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

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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.