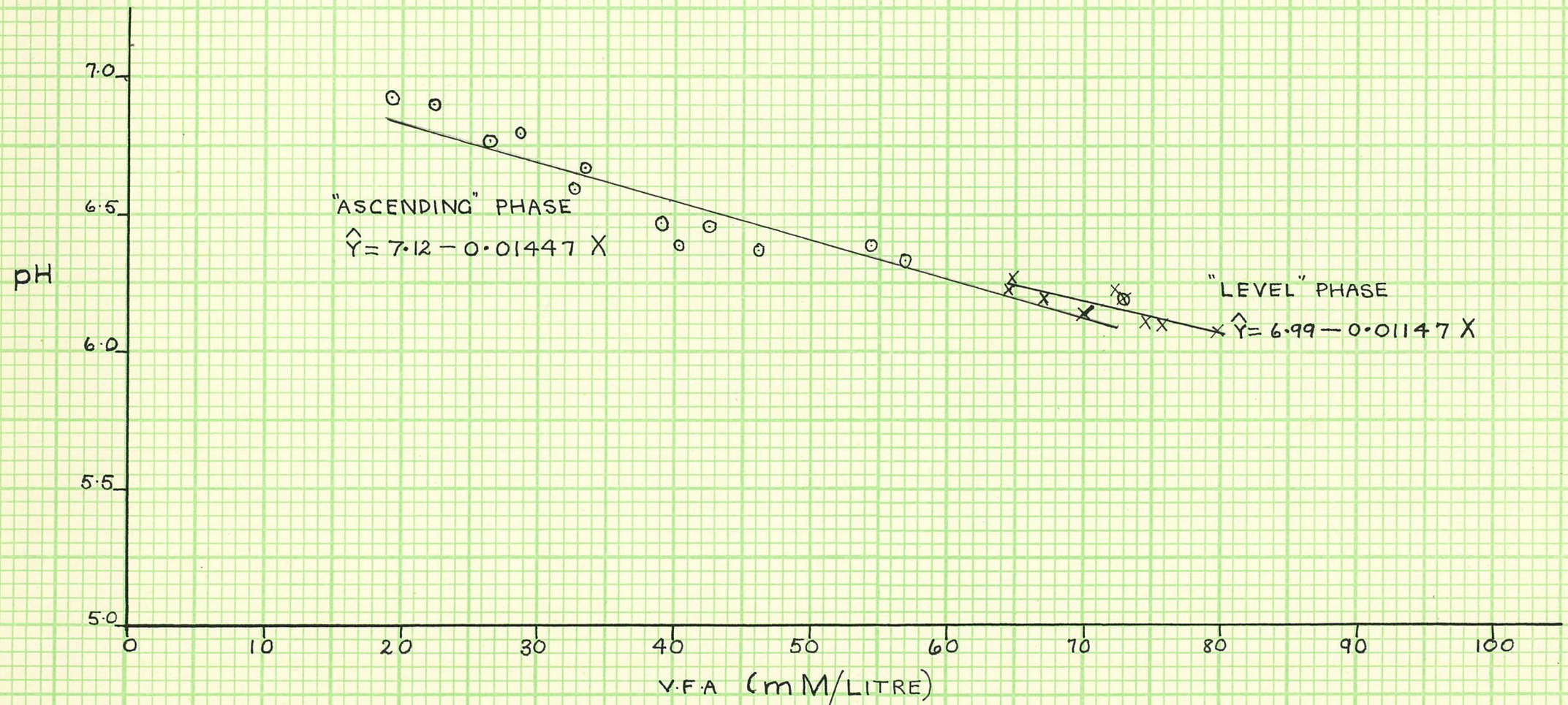


FIGURE 8a CALF 5

REGRESSION OF pH ON V.F.A. CONCENTRATION



inserted. Despite this, the cannula came out at 16 days and was replaced with a cut down milk inflation. The cannula in calf 3 stayed in place until 32 days but those in calves 5 and 6 came out at 16 and 17 days respectively. The replacement cannulas also came out on several occasions resulting in some loss of rumen contents. Where this occurred it is noted in the tables of results (see appendices III - VI.)

pH and VFA concentration of rumen contents.

The results of pH determinations and VFA analyses of rumen contents for calves in experiment II are presented in figures 6, 7, 8 and 9 and in appendices III, IV, V and VI. The patterns of VFA concentration were similar to those obtained in the preliminary trial, but the initial levels of VFA are slightly higher and they commence to increase at an earlier age.

Marked day to day differences occurred. Some of these fluctuations may have been due to loss of rumen contents when the cannulas came out but even when these occasions (as indicated in the appendices) are ignored, considerable day to day fluctuations are still evident. The levels of VFA concentration attained by about 3 weeks of age were within the range of VFA concentration found from then on.

Changes in colour and consistency of the rumen contents bore the same relationship to the VFA concentrations as was found in experiment I.

The pH of the rumen contents was seen to bear an inverse relationship to the VFA concentration. Early rumen samples had a pH near neutrality and fell generally to between pH 6.0 and 6.5 as the VFA concentration rose to the higher levels. Day to day fluctuations closely followed the day to day fluctuations in VFA concentration. In calf 4, the pH was somewhat higher for a start than in the other calves and fell to a lower level, fluctuating generally below pH 6.0.

In order to further investigate the relationship between pH and VFA concentration, the regression of pH on VFA concentration

was calculated for each calf for "ascending" and "level" phases of VFA concentration. The age spans included in each phase are shown in table III.

TABLE III.

AGE SPANS INCLUDED IN REGRESSION ANALYSES.

Calf No.	"Ascending" phase		"Level" phase	
	Days of age (inclusive)	Number of observations	Days of age (inclusive)	Number of observations
3	11 - 20	10	20 - 47	27*
4	13 - 24	11*	24 - 46	23
5	9 - 21	12*	21 - 29	9
6	7 - 19	12*	19 - 27	9

* One observation deleted from analysis because of excessive loss of rumen contents when the Vulcathene cannula came out.

These age spans were selected on the assumption that the "level" phase represented the ruminant stage and the "ascending" phase represented the stage of transition from non ruminant to ruminant. If there was any physiological difference in the relationship between pH and VFA concentration between these two stages it might have been expected that different regression coefficients would be obtained for the two stages. The regression coefficients obtained, together with their standard errors and results of tests for significance of these coefficients and the differences between them are presented in table IV. The analyses of regression are found in appendices VII to XI and the regression equations with graphical representation are shown in figures 6a, 7a, 8a and 9a.

T A B L E IV.

REGRESSION COEFFICIENTS, STANDARD ERRORS AND RESULTS OF TESTS OF SIGNIFICANCE.

Calf No.	b ± S.E.b "Ascending" phase	"Level" phase	Difference from common regression
3	-0.008388** ±0.00184	-0.009975** ± 0.002102	N.S.
4	-0.01704** ± 0.00424	-0.01369** ±0.00307	N.S.
5	-0.01446** ±0.00168	-0.01147** ±0.00275	N.S.
6	-0.01075** ±0.00121	-0.006158 ±0.005718	N.S.
Difference from common regression	N.S.	N.S.	

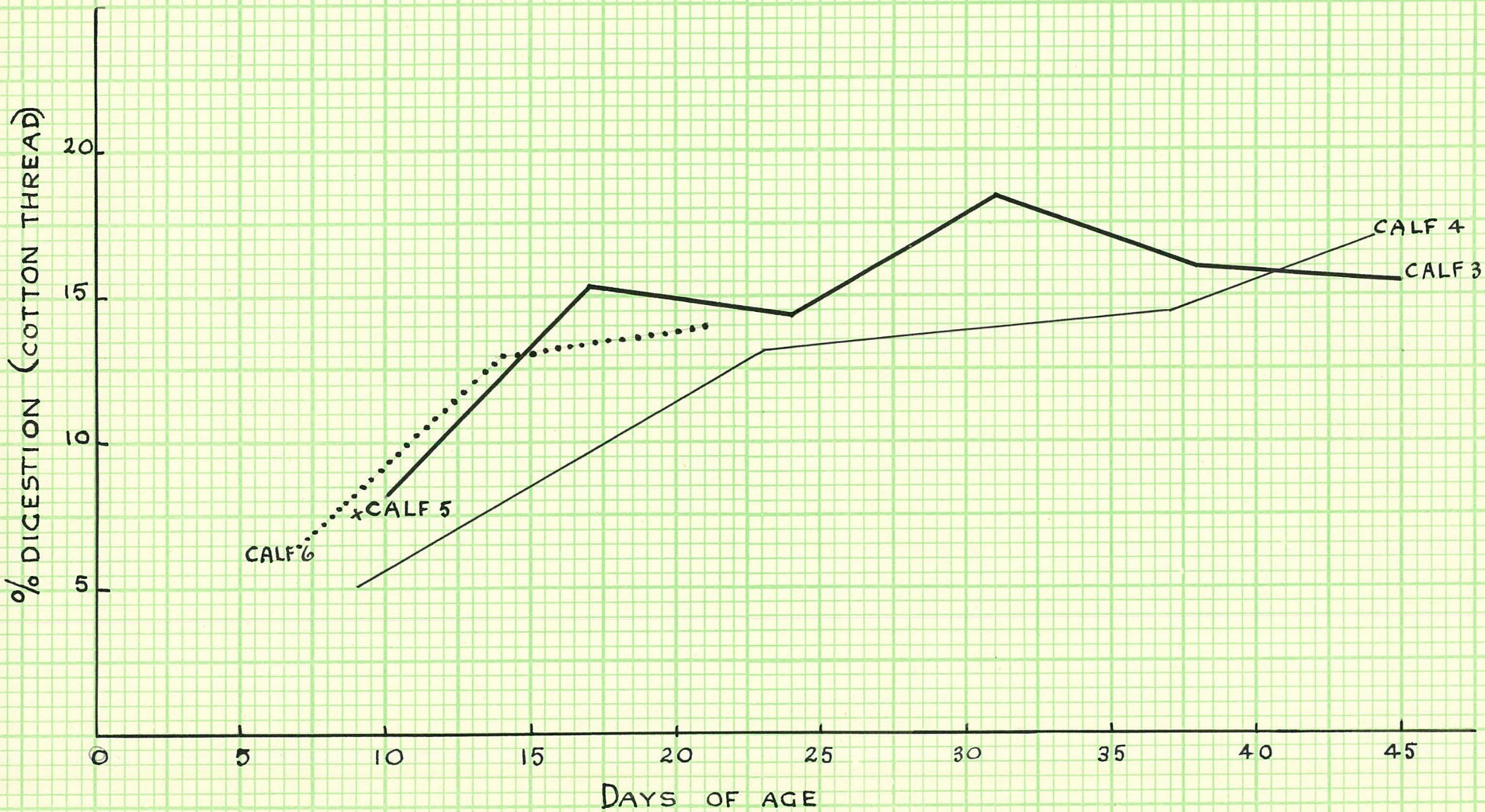
** Highly significant ($P < .001$)

N.S. Not statistically significant.

With the exception of the "level" phase regression coefficient for calf 6 which was not statistically significant, all regression coefficients were highly significant ($P < .001$). Differences between "ascending" and "level" phase regression coefficients for individual calves, and differences between all "ascending" and all "level" phase regression coefficients were not statistically significant.

FIGURE 10

CELLULOSE (COTTON THREAD) DIGESTION x AGE



The estimation of cellulose digestion.

Less information than had been hoped was obtained from the estimation of the cellulose digesting ability of rumen contents by measuring the loss of weight of loops of cotton thread suspended in the rumen for 24 hour periods. On several occasions, with the cotton threads suspended in the rumen, the cannula came out, taking with it the cotton threads. The results which were obtained are presented in figure 10 and appendix XII, blank spaces in the appendix indicating that the cotton loops had come out of the rumen.

These results indicated that the rumen contents were capable of digesting cellulose by the time calves were about a week old and that there was some increase in the ability to digest cellulose to 2-3 weeks of age. After this there did not appear to be any further increase in cellulose digesting ability.

Times spent in grazing and ruminating and diurnal variation in pH and VFA concentration of rumen contents.

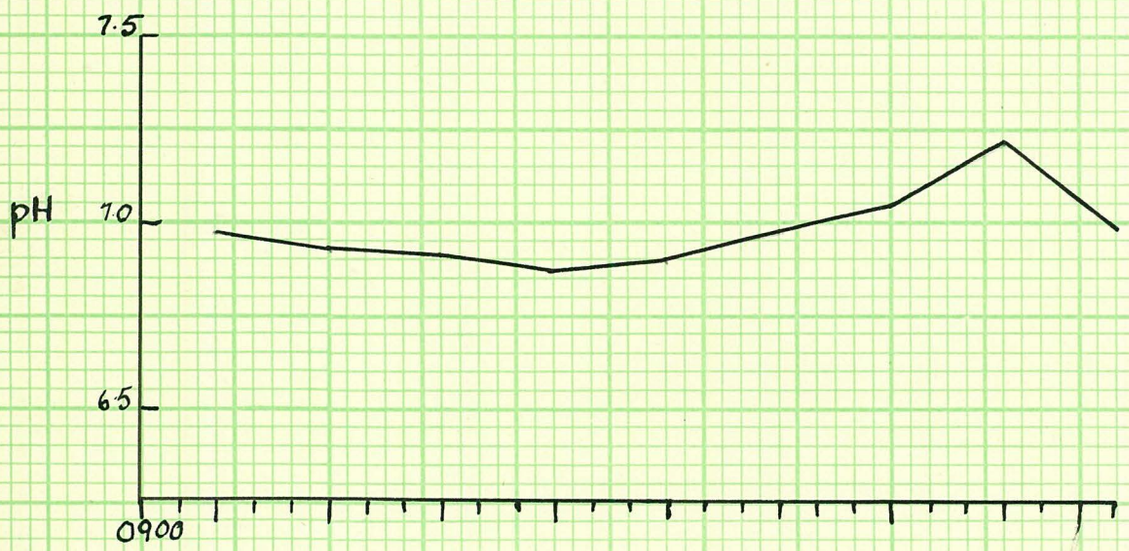
All calves had ingested some pasture by five days of age although at this stage there appeared to be little conscious effort to graze. It was observed that calves nosed around in the grass, inevitably getting some blades of it into their mouths. Such observations might be more aptly described as nibbling or "chewing at grass" rather than grazing but nevertheless some pasture was ingested as was evidenced by the rumen contents.

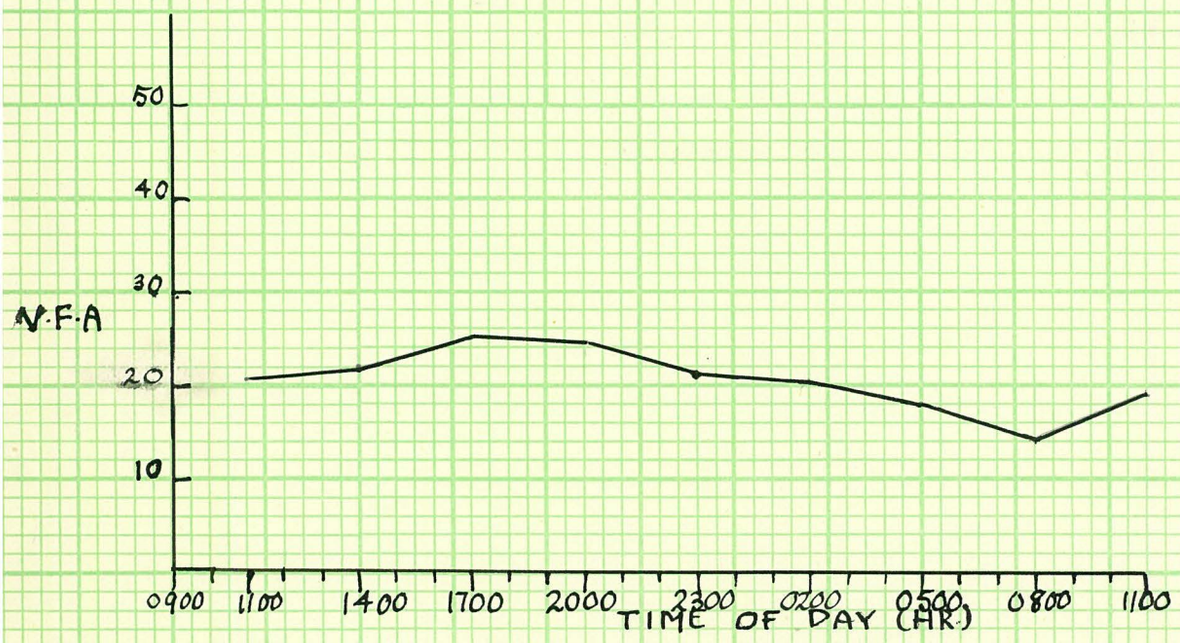
The first observations of what was considered to be true grazing were at 19, 18, 18 and 16 days respectively for calves 3, 4, 5 and 6. There was a rapid increase in the time spent "grazing" from approximately 30 minutes at 9-12 days to at least 4 hours at about 3 weeks of age. In the two calves which were observed at older stages (Nos. 3 & 4) a further increase in grazing time to 5-6½ hours was noted.

All calves had commenced to ruminate by 9-12 days, spending between 1 hour 5 minutes and 2 hours 35 minutes in this activity although one calf (no.4) spent 5½ hours ruminating at 11 days. A general increase in time spent ruminating was observed and by

FIGURE 16 CALF 6.

DIURNAL VARIATION IN V.F.A. CONCENTRATION AND pH OF RUMEN LIQUOR AT 10 DAYS OF AGE





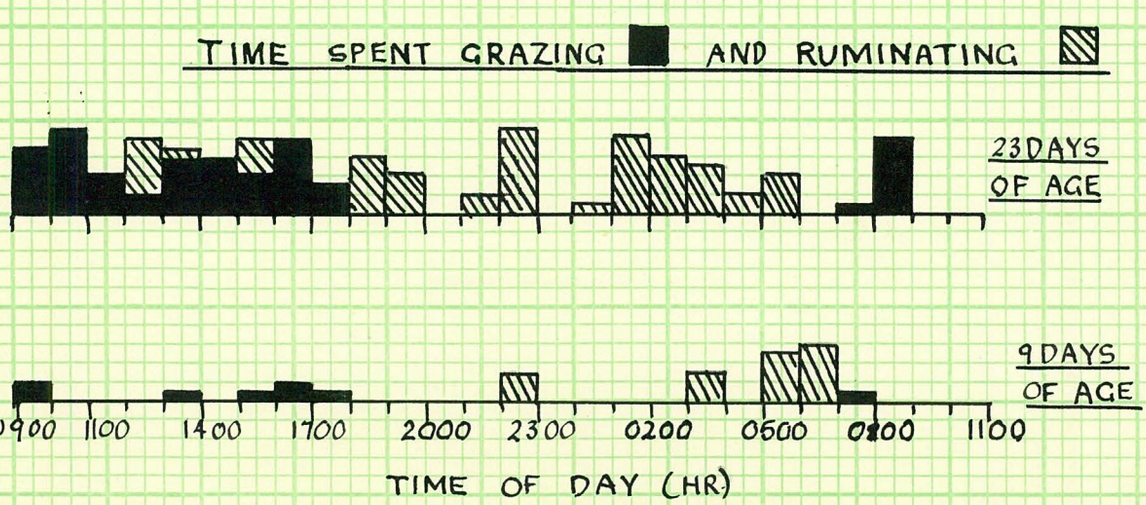
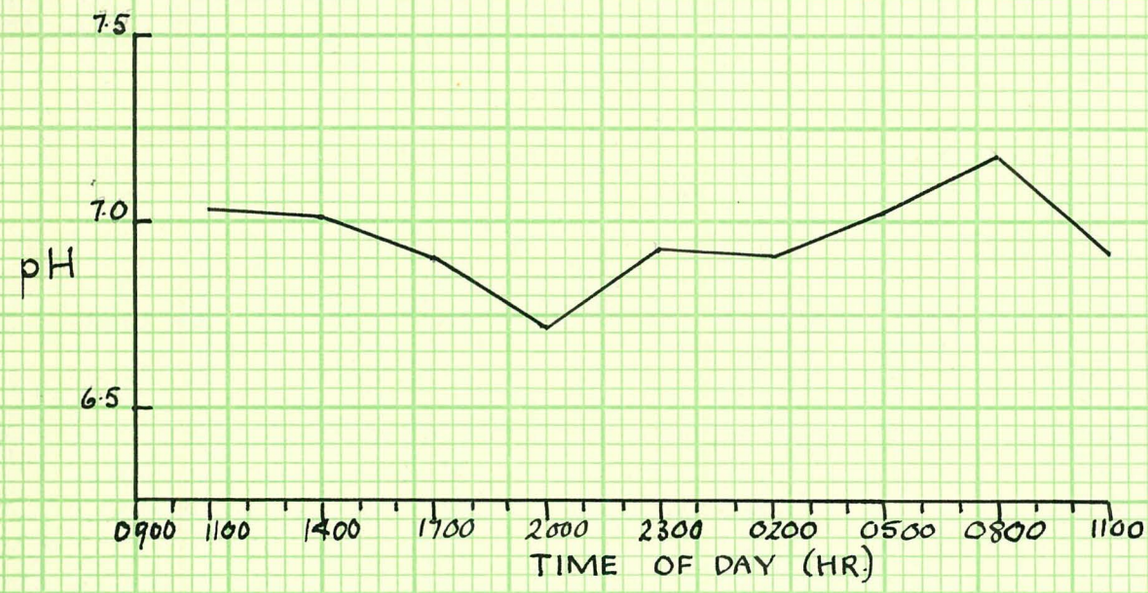
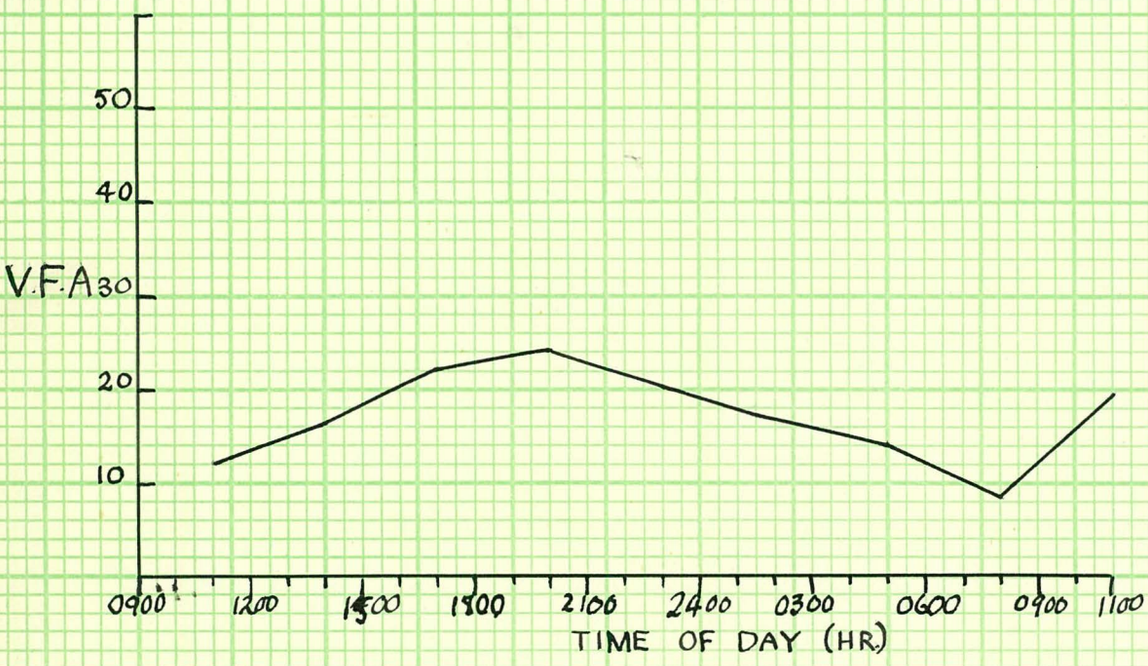


FIGURE 15 — CALF 5

DIURNAL VARIATION IN V.F.A. CONCENTRATION AND pH OF RUMEN LIQUOR AT 10 DAYS OF AGE





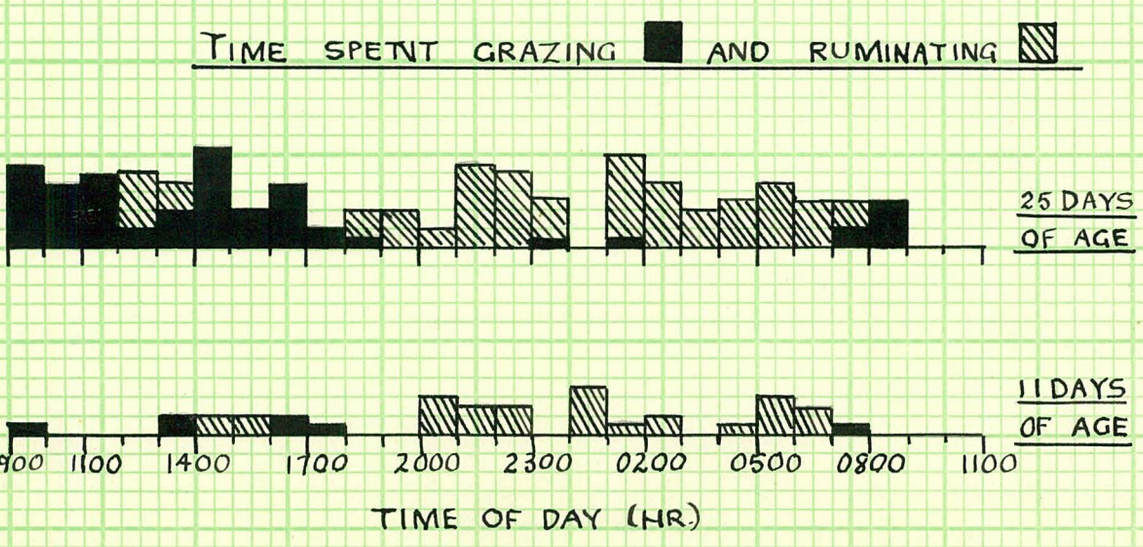
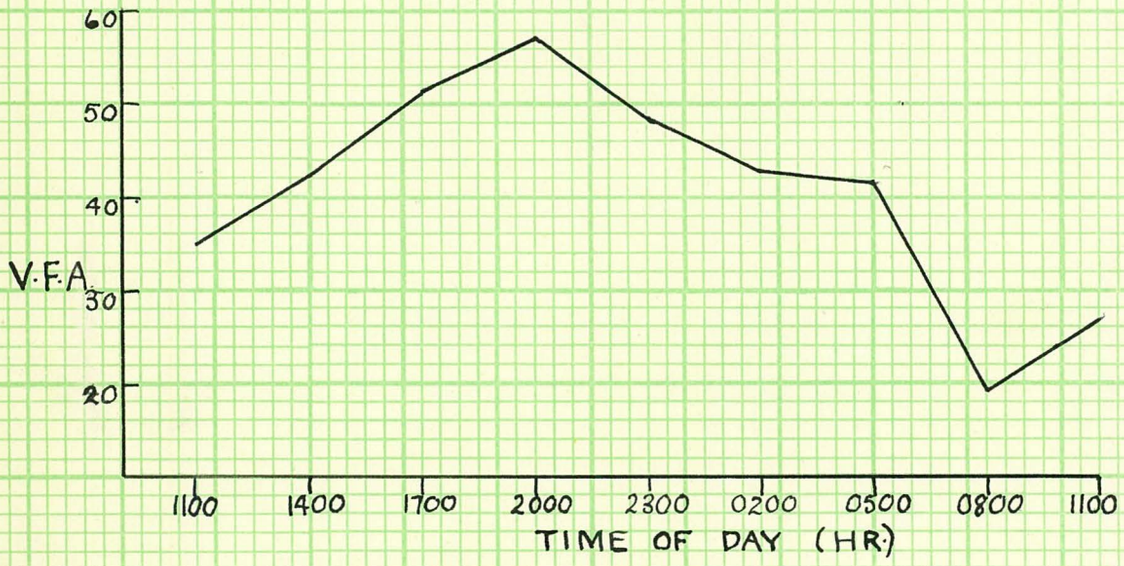


FIGURE 14 — CALF 4

DIURNAL VARIATION IN V.F.A. CONCENTRATION AND pH OF RUMEN LIQUOR AT 19 DAYS OF AGE.



TIME SPENT GRAZING ■ AND RUMINATING ▨

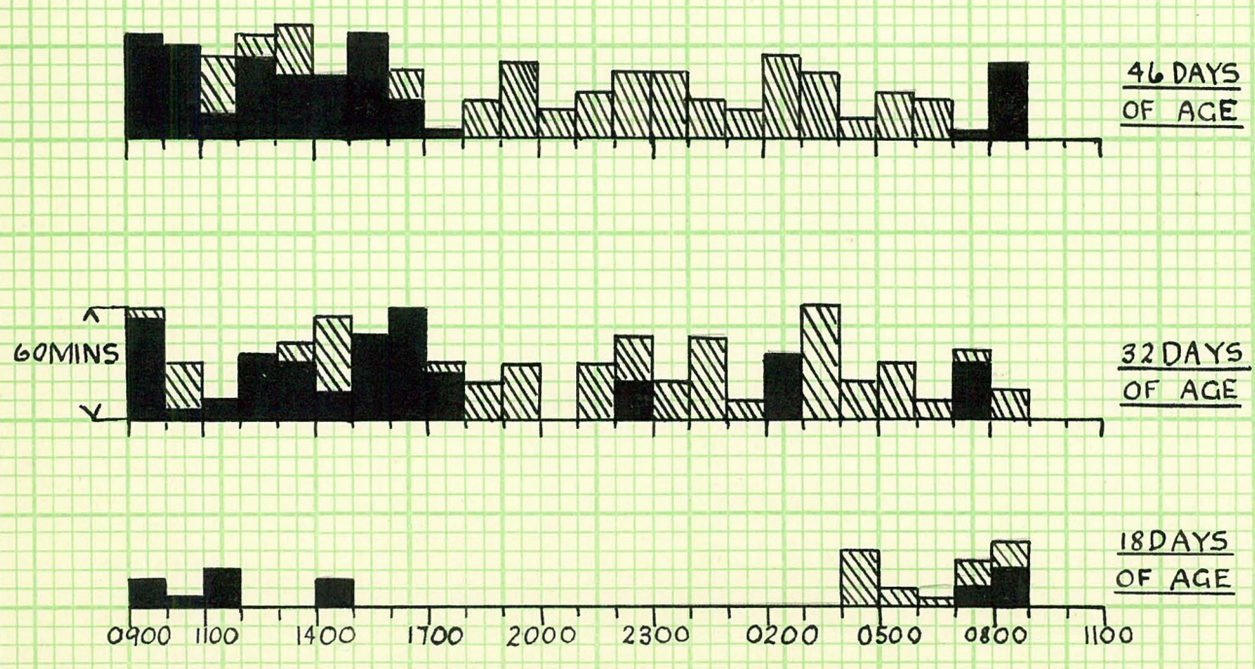
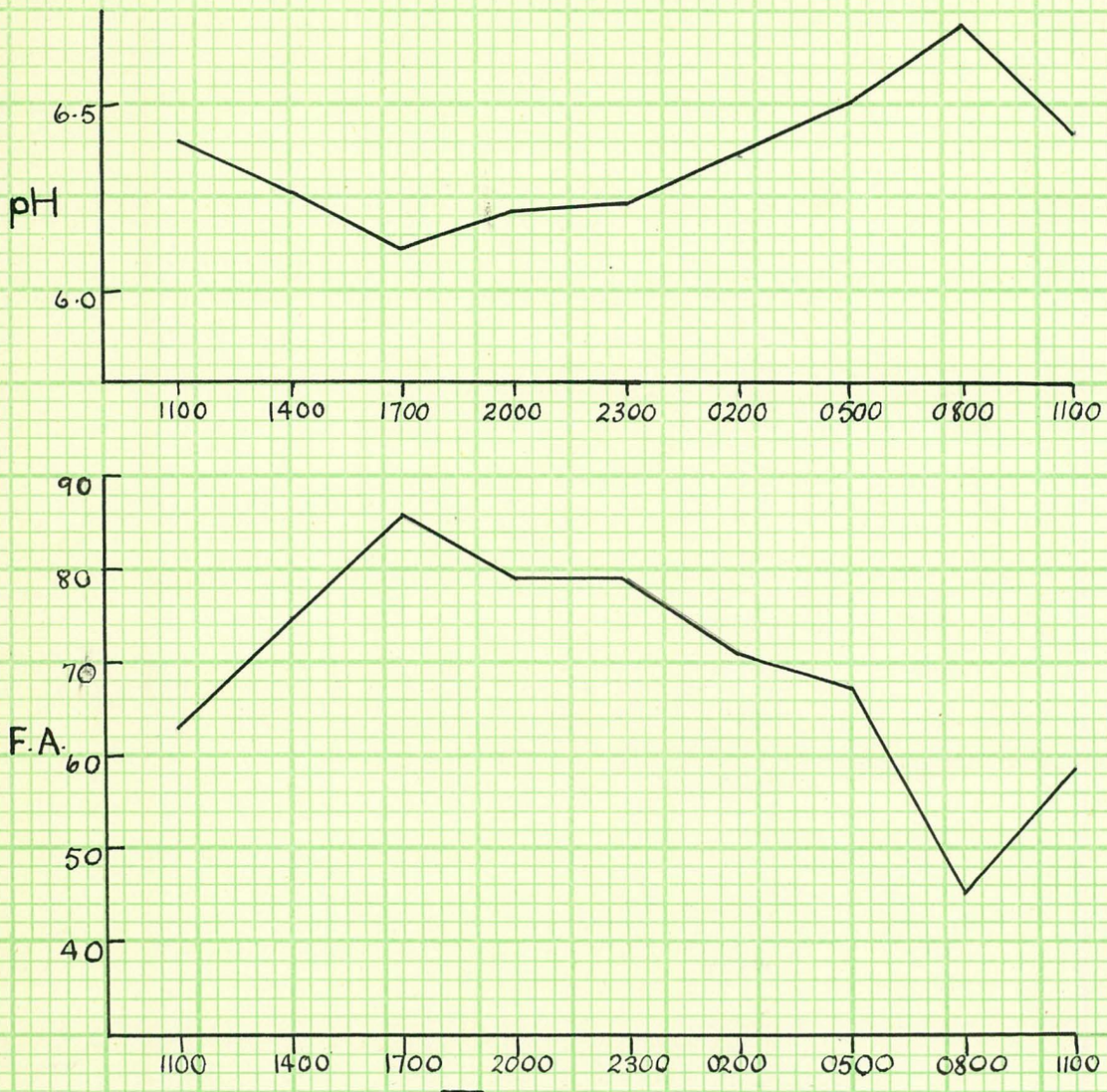


FIGURE 13 — CALF 3

DIURNAL VARIATION IN V.F.A. CONCENTRATION AND pH OF RUMEN LIQUOR AT 20 DAYS OF AGE.



TIME SPENT GRAZING ■ AND RUMINATING ▨

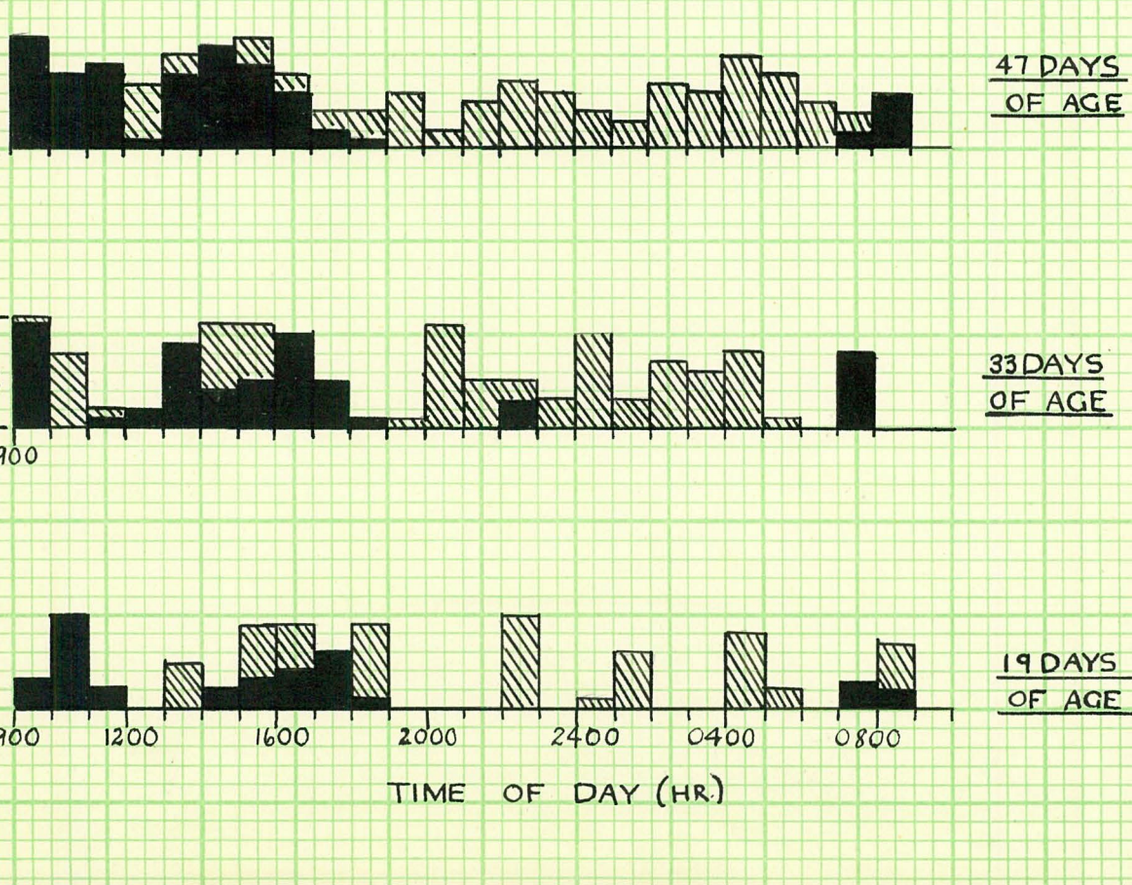


FIGURE 11

TIME PER DAY SPENT GRAZING X AGE

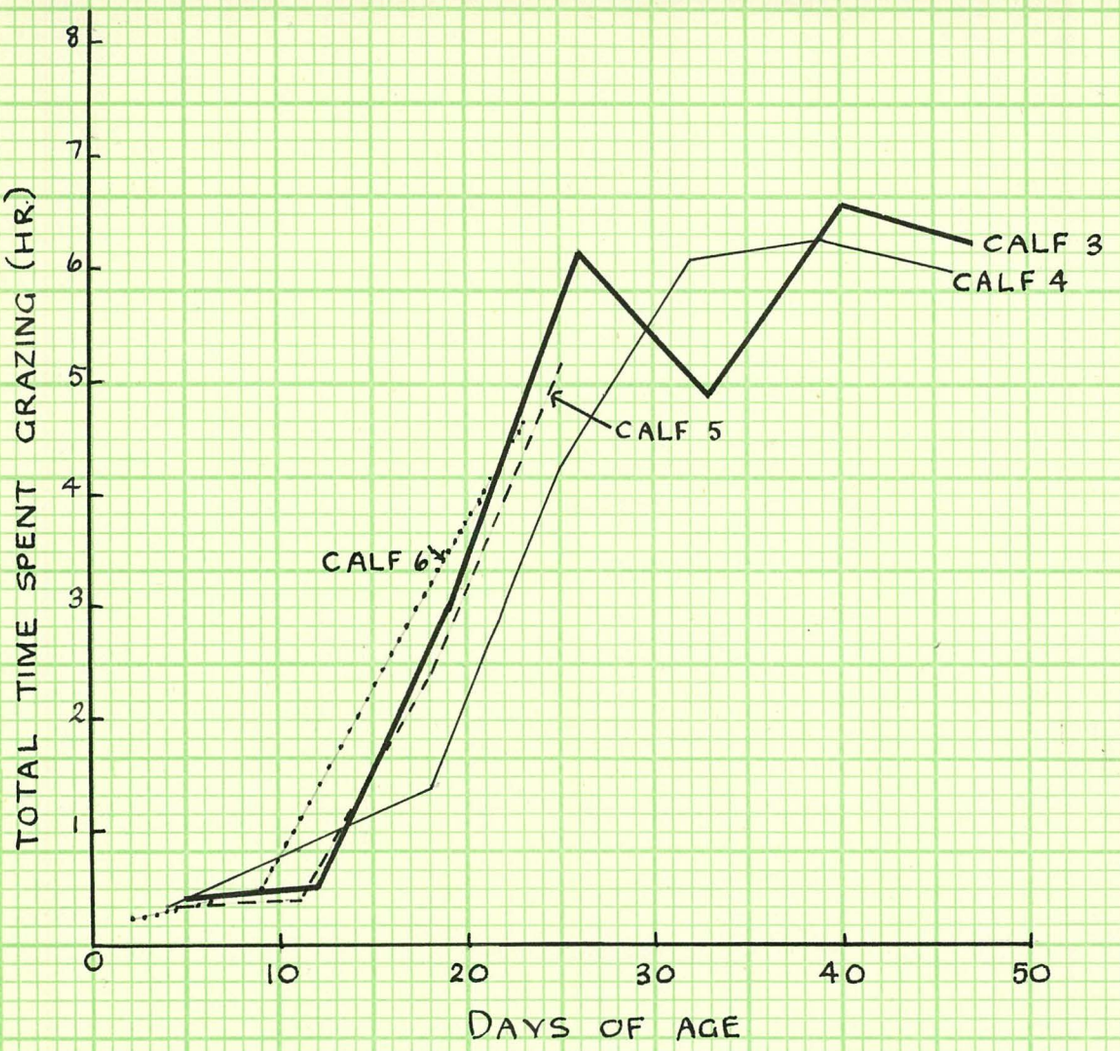
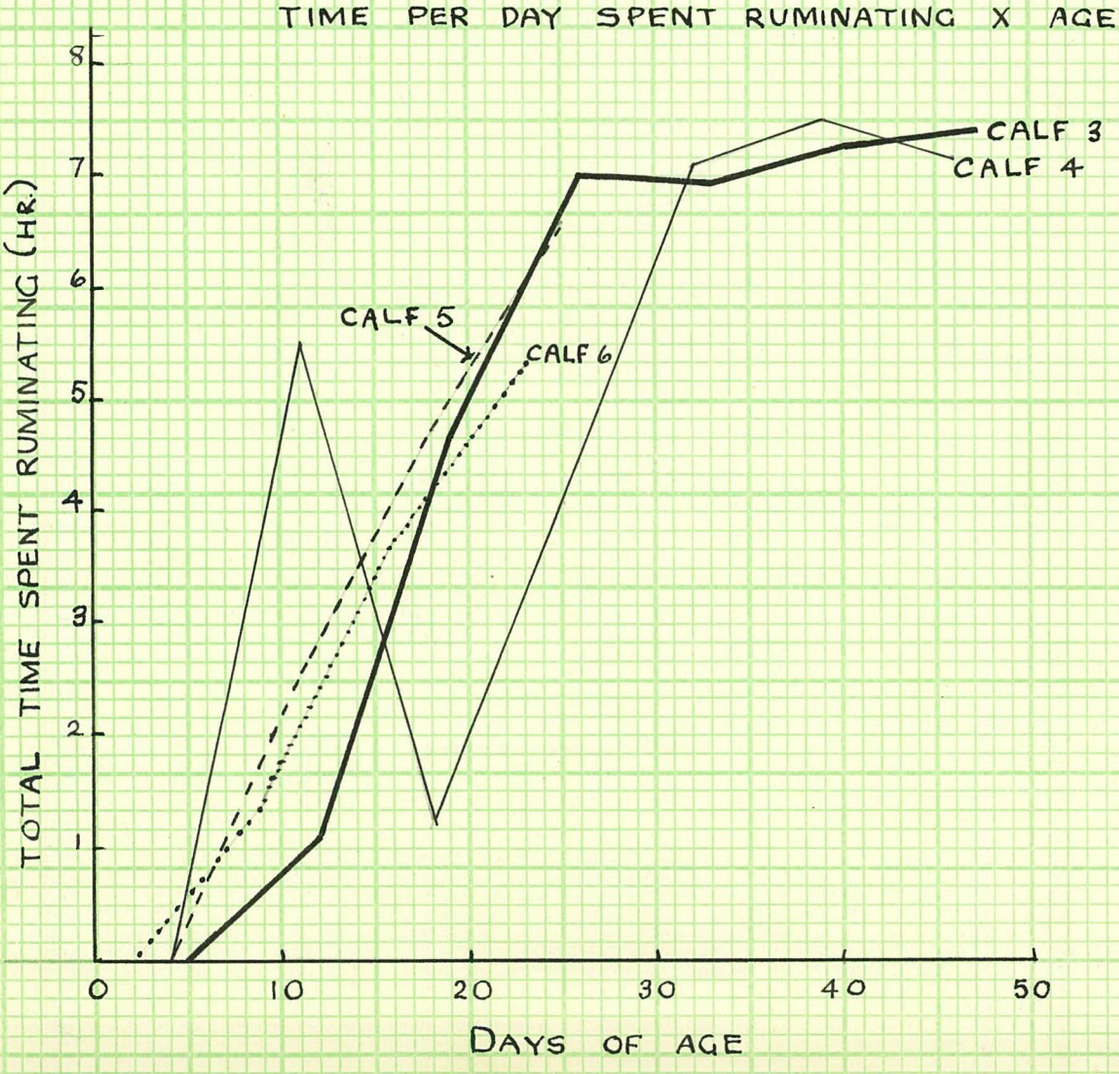


FIGURE 12

TIME PER DAY SPENT RUMINATING X AGE



3 weeks of age all except calf 4 spent over 5 hours ruminating. Although calf 4 spent 5½ hours ruminating at 11 days it only ruminated for 1 hour 15 minutes and 4 hours 5 minutes at 18 and 25 days. However from 32 days on, this calf ruminated for at least 7 hours as did calf 3. Calves 5 and 6 were not observed at these ages. Total times spent grazing and ruminating are presented in figures 11 and 12 and in appendix XIII.

Fortnightly grazing and ruminating patterns are graphed in figures 13 - 16 together with a representative curve of diurnal variation in pH and VFA concentration of rumen liquor for each calf. The diurnal variation figures are set out in appendices IIIa, IVa, Va and VIa.

It was noted that although the calves had continuous access to pasture they grazed almost exclusively by daylight and ruminated mostly at night. Occasionally a calf would consume some of its hay bedding during the night and on one occasion calves 3 and 4 grazed for a period at about 0200 hours (not shown in the graphs presented).

The patterns of diurnal variation in pH and VFA concentration were in all cases similar to those found in experiment I.

D I S C U S S I O N

MATERIALS AND METHODS

Fistulation

Although the operations appeared to be successful and to cause little discomfort to the calves, there was one possible drawback in the technique as performed in these animals. The type of cannula used was not ideal, as an imperfect fit was obtained in the fistula and it became displaced on several occasions resulting in some loss of rumen contents. Because of the imperfect fit of the cannula, some leakage of rumen contents occurred continually and concomitantly it was most likely that there was some entry of air into the rumen. Because of this, the development of rumen function may not have followed a normal pattern. However, the production of volatile fatty acids (VFA) was not prevented and no serious impairment to rumen function was indicated..

The Sampling of Rumen Contents

It was assumed that some layering of rumen contents might occur, resulting in different VFA and pH values for different parts of the rumen (see Bryant 1961). Although this was not investigated, care was taken to sample from the same position each time. As it was found that considerable diurnal variation occurred, daily samples were taken at the same time each day, this sampling time being at or near the time when peak VFA values occurred as disclosed by the diurnal variation results. This time (1700 hrs.) suited the daily routine and it was felt that at this time each day the rumen fermentation was exhibiting its peak capability in terms of VFA production.

pH determination

The pH determined from the rumen samples may not have been the true pH of the rumen contents *in situ*. Although pH was determined within 5 minutes of collection of the sample, and

duplicate determinations agreed closely, any changes in pH due to exposure to air (Turner & Hodgetts 1) could have occurred before such measurements were made, especially as the method of obtaining liquor from the rumen contents gave ample opportunity for aeration of the sample. Thus, although a large change was observed in the pH of rumen liquor between one and four weeks, this change may not have been a true representation of the pH as it occurred *in situ*.

Even if it was known that the pH obtained for each sample was the true pH of the rumen contents at that time, the question remains as to whether or not the pH trend was the same as would have occurred had the animals remained intact. Air entering the rumen may have upset the normal situation resulting in a different pH (see discussion of results).

Volatile Fatty Acid Measurement

The method used for the estimation of concentration of volatile fatty acids (VFA) in rumen liquor appears to be quite reliable at least for the higher concentrations of VFA (Bryant 1961). When titrating against low concentrations of VFA a very dilute (.02N) solution of NaOH was used and considerable care was necessary to reduce interference by carbon dioxide. Aeration of the sample distillate with carbon dioxide free air for two minutes before and during titration was found to result in a lower titration figure than without such aeration, and close agreement between duplicates was obtained. Whether or not the lower levels of VFA were accurately determined, the magnitude of the changes obtained was such that it was reasonably certain that marked changes in VFA concentration did occur as the animals aged.

Estimation of Cellulose Digestion

The estimation of cellulose digestion by measuring the loss of weight of loops of cotton thread was employed to gain additional evidence for microbial fermentation. The

high levels of VFA observed were probably in themselves sufficient evidence that plant cellulose was being broken down, but the loss of weight of cotton threads was confirmatory evidence that cellulose was being digested, though the extent of such digestion may not have been accurately determined by this means.

The method proved to be quite practicable. However, in this study, the ill fitting cannulas were a problem, as they came out on several occasions when the cotton threads were suspended in the rumen.

RESULTS

The results of this study show that a marked increase in VFA concentration of rumen contents may occur in pasture fed calves by the time they are 3 - 4 weeks old. In the calves under observation this increase took place in the space of approximately one week.

The main requirements for the production of VFA (and other fermentation end products) are a fermentable substrate, inoculation with micro-organisms capable of fermenting that substrate and a suitable environment. In this case it was obvious that pasture provided the substrate since there was no evidence that milk entered the rumen in significant quantity. Inoculation and the establishment of a suitable environment appear to have been relatively instantaneous, as the increase in VFA concentration coincided closely with the increase in grazing time.

The changes in pH of the rumen contents, as measured from the samples taken, bore an inverse relationship to the VFA concentration, and by the time the calves were about 3 weeks of age, the pH had reached a relatively constant level. The fact that the regression coefficients were highly significant statistically (with the exception of the regression coefficient for "level" phase, calf No.6) indicates that there was a close association between pH and VFA concentration. For each sample, pH seems likely to be dependent to some extent on the VFA concen-

tration. However, the calculation of regression of pH on VFA concentration is not intended to imply a dependent relationship in this direction, but was mainly for purposes of comparison. It is realized that in terms of the dynamics of rumen function, pH is likely to be a determining factor in the VFA levels attained, and that many other factors may be involved.

Since there was no significant difference between the regressions for all calves, and no significant differences between "level" and "ascending" phase regressions for each calf, this would seem to indicate that there was no great dissimilarity between the fermentation setup in each phase.

The diurnal pattern of VFA concentration and pH appears to have been closely related to the pattern of grazing. The calves grazed almost exclusively during the hours of daylight, and during this period VFA concentration increased. The VFA concentration did not fall very much until well after the last intake of pasture, probably due to the fact that there was still a considerable amount of unfermented substrate in the rumen. As time went on, less unfermented pasture would be available, resulting in a decline in VFA levels until more pasture was ingested.

Since cellulose digestion was shown to occur, and a relatively highVFA concentration was attained by 3 - 4 weeks, then despite the uncertainty of the pH measurements, it was indicated that a fermentation similar to that found in mature animals was in progress by the time the calves were one month of age. It is suggested that this adult type fermentation commenced as early as 2 weeks of age, differing mainly in its quantitative aspects and that at this age the main reason for this quantitative difference is the lack of sufficient pasture or other dry feed in the rumen.

The results of this study are in general agreement with those of Godfrey (1961) for pasture fed calves and with those of other workers (e.g. Lengemann and Allen 1959) for calves fed other diets. Wardrop and Coombe (1961) also obtained

similar results for pasture fed lambs. The main difference is that in this study a somewhat earlier increase in fermentative activity is indicated. It is suggested that this could be due to an earlier increase in pasture intake.

Wardrop and Coombe (1961) divided the development of rumen function in lambs into three stages, viz :

(i) 0 - 3 weeks; non ruminant stage.

(ii) 3 - 8 weeks; transition stage.

(iii) 8 weeks onwards; "adult" ruminant stage.

These stages appear to correspond with the pre ascending, ascending and level phases respectively of VFA concentration in the present study.

Several reasons for the presence of a transition stage were suggested, amongst which the dependence on quantity of substrate was not fully accounted for. In fact Wardrop and Coombe argued that if the development of the microflora was practically instantaneous and was dependent on the presence of the "right" substrate (Preson et al 1957) then there would be no transition phase.

However, it seems that this transition stage is a quantitative rather than a qualitative question. Some rumen fermentation appears to occur provided there is some fermentable substrate to work on, and pasture would seem to be one "right" substrate. Other dry feeds such as hay and grains also appear to provide a "right" substrate as VFA production occurs on these rations. The actual level of VFA (and presumably other fermentation end products) appears to be dependent on the amount of substrate. Possibly, then, calves have the potential of rumen fermentation from birth and the only barrier to this is the ingestion of suitable rumen-entering feed. Whether or not they would be able to make use of fermentation end products at such an early age is another question.

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APPENDIX I.

EXPERIMENT I. CALF NO.1.

CONCENTRATION OF VFA IN RUMEN LIQUOR.

Age (days)	VFA conc. (mM/litre of rumen liquor) 1	2	Average
7	Calf fistulated		
9	8.5	8.7	8.6
11	4.2	3.8	4.0
13	8.4	8.7	8.6
15	7.0	7.1	7.1
17	9.2	9.2	9.2
19	18.0	17.9	18.0
21	31.6	31.7	31.6
23	18.1	17.9	18.0
25	68.1	68.0	68.1
27	78.5	78.5	78.5
29	79.1	79.0	79.1
31	87.2	87.3	87.3
33	84.2	84.5	84.3
35	72.8	71.9	72.4
37	91.1	92.2	91.6
39	86.6	86.5	86.5
41	86.7	86.8	86.8
43	80.2	80.4	80.3
45	84.7	84.6	84.7
47	87.2	86.0	86.6
49	86.4	86.6	86.5

APPENDIX Ia.

EXPERIMENT I. CALF NO.1.

DIURNAL VARIATION OF VFA CONCENTRATION.

Age (days)	Time of sampling (hr)	VFA conc. (mM/litre of rumen liquor) 1	2	Average
21	0815	18.5	18.3	18.4
	1115	19.9	20.1	20.0
	1415	23.3	23.0	23.1
	1715	31.6	31.7	31.6
	2015	33.0	33.2	33.1
	2315	27.0	26.9	26.9
22	0215	20.2	20.3	20.3
	0515	17.5	17.4	17.4
	0815	18.8	18.4	18.6
23	0830	18.9	18.7	18.8
	1130	19.3	19.2	19.2
	1430	19.9	20.1	20.0
	1730	17.9	18.1	18.0
	2030	24.1	26.1	25.1
	2330	25.3	24.9	25.1
24	0230	27.5	27.6	27.6
	0530	27.3	27.0	27.1
	0830	27.8	27.9	27.8
49	1400	81.3	79.9	80.6
	1700	86.4	88.3	87.3
	2000	88.4	86.5	87.5
	2300	79.3	80.2	79.8
50	0200	67.2	68.5	67.8
	0500	49.2	50.8	50.0
	0800	49.5	50.2	49.9
	1100	65.5	64.2	64.8
	1400	72.7	72.5	72.6

APPENDIX II.

EXPERIMENT 1. CALF NO.2.

CONCENTRATION OF VFA IN RUMEN LIQUOR.

Age (days)	VFA conc. (mM/litre of rumen liquor) 1	2	Average
11	Calf fistulated		
13	11.6	11.5	11.5
15	12.2	12.0	12.1
17	37.4	37.9	37.6
19	55.4	56.2	55.8
21	80.2	81.7	80.9
23	77.9	77.8	77.9
25	85.2	84.0	84.6
27	71.7	73.0	72.4
29	73.0	72.0	72.5
31	80.8	81.1	81.0
33	76.3	78.3	77.3
35	88.7	89.7	89.2
37	81.5	80.0	80.7
39	80.0	79.0	79.5
41	69.7	71.0	70.3
43	78.2	78.5	78.3
45	83.4	84.0	83.7

APPENDIX IIa.

EXPERIMENT I. CALF NO.2.

DIURNAL VARIATION OF VFA CONCENTRATION.

Age (days)	Time of sampling (hr)	VFA conc. (mM/litre of rumen liquor) 1	2	Average
17	0815	19.9	20.2	20.1
	1115	23.0	22.8	22.9
	1415	26.7	27.3	27.0
	1715	37.4	37.9	37.7
	2015	46.4	45.5	45.9
	2315	40.9	41.3	41.1
18	0215	37.2	37.7	37.5
	0515	28.4	29.0	28.7
	0815	19.9	19.8	19.8
19	0830	31.5	31.7	31.6
	1130	34.0	33.6	33.7
	1430	40.6	41.3	40.9
	1730	55.4	56.2	55.8
	2030	65.3	64.4	64.9
	2330	63.8	63.0	63.4
20	0230	57.4	58.0	57.7
	0530	46.7	46.2	46.5
	0830	37.2	36.8	37.0
45	1400	75.4	76.8	76.1
	1700	83.4	84.0	83.7
	2000	82.3	83.1	82.7
	2300	74.5	75.0	74.8
46	0200	76.0	74.0	75.0
	0500	59.2	60.5	59.8
	0800	57.0	58.4	57.7
	1100	70.5	72.2	71.4
	1400	79.4	79.2	79.3

APPENDIX III.

EXPERIMENT II. CALF NO.3.

DAILY pH AND VFA CONCENTRATION OF RUMEN LIQUOR.

Age (days)	VFA concentration (mM/litre of rumen liquor)			pH		
	1	2	Av.	1	2	Av.
4	9.0	9.0	9.0	7.08	7.08	7.08
5	13.8	13.9	13.9	6.98	6.98	6.98
6	13.2	13.3	13.2	6.96	6.96	6.96
7	----------Calf fistulated----------					
8	----------					
9	19.6	19.8	19.7	6.92	6.94	6.93
10	5.9	5.8	5.9	6.88	6.90	6.89
11	13.4	13.4	13.4	6.80	6.80	6.80
12	12.6	12.2	12.4	6.80	6.82	6.81
13	31.4	32.6	32.0	6.74	6.74	6.74
14	27.7	27.4	27.5	6.36	6.34	6.35
15	42.5	43.3	42.9	6.34	6.34	6.34
16	47.6	47.4	47.5	6.42	6.46	6.44
17	69.9	70.0	70.0	6.18	6.20	6.19
18	59.0	59.0	59.0	6.22	6.26	6.24
19	73.7	73.9	73.8	6.32	6.38	6.35
20	86.1	85.8	85.9	6.12	6.18	6.15
21	82.5	82.1	82.3	6.24	6.26	6.25
22	60.0	60.2	60.1	6.58	6.60	6.59
23	78.0	78.1	78.0	6.48	6.52	6.50
24	89.0	89.2	89.1	6.22	6.28	6.25
25	100.9	100.6	100.7	6.28	6.30	6.29
26	92.4	92.0	92.2	6.32	6.30	6.31
27	90.0	90.4	90.2	6.30	6.30	6.30
28	72.4	72.5	72.5	6.36	6.38	6.37
29	80.2	80.7	80.4	6.30	6.32	6.31
30	104.0	104.4	104.2	6.10	6.12	6.11

APPENDIX III. (cont'd)

Age (days)	VFA concentration (mM/litre of rumen liquor) 1	2	Av.	pH 1	2	Av.
31	91.6	91.6	91.6	6.26	6.28	6.27
32 (b)	25.6	25.6	25.6	6.76	6.82	6.79
33	78.9	78.9	78.9	6.58	6.62	6.60
34 (c)	74.3	74.2	74.3	6.66	6.68	6.67
35	82.0	82.2	82.1	6.56	6.56	6.56
36	96.7	96.7	96.7	6.28	6.30	6.29
37	93.8	94.2	94.0	6.30	6.34	6.32
38	92.1	92.0	92.0	6.32	6.32	6.32
39	93.6	93.8	93.7	6.28	6.32	6.30
40	85.2	85.3	85.2	6.34	6.36	6.35
41	86.8	86.9	86.9	6.26	6.26	6.26
42 (c)	80.1	79.9	80.0	6.34	6.40	6.37
43	81.0	81.1	81.1	6.38	6.42	6.40
44	84.8	84.9	84.9	6.28	6.28	6.28
45	80.9	80.9	80.9	6.32	6.36	6.34
46	81.9	82.1	82.0	6.32	6.34	6.33
47	89.3	89.2	89.2	6.26	6.28	6.27

(b) Vulcathene cannula forced out; replaced by milk inflation. Considerable loss of rumen contents.

(c) Milk inflation forced out; slight loss of rumen contents.

APPENDIX IIIa.

EXPERIMENT II. CALF NO.3.

DIURNAL VARIATION OF pH AND VFA CONCENTRATION IN RUMEN LIQUOR.

Age (days)	Time of Sampling (hr)	VFA concentration (mM/litre of rumen liquor)			pH		
		1	2	Av.	1	2	Av.
20	1100	63.1	63.2	63.2	6.40	6.40	6.40
	1400	74.6	75.0	74.8	6.24	6.28	6.26
	1700	85.8	86.1	85.9	6.12	6.18	6.15
	2000	79.0	79.0	79.0	6.22	6.22	6.22
	2300	79.0	78.9	79.0	6.26	6.22	6.24
21	0200	70.6	70.9	70.8	6.34	6.38	6.36
	0500	67.8	67.6	67.7	6.50	6.50	6.50
	0800	45.1	45.0	45.1	6.70	6.74	6.72
	1100	58.5	58.3	58.4	6.42	6.42	6.42
34	1100	60.6	60.9	60.8	6.72	6.74	6.73
	1400	70.9	70.8	70.9	6.66	6.66	6.66
	1700	74.2	74.3	74.3	6.66	6.68	6.67
	2000	71.0	71.0	71.0	6.66	6.70	6.68
	2300	68.7	68.6	68.7	6.74	6.70	6.72
35	0200	58.0	57.8	57.9	6.78	6.78	6.78
	0500	50.7	50.9	50.8	6.86	6.88	6.87
	0800	48.2	48.1	48.1	6.90	6.92	6.91
	1100	64.0	64.4	64.2	6.72	6.72	6.72

APPENDIX IV.

EXPERIMENT II. CALF NO.4.

DAILY pH AND VFA CONCENTRATION OF RUMEN LIQUOR.

Age (days)	VFA concentration (mM/litre of rumen liquor) 1	2	Av.	pH 1	2	Av.
3	14.1	14.1	14.1	7.18	7.18	7.18
4	12.1	12.2	12.2	7.26	7.26	7.26
5	13.3	13.2	13.2	7.20	7.20	7.20
6	----------Calf fistulated----------					
7	----------					
8	23.3	23.6	23.5	7.24	7.24	7.24
9	13.6	13.4	13.5	7.22	7.24	7.23
10	16.1	15.8	16.0	7.16	7.16	7.16
11	15.1	15.2	15.2	6.98	7.00	6.99
12 (a)	----------					
13	15.7	15.0	15.3	7.00	7.02	7.01
14	20.3	20.8	20.5	7.20	7.14	7.17
15	33.7	34.0	33.8	6.48	6.50	6.49
16 (b)	9.0	8.9	9.0	5.80	5.82	5.89
17	24.0	23.8	23.9	5.90	5.92	5.91
18	36.7	36.8	36.7	6.00	6.02	6.01
19	51.9	51.6	51.8	5.80	5.84	5.82
20 (c)	44.4	44.4	44.4	6.02	6.08	6.05
21	50.4	50.0	50.1	6.10	6.08	6.09
22	69.0	69.1	69.0	5.96	5.98	5.97
23	79.0	78.9	78.9	5.58	5.62	5.60
24	97.3	97.7	97.5	5.40	5.40	5.40
25	88.4	88.6	88.5	5.66	5.68	5.67
26	90.0	89.9	89.9	5.68	5.70	5.69
27	92.0	92.7	92.4	5.60	5.62	5.61
28	83.0	83.0	83.0	5.92	5.90	5.91
29	84.4	84.6	84.5	6.04	6.10	6.07
30 (c)	75.0	75.1	75.0	5.98	6.06	6.02

APPENDIX IV.(cont'd)

Age (days)	VFA concentration (mM/litre of rumen liquor) 1	2	Av.	pH 1	2	Av.
31	95.3	95.1	95.2	5.86	5.88	5.87
32 (c)	88.1	88.3	88.2	5.92	5.98	5.95
33	99.1	98.8	99.0	5.76	5.78	5.77
34	94.1	93.8	93.9	5.80	5.80	5.80
35	95.0	95.0	95.0	5.74	5.76	5.75
36	101.1	101.5	101.3	5.66	5.70	5.68
37	85.0	84.9	84.9	5.86	5.88	5.87
38	82.9	83.1	83.0	5.90	5.90	5.90
39	88.6	88.6	88.6	5.80	5.84	5.82
40	88.8	88.4	88.6	5.76	5.80	5.78
41	97.9	97.8	97.9	5.74	5.76	5.75
42 (c)	74.1	73.8	74.0	5.98	6.02	6.00
43	80.4	80.3	80.3	5.90	5.90	5.90
44	78.0	78.2	78.1	5.88	5.92	5.90
45	86.5	86.2	86.3	5.80	5.80	5.80
46	86.0	86.0	86.0	5.86	5.88	5.87

(a) Vulcathene cannula partially out; Reinforcing stitches inserted. No sample taken.

(b) Vulcathene cannula forced out; replaced by milk inflation. Considerable loss of rumen contents.

(c) Milk inflation forced out; slight loss of rumen contents.

APPENDIX IVa.

EXPERIMENT II. CALF NO.4.

DIURNAL VARIATION OF pH AND VFA CONCENTRATION IN RUMEN LIQUOR.

Age (days)	Time of Sampling (hr)	VFA concentration (mM/litre of rumen liquor) 1	2	Av.	pH 1	2	Av.
19	1100	35.1	35.0	35.0	6.60	6.60	6.60
	1400	42.4	42.6	42.5	6.08	6.12	6.10
	1700	51.9	51.6	51.8	5.80	5.84	5.82
	2000	57.4	57.2	57.3	6.42	6.42	6.42
	2300	48.5	48.1	48.3	6.74	6.74	6.74
20	0200	42.6	42.9	42.8	6.96	7.00	6.98
	0500	41.5	41.9	41.7	7.22	7.18	7.20
	0800	19.2	19.2	19.2	7.04	7.04	7.04
	1100	26.7	26.9	26.8	6.80	6.82	6.81
33	1100	73.0	72.6	72.8	6.08	6.10	6.09
	1400	86.5	86.4	86.4	5.88	5.90	5.89
	1700	98.8	99.1	99.0	5.76	5.78	5.77
	2000	98.0	98.1	98.0	5.80	5.80	5.80
	2300	87.6	88.0	87.8	5.86	5.84	5.85
34	0200	80.3	80.2	80.2	6.00	6.04	6.02
	0500	67.9	67.7	67.8	6.30	6.34	6.32
	0800	66.1	66.0	66.1	6.32	6.34	6.33
	1100	77.4	77.2	77.3	5.98	6.00	5.99

APPENDIX V.

EXPERIMENT II. CALF NO.5.

DAILY pH AND VFA CONCENTRATION IN RUMEN LIQUOR.

Age (days)		VFA concentration (mM/litre of rumen liquor) 1	2	Av.	pH 1	2	Av.
4		18.5	18.7	18.6	6.90	6.90	6.90
5		19.1	19.3	19.2	6.84	6.88	6.86
6		----------Calf fistulated----------					
7		----------					
8		23.0	23.0	23.0	7.00	7.00	7.00
9		19.1	19.2	19.2	6.92	6.92	6.92
10		26.4	26.6	26.5	6.76	6.78	6.77
11		28.6	28.9	28.8	6.78	6.80	6.79
12		22.2	22.1	22.2	6.88	6.92	6.90
13		33.2	33.5	33.4	6.66	6.68	6.67
14		32.8	32.7	32.8	6.58	6.58	6.58
15		42.5	42.7	42.6	6.44	6.46	6.45
16	(b)	38.8	39.1	39.0	6.46	6.48	6.47
17		40.2	40.2	40.2	6.38	6.38	6.38
18		46.0	46.2	46.1	6.38	6.36	6.37
19		57.0	57.1	57.0	6.34	6.32	6.33
20	(c)	54.4	54.6	54.5	6.36	6.40	6.38
21		72.8	73.1	73.0	6.18	6.18	6.18
22		67.0	67.1	67.1	6.18	6.20	6.19
23	(c)	64.9	64.8	64.9	6.28	6.26	6.27
24		72.1	72.5	72.3	6.22	6.22	6.22
25		79.9	79.8	79.9	6.06	6.08	6.07
26		74.6	64.8	74.7	6.08	6.12	6.10
27		75.9	75.9	75.9	6.08	6.10	6.09
28	(c)	64.5	64.4	64.5	6.22	6.24	6.23
29		69.9	70.1	70.0	6.14	6.14	6.14

(b) Vulcathene cannula forced out; replaced by milk inflation. Considerable loss of rumen contents.

(c) Milk inflation forced out; slight loss of rumen contents.

APPENDIX Va.

EXPERIMENT II. CALF NO.5.

DIURNAL VARIATION OF pH AND VFA CONCENTRATION IN RUMEN LIQUOR.

Age (days)	Time of Sampling (hr)	VFA concentration (mM/litre of rumen liquor) 1	2	Av.	pH 1	2	Av.
12	1100	12.1	12.2	12.1	7.02	7.04	7.03
	1400	16.2	16.2	16.2	7.00	7.02	7.01
	1700	22.2	22.1	22.2	6.88	6.92	6.90
	2000	24.2	23.8	24.0	6.72	6.72	6.72
	2300	20.0	19.8	19.9	6.94	6.92	6.93
13	0200	17.4	17.2	17.3	6.90	6.94	6.92
	0500	14.2	14.3	14.2	7.00	7.04	7.02
	0800	8.5	8.4	8.4	7.16	7.18	7.17
	1100	19.9	19.6	19.8	6.94	6.90	6.92
26	1100	56.2	55.8	56.0	6.50	6.52	6.51
	1400	65.6	65.8	65.7	6.34	6.30	6.32
	1700	74.6	74.8	74.7	6.08	6.12	6.10
	2000	70.0	70.1	70.1	6.20	6.22	6.21
	2300	66.9	66.5	66.7	6.42	6.42	6.42
27	0200	58.2	58.2	58.2	6.38	6.36	6.37
	0500	55.4	55.2	55.3	6.58	6.60	6.59
	0800	50.1	50.2	50.1	6.72	6.68	6.70
	1100	62.5	62.8	62.7	6.38	6.40	6.39

APPENDIX VI.

EXPERIMENT II. CALF NO.6.

DAILY pH AND VFA CONCENTRATION OF RUMEN LIQUOR.

Age (days)		VFA concentration (mM/litre of rumen liquor) 1	2	Av.	pH 1	2	Av.
3		16.4	16.3	16.3	7.00	7.02	7.01
4		11.3	11.1	11.2	7.16	7.16	7.16
5		----------Calf fistulated----------					
6		8.8	8.8	8.8	7.10	7.10	7.10
7		10.5	10.4	10.5	7.00	7.00	7.00
8		18.8	19.0	18.9	6.96	6.98	6.97
9		27.1	27.0	27.0	6.98	7.02	7.00
10		24.8	24.9	24.9	6.94	6.90	6.92
11		23.0	23.3	23.1	6.90	6.90	6.90
12		26.3	26.5	26.4	6.84	6.82	6.83
13		38.8	38.8	38.8	6.70	6.74	6.72
14		36.4	36.4	36.4	6.76	6.78	6.77
15		46.7	46.9	46.8	6.56	6.52	6.54
16		65.1	65.0	65.0	6.52	6.52	6.52
17	(b)	51.2	51.0	51.1	6.46	6.48	6.47
18		62.0	62.4	62.2	6.32	6.34	6.33
19		77.0	77.1	77.0	6.42	6.42	6.42
20		88.1	88.2	88.2	6.38	6.36	6.37
21		97.9	97.5	97.7	6.16	6.18	6.17
22	(c)	87.2	87.2	87.2	6.16	6.16	6.16
23		89.0	89.0	89.0	6.10	6.14	6.12
24		78.4	78.6	78.5	6.16	6.12	6.14
25		86.8	86.9	86.9	6.22	6.24	6.23
26		91.1	90.9	91.0	6.18	6.18	6.18
27	(c)	81.2	81.2	81.2	6.22	6.24	6.23

(b) Vulcathene cannula forced out; replaced by milk inflation. Considerable loss of rumen contents.

(c) Milk inflation forced out; slight loss of rumen contents.

APPENDIX VIa.

EXPERIMENT II. CALF NO.6.

DIURNAL VARIATION OF pH AND VFA CONCENTRATION IN RUMEN LIQUOR.

Age (days)	Time of Sampling (hr)	VFA concentration (mM/litre of rumen liquor) 1	2	Av.	pH 1	2	Av.
10	1100	20.6	20.5	20.6	6.96	6.98	6.97
	1400	22.0	22.2	22.1	6.92	6.94	6.93
	1700	24.8	24.9	24.9	6.94	6.90	6.92
	2000	24.0	24.4	24.2	6.88	6.86	6.87
	2300	21.3	21.3	21.3	6.90	6.90	6.90
11	0200	20.7	20.1	20.4	6.96	7.00	6.98
	0500	17.8	17.9	17.9	7.04	7.06	7.05
	0800	14.2	13.8	14.0	7.20	7.22	7.21
	1100	19.1	19.0	19.1	7.00	6.98	6.99
24	1100	66.0	65.8	65.9	6.34	6.36	6.35
	1400	72.8	72.8	72.8	6.26	6.26	6.26
	1700	78.4	78.6	78.5	6.16	6.12	6.14
	2000	78.8	79.2	79.0	6.12	6.08	6.10
	2300	76.7	76.9	76.8	6.42	6.42	6.42
25	0200	71.1	71.0	71.1	6.48	6.50	6.49
	0500	69.0	69.2	69.1	6.50	6.52	6.51
	0800	59.4	59.2	59.3	6.70	6.68	6.69
	1100	69.2	69.5	69.4	6.40	6.40	6.40

APPENDIX VIII.

EXPERIMENT II. CALF NO.3.

ANALYSES OF REGRESSION OF pH ON VFA CONCENTRATION FOR "ASCENDING" AND "LEVEL" PHASES OF VFA CONCENTRATION.

Y = pH X = VFA concentration

Regression (a) - ascending phase.

$$\hat{Y} = \bar{y} + b\,(X - \bar{x})$$

$$= 6.44 - 0.008388\,(X - 46.4)$$

$$= 6.83 - 0.008388X$$

S.E.b = $\pm$ 0.00184 t = 4.559

Regression (b) - level phase

$$\hat{Y} = \bar{y} + b\,(X - \bar{x})$$

$$= 6.35 - 0.009975\,(X - 85.5)$$

$$= 7.20 - 0.009975X$$

S.E.b = $\pm$ 0.002102 t = 4.745

Test of significance of deviations from individual and common regression lines.

Source	df.	Sx^2	Sxy	Sy^2	Errors of estimate df.	SS.	M.S.	F.
Reg.(a)	9	5849.74	-49.07	0.57	8	0.1584		
Reg.(b)	26	2191.55	-21.86	0.46	25	0.2420		
Deviations from individual regressions					33	0.4004		
Common Reg.	35	8041.29	-70.93	1.03	34	0.4044	0.0121	0.331
					1	0.0040	0.0040	N.S.

APPENDIX VIII.

EXPERIMENT II. CALF NO.4.

ANALYSES OF REGRESSION OF pH ON VFA CONCENTRATION FOR "ASCENDING" AND "LEVEL" PHASES OF VFA CONCENTRATION.

Y = pH X = VFA concentration

Regression (a) - "ascending" phase.

$$\hat{Y} = \bar{y} + b\,(X - \bar{x})$$

$$= 6.14 - 0.01704\,(X - 47.4)$$

$$= 6.95 - 0.01704X$$

S.E.b = ± 0.00424 t = 4.016

Regression (b) - "level" phase.

$$\hat{Y} = \bar{y} + b\,(X - \bar{x})$$

$$= 5.82 - 0.01369\,(X - 88.3)$$

$$= 7.03 - 0.01369X$$

S.E.b = ± 0.00307 t = 4.455

Test of significance of deviations from individual and common regression lines.

Source	df.	Sx^2	Sxy	Sy^2	Errors of estimate df.	SS.	M.S.	F.
Reg. (a)	10	6610.17	-112.63	2.99	9	1.0710		
Reg. (b)	22	1244.64	- 17.04	0.48	21	0.2467		
Deviations from individual regressions					30	1.3177		
Common Reg.	32	7854.81	-129.67	3.47	31	1.3294	0.0428	0.273
					1	0.0117	0.0117	N.S.

APPENDIX IX.

EXPERIMENT II. CALF NO.5.

ANALYSES OF REGRESSION OF pH ON VFA CONCENTRATION FOR "ASCENDING" AND "LEVEL" PHASES OF VFA CONCENTRATION.

Y = pH X = VFA concentration

Regression (a) - "ascending" phase.

$$\hat{Y} = \bar{y} + b (X - \bar{x})$$

$$= 6.55 - 0.01447 (X - 39.6)$$

$$= 7.12 - 0.01447X$$

S.E.b $= \pm 0.00168$ $t = 8.603$

Regression (b) - "level" phase.

$$\hat{Y} = \bar{y} + b (X - \bar{x})$$

$$= 6.17 - 0.01147 (X - 71.4)$$

$$= 6.99 - 0.01147X$$

S.E.b $= \pm 0.00275$ $t = 4.171$

Test of significance of deviations from individual and common regression lines.

Source	df.	Sx^2	Sxy	Sy^2	df.	Errors of estimate SS.	M.S.	F
Reg. (a)	12	2784.13	-40.30	0.67	11	0.0867		
Reg. (b)	8	217.06	-2.49	0.04	7	0.0115		
Deviations from individual regressions					18	0.0982		
Common Reg.	20	3001.19	-42.79	0.71	19	0.0999	0.0053	0.321
					1	0.0017	0.0017	N.S.

APPENDIX X.

EXPERIMENT II. CALF NO.6.

ANALYSES OF REGRESSION OF pH ON VFA CONCENTRATION FOR "ASCENDING" AND "LEVEL" PHASES OF VFA CONCENTRATION.

Y = pH X = VFA concentration

Regression (a) - "ascending" phase.

$$\hat{Y} = \bar{y} + b\,(X - \bar{x})$$

$$= 6.74 - 0.01075\,(X - 38.1)$$

$$= 7.15 - 0.01075X$$

S.E.b $= \pm 0.00121$ $t = 8.870$

Regression (b) - "level" phase.

$$\hat{Y} = \bar{y} + b\,(X - \bar{x})$$

$$= 6.22 - 0.006158\,(X - 86.3)$$

$$= 6.75 - 0.006158X$$

S.E.b $= \pm 0.005718$ $t = 1.077$

Test of significance of deviations from individual and common regression lines.

Source	df.	Sx^2	Sxy	Sy^2	Errors of estimate df.	SS.	M.S.	F.
Reg. (a)	11	4686.44	-50.36	0.61	10	0.0688		
Reg. (b)	8	337.46	- 2.08	0.09	7	0.0772		
Deviations from individual regressions					17	0.1460		
Common Reg.	19	5023.90	-52.44	0.70	18	0.1526	0.0085	0.776
					1	0.0066	0.0066	N.S.

APPENDIX XI.

EXPERIMENT II. CALF NOS.3,4,5 AND 6.

TESTS OF SIGNIFICANCE OF DEVIATIONS FROM INDIVIDUAL AND COMMON REGRESSION LINES FOR "ASCENDING" AND "LEVEL" PHASES OF VFA CONCENTRATION.

I. "Ascending" phase regressions.

Source	df.	Sx^2	Sxy	Sy^2	Errors of estimate df.	SS.	M.S.	F.
Reg.3a	9	5849.74	- 49.07	0.57	8	0.1584		
Reg.4a	10	6610.17	-112.63	2.99	9	1.0710		
Reg.5a	12	2784.13	- 40.30	0.67	11	0.0867		
Reg.6a	11	4686.44	- 50.36	0.61	10	0.0688		
Deviations from individual regressions					38	1.4849		
Common Reg.	42	19930.48	-252.36	4.84	41	1.6446	0.0401	1.327
					3	0.1597	0.0532	N.S.

II. "Level" phase regressions.

Source	df.	Sx^2	Sxy	Sy^2	Errors of estimate df.	SS.	M.S.	F.
Reg.3b	26	2191.55	-21.86	0.46	25	0.2420		
Reg.4b	22	1244.64	-17.04	0.48	21	0.2467		
Reg.5b	8	217.06	- 2.49	0.04	7	0.0115		
Reg.6b	8	337.46	- 2.08	0.09	7	0.0772		
Deviations from individual regressions					60	0.5774		
Common Reg.	64	3990.71	- 43.47	1.07	63	0.5965	0.0095	0.674
					3	0.0191	0.0064	N.S.

APPENDIX XII.

EXPERIMENT II. CALF NOS.3,4,5 & 6.

CELLULOSE DIGESTION IN THE RUMEN AS ESTIMATED BY LOSS OF WEIGHT OF COTTON THREAD.

	Age (days)	% Cellulose (cotton thread) digestion 1	2	3	Av.	Corrected Av.
Calf No.3	10	8.14	8.19	8.24	8.19	8.10
	17	15.50	15.42	15.67	15.53	15.45
	24	14.69	14.39	14.21	14.43	14.32
	31	18.56	18.72	18.28	18.52	18.45
	38	16.12	15.97	16.15	16.08	16.00
	45	15.47	15.72	15.67	15.62	15.51
Calf No.4	9	5.12	5.16	5.02	5.10	5.01
	16	-	-	-	-	-
	23	13.06	13.24	13.39	13.23	13.12
	30	-	-	-	-	-
	37	14.58	14.51	14.59	14.56	14.48
	44	16.92	17.21	17.20	17.11	17.00
Calf No.5	9	7.60	7.61	7.50	7.57	7.50
	16	-	-	-	-	-
	23	-	-	-	-	-
Calf No.6	7	6.71	7.03	6.54	6.56	6.49
	14	13.06	13.18	12.94	13.06	12.98
	21	14.18	14.02	14.16	14.12	14.01

APPENDIX XIII.

EXPERIMENT II. CALF NOS.3,4,5 & 6.

TOTAL TIME SPENT IN GRAZING AND RUMINATING OVER A 24 HOUR PERIOD OF OBSERVATION ONCE WEEKLY.

	Age of Calf (days)	Total time grazing (hr:min)	Total time ruminating (hr:min)
Calf No.3	5	0:25	-
	12	0:30	1:05
	19	3:00	4:40
	26	6:10	7:00
	33	4:55	6:55
	40	6:35	7:15
	47	6:15	7:25
Calf No.4	4	0:20	-
	11	0:50	5:30
	18	1:25	1:15
	25	4:15	4:05
	32	6:05	7:05
	39	6:20	7:30
	46	6:00	7:00
Calf No.5	4	0:20	-
	11	0:25	2:35
	18	2:25	4:45
	25	5:10	6:35
Calf No.6	2	0:15	-
	9	0:30	1:25
	16	2:35	3:45
	23	4:40	5:20