Selection for and against susceptibility to bloat in dairy cows — a review

F. R. M. COCKREM, J. T. MCINTOSH and R. MCLAREN

Ruakura Animal Research Station
Ministry of Agriculture and Fisheries, Hamilton

ABSTRACT

A selection experiment which has resulted in dairy cows of extremes of susceptibility to bloat is reviewed.

The low susceptibility cows had a higher liquid milk production and a lower milkfat percentage than the high susceptible ones. They showed little or no bloat on highly potent pastures.

Comparison of the herds was directed towards physiological differences and relationships which could explain the origin of low susceptibility and lead to phenotypic definition useful for selection.

Some differences were found in proportions of salivary proteins and progress has been made in their separation, definition and measurement.

In vitro experiments have not indicated sufficient differences in gas or foam production per litre of rumen fluid to explain bloat grade variation but observation followed by direct experiment has shown that a greater volume of rumen fluid is associated with a high susceptibility to bloat. This could form a basis for commercial selection if appropriate measurement methods can be devised.

INTRODUCTION

Cockrem (1975) and McIntosh (1975) described plans for the investigation of the observed variation in bloat susceptibility amongst dairy cows in the context of the genetic component of this variation. The planned selection for high susceptibility (HS) and low susceptibility (LS) to bloat has now gone on for 10 years. The results of this selection are reviewed in terms of the differences in susceptibility which have arisen and possible causes of it indicated by other differences which have arisen between the 2 lines.

Possible approaches to practical solutions of the bloat problem will be discussed in the context of these new data.

The Selection Experiment

The founder herd was the identical-twin herd at Ruakura No. 4 Dairy which had been used for bloat drenching trials and was thus well classified for bloat susceptibility under the grading system described and tested by Cockrem and McIntosh (1976). This system was used throughout the experiment. The herd was divided by grades in 1972 into HS and LS founder cows and for the first 4 years they were mated to Dairy Board bulls of the appropriate susceptibility estimated according to progeny test reports. Jersey and Friesian bulls were used alternatively. From 1974 yearling bulls were used on yearling cows. The bulls were selected on their own phenotypes obtained at 6 months (autumn bloat) and 1 year of age. The herds were closed in 1976, the selected bulls being used as yearlings with yearling cows and as 2-year-olds with the older cows. Some selection was done on cows at 4 years of age and selection started on the heifers in 1981.

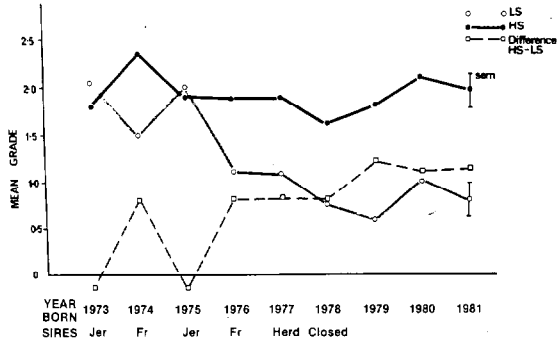

FIG. 1 The means of bloat susceptibility (see text) for cows of the HS and LS herds by their date of birth.

The results for the cows are shown in Fig. 1, where the bloat grade or susceptibility is the average of all available grades for days when at least 20% of the HS herd were grade 2.5 or above. This somewhat arbitary system was introduced to ensure that the LS grades of the animals were reflecting a reasonable challenge. The differences between the 2 herds were clearcut and arose mainly from reductions in susceptibility in the LS herd in 1976 and in daughters and granddaughters of the LS Friesian bulls.

While it is not possible from the present data to distinguish between the actions of a single, a few, or

many genes (Elston, 1981) with certainty, the sudden change together with pedigree examination and some subsequent ratios (McIntosh and Cockrem, 1982) suggest that a single locus for low susceptibility is a reasonable minimum hypothesis. This may be separate genetically from the variation amongst animals of high and intermediate susceptibility. The problem and data are discussed by Cockrem and McIntosh (in preparation).

The core of the genetic problem is the identification of the phenotype corresponding to the various genotypes. However, we know that the herds differ genetically whatever the exact basis, and therefore the correlated changes can be examined between the herds. This gives the opportunity to investigate the underlying physiology, knowledge of which could lead to measurement methods to identify the LS genotype.

Saliva Studies

Work by Clarke *et al.* (1974) indicated a possibility that salivary protein(s), designated band 4 from electrophoretic studies, could be implicated in bloat susceptibility. Subsequent work by McIntosh (1978) established quantitative techniques (McIntosh *et al.*, 1982) and some susceptibility relationships with bands 4, 6 and 7 (McIntosh and Cockrem, 1977). The band nomenclature used in this review and all the present authors' papers is that originally agreed (Reid *et al.*, 1974) and is such that band 6 will crossreact with antibovine serum albumin (McIntosh, 1975). It does not correspond with that used by Jones *et al.* (1982) where the band designated 7 crossreacts with 'antibodies raised against bovine serum' and is presumably band 6.

The rate of saliva flow has also been implicated in bloat by a number of authors (reviewed by Clarke and Reid, 1974) and this agreed with the greater saliva flow in LS cows which was found in our initial work (McIntosh and Cockrem, 1977). This saliva flow difference has not been maintained during selection but there is still evidence that bands 4, 6, 7 and 8 are implicated. The band proportions are also affected by the pasture being fed (Cockrem and McIntosh, 1978) and, in addition, whereas repeatable results were obtained with cows which had become used to the bit sampling procedure, sampling under field conditions was less successful. In cows fitted with oesophageal fistulae so that samples could be collected either with, or as an alternative to, the standard sampling with a suction bit, there was a correlation between saliva flows by the 2 methods although absolute amounts were not the same. There were also relationships for the proportions of the salivary proteins, although there were 2 major absolute differences. Firstly, there were greater amounts of band 4 and 6 in the oesophageal samples. Secondly, they contained less of the major band 7. This resulted from an apparent change of this band to band 8. The ratio of band 4 to bands 7 and 8 was related to susceptibility for cows when fed on clover but not when fed on hay. The bit sample could be used to predict the oesophageal sample which presumably in turn represents what actually reaches the rumen. The higher ratio was from HS cows fed clover or grass.

For detailed work on saliva, D. H. Carr of Massey University has developed a technique for temporary cannulation of individual glands. Collections are then made under sympathetic or parasympathetic nerve stimulation. This has established the origin of the various bands and shown that they are not unique to specific glands. Because of effect on the autonomic, system, the handling of the animals in routine sampling could be the source of sampling errors and these measurements under stimulation give a basis for experiments on the sampling methods. We are now looking for appropriate conditions, if such exist, whereby repeatable relationships with susceptibility might be obtained.

Such a test of sampling methods would require the measurement of a large number of samples, as would the final practical application and therefore considerable attention has been given to quicker measurement methods than those provided by the electrophoretic techniques. Separation methods to obtain the pure proteins have been developed so that antibodies can be prepared. The availability of specific antibodies means that techniquies such as nephelometry or ELISA are available for fast accurate measurement of actual concentrations. Work is also being done on the production of monoclonal antibodies with their increased specificity and repeatability.

A more direct approach is to characterise the proteins and determine their actual functions in the rumen (if any) or in relation to the functioning of the salivary glands. A possible example of a direct effect arises from the knowledge that band 6 has a similar molecular weight to bovine serum albumin and cross-reacts with its antibody. Bovine serum albumin has considerable foam stabilising properties and is more slowly metabolised in the rumen than other proteins (Nugent and Mangan, 1978). By competing for sites of proteolysis it could also slow the breakdown of other possible foam producing proteins.

In Vitro **Experiments**

There are 3 main sources of variation associated with the bloat grade attained by an animal: firstly, the saliva, of which both quantity and quality will interact with feed from the previous 24 or 48 hours to produce rumen fluid; this rumen fluid then ferments the current feed in the presence of the current saliva to form gas which can then be trapped in a foam which will have a certain persistence. Thus for bloat to occur these 3

sources of variation, rumen fluid, current feed and saliva must combine to form sufficient gas which must be trapped in foam for long enough for the animal to inflate. While there has been considerable speculation on these causal components (e.g., Reid *et al.*, 1974) there have been few experiments designed to examine all the factors together.

In order to test these factors experimentally, 2 HS and 2 LS cows were fitted with rumen fistulae so as to obtain rumen fluid based on different feeds and on the different salivas produced by these animals. An *in vitro* technique was developed using strained rumen fluid and pasture juice or other substrates together with an artificial saliva buffer which could be substituted with salivas of specific origin. Response was measured as gas production (ml), foam production (ml) and the amount of liquid taken up by the foam. This last measurement is highly correlated with the stability of the foam (Cooney, 1974). A full description of the technique and results from it is to be published shortly by Cockrem, McLaren and McIntosh.

In 1981, initial experiments incubated rumen fluid from the 4 cows with juice from the pasture being grazed or with pure clover or grass sampled from other paddocks. The occurrence of bloat on the paddock being grazed was noted. This was done every week from June to the end of October but only the key results into and through the bloat season have been analysed so far.

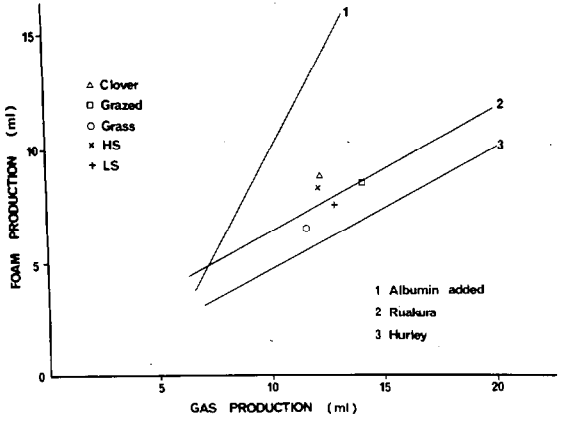

FIG. 2 The regressions of total foam (y) on total gas production (x) after 90 minutes *in vitro* fermentation together with mean values.

In 1 trial bovine serum albumin, at the concentrations expected from saliva, was used instead of a pasture juice. Glucose was also added as an additional cross-treatment to increase gas production. This tested whether substrate or rumen fluid (as micro-organism population) was limiting gas production and also whether gas production or foam potential was limiting foam production. Results expressed as the regression of foam volume on gas volume are shown in Fig. 2. The slopes of the regressions were similar for all the subgroupings of juices, susceptibility and whether the pastures grazed were causing bloat or not, except that the line for clover/non-bloating was slightly steeper. Non-bloating in this context refers to the rumen fluid and not necessarily the clover itself which was not from the paddock being grazed. The addition of albumin led to a steeper line and a lower gas production. A similar slope was also found in a group of 10 clover fed calves whose rumen fluid was fermented with clover juice in preliminary trials of the technique at Hurley (UK). The slopes for all individual animals were also similar in the Hurley trial. The position of the lines (i.e., foam production at constant gas production) was such that HS rumen fluid produced slightly more foam than that from LS cows and clover juice more than grass juice. Rumen fluid from cows on bloat-potent pasture also produced more foam per unit of gas for all the juices being fermented, including grass. There were negligible gas production differences and the order of differences in actual foam volumes was insufficient to account for the observed bloat grade differences of 1.5. Calculation of the gas required to inflate a cow containing 30 litres of rumen fluid to observed bloat grades and reported pressures (see Clarke and Reid, 1974) indicated that the 20 ml of rumen fluid used *in vitro* would have had to produce 5 ml more foam (cf. < 1 ml actually produced) to account for a bloat grade difference of 1.

The calculations also showed that at the same production of gas per unit volume of rumen fluid, then a cow with rumen contents filling 0.5 of its rumen would have at least 1 grade less than a cow with rumen fluid comprising 0.75 of its rumen size. While sampling the cows for the *in vitro* work it was observed that the level of digesta was consistently lower in LS cows relative to the fistula. Estimates indicated possible volume differences of an order which could account for observed HS/LS differences in bloat grade and it was decided to test this possible relationship experimentally.

Rumen Fluid Exchange Experiments

This experiment was planned to test the effect on degree of bloat of volume of rumen digesta in conjunction with the effects of the rumen fluid itself (HS or LS) and the susceptibility of the cow containing it. After overnight starvation the level of rumen contents was estimated, they were pumped out, the volume measured and the contents replaced in the same or another cow. At the same time the volumes were adjusted to large or small according to the plan shown in Table 1. The new level was also estimated. The whole experiment was replicated by replacing HS1 with HS2 and LS1 with the cow LS2 shown in the table. After the exchange, which took about 30

TABLE 1 The plan for the experiment showing origin of rumen contents after adjusting their levels. The experiments were replicated by interchanging HS1 with HS2 and LS1 with LS2.

Experiment type		1		2		3	
Volume		Large	Small	Large	Small	Large	Small
Host cow	HS	OWN	HS2	OWN	HS2†	LS1 + LS2	LS1*
	LS	LS1 + LS2	LS2 + HS2	LS1 + LS2	LS2*	HS1	HS2†

* Very small volume.
† Some discarded.

minutes, the animals were grazed on lucerne pasture and graded for the maximum bloat attained.

Both the LS cows reached a grade of 3, a new experience for them, and bloat was associated with the greater volumes of rumen fluid regardless of the susceptibility of the host or the donor cow. Because the potency of the lucerne could vary daily the results in Table 2 are summarised as differences in grades for large volume to small volume. The results are consistent and independent of animal susceptibility. The variance of bloat grade accounted for by rumen digesta volume was 64% and that of the eye-level estimates by actual volume, 61%. Using this latter regression on all the level estimates obtained over the 4 months sampling then, after 12 hours starvation, with access to water, the HS cows had about 40 litres of rumen fluid and the LS cows, 30 litres. Total rumen size was about 50 litres.

TABLE 2 Bloat grade differences from the experiments in Table 1 expressed as those from the large volume treatment less those from the small volume treatment.

Experiment type	1		2		3	
Host cow	HS	LS	HS	LS	HS	LS
Replicate 1	1.5	1.5	1.5	0.5	2.0	1.5
2	1.5	1.0	1.0	1.5	1.5	2.0
Mean	1.5	1.25	1.25	1.0	1.75	1.75

As the test on lucerne was after the exchange of rumen contents then all the cows would have added their own saliva to the mix at this stage. Thus LS cows with HS or LS rumen contents and LS saliva bloated to a level normally associated with HS animals. Given confirmation on a larger number of animals this means that for the LS animals that we have bred neither the origin of the saliva or the rumen fluid was of importance in determining differences between animals. It does *not* test the importance of these factors for pasture or other environmental differences (e.g., the effect of clover on the secretion of bands 4 and 6 (McIntosh, 1978)).

The observations on the volume differences and their significance have put into perspective some earlier data (being prepared for publication) on blood levels of albumin and haematocrits which, in the absence of anemia, are inversely related to blood volume (Ulrych, 1973). LS cows had a higher haematocrit (lower blood volume) particularly in the spring and overall there was a negative relationship between haematocrit and bloat susceptibility.

Production Differences

These have been consistent over the last few years with HS cows having a lower liquid milk production but a higher milkfat content so that total milkfats are fairly similar. Because of the mixed Jersey Friesian origin a detailed analysis is needed to test a possible sire × breed founder effect. However this is unlikely to be important because the animals of the 2 origins will have been successively crossed as part of the plan to avoid inbreeding. The differences do not arise from differences in the indices of the founder bulls. They are now being examined in relation to feed intakes, digestibility and complete balance experiments.

The Physiological Factors Involved

The relevance of these new results to our understanding of the animal factors in bloat would appear to lie in the dynamic fluid balance of the animal. The key aspects of this shown in Fig. 3 are:

1. The high saliva flow associated with feeding can lower plasma volume and increase its osmolality (and haematocrit) (Dooley and Williams, 1976). Parotid gland but not mandibular gland flow is

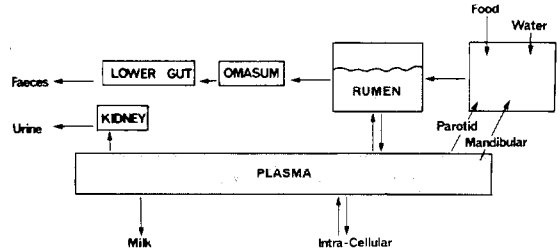

FIG. 3 The main compartments involved in water balance in the ruminant.

reduced by a feedback effect which is probably acting through osmoreceptors (Carr and Titchen, 1978).
2. Parotid flow is isotonic with plasma while mandibular flow is hypotonic. The major other inputs to the rumen fluid which may affect its osmolality are sodium and potassium in the feed, but fermentation products such as volatile fatty acids could also be important.
3. Water exchange across the rumen wall (and hence 1 possible origin of level effects) will depend on the relative osmolalities of rumen fluid and plasma. Sodium, potassium, urea and volatile fatty acids will also exchange by gradients or active transport.
4. Plasma osmolality will also be affected by loss of sodium and potassium relative to water loss through the kidney. The sodium is under active homeostatic control (by readsorption) but not potassium. Water loss is related to adjustment of blood volume and blood pressure and depends upon a complex control system involving vasopressin, kallakreins and the angiotensin-renin system (Ganong, 1981).
5. Kallakreins occur in saliva, and, in the mouse, the mandibular gland is an important source of renin (Bing *et al.*, 1980).

It is clear that the genetic control which shows as differences in relative volumes of rumen digesta could arise in a large number of ways, including the products of the salivary glands. Amongst environmental effects, as well as substrates for gas production, and foaming or foam stabilising agents, both relative and absolute amounts of sodium and potassium must also be considered. In this context a high potassium diet can lead to a decreased saliva flow (Beal *et al.*, 1975) and also to a preferential loss of sodium from the rumen with its subsequent excretion by the kidney and the possibility of an induced sodium deficiency (Warner and Stacey, 1972). However changes from a low to a high sodium feed leads to sodium retention and greater rumen volume and vice versa (Dobson *et al.*, 1966). These results suggest that a high potassium but not necessarily a low sodium might predispose to bloat but this could well be a gross oversimplification of a complex system. P. W. Young (pers. comm.) in a switchback trial on high and low sodium pastures, both with high potassium, found no difference in the proportion of cows with obvious bloat.

Possible Practical Applications

The best practical solutions to problems arise from a clear understanding of the mechanisms involved. We are some way from this point with bloat but the knowledge obtained from the selected LS cows suggests some empirical approaches which could be worth trying.

Pasture management to avoid the combination of factors which cause bloat would seem a desirable approach. However as we have little or no idea of the factors leading to rapid gas production and foam formation, even an empirical approach is not possible. Even if we knew the factors our knowledge of pasture management itself is still so empirical that it is unlikely that methods could be developed.

As cows which do not bloat can be obtained genetically apparently without loss of production, then methods for selecting these animals would appear to be the best use of limited resources. Direct selection on potent pastures as we have practised it is not very efficient. In the early stages there may be some mortality if many animals are being tested at once but more important is the variability of potency of pasture for bloat. This can be overcome in part by using 'standard' animals, but years in which very little bloat occurs can cause a break in the records. Also the very success of selection for LS animals means a requirement for more potent pasture to ensure that they have been adequately tested. The comparison of HS *v* LS has been an important aspect overcoming this problem in our research but only LS are required in practice.

Various ways of identifying phenotypes and hence genotypes are being worked on as resources permit and these, in summary, are:

1. Measurement of the relative proportions, absolute production, or concentrations, of particular salivary proteins. This still seems a likely system if some sampling and measurement problems can be overcome.
2. Saliva flow rates. Unlikely as a practical proposition and doubt exists as to relationships to susceptibility in our selected animals.
3. Use of a synthetic (and hence standardised) feed to cause mild bloat which could be measured by girth behind the last rib. If the key variable is the total gas production from differing volumes of rumen fluid, then using natural or artificial build up of foaming agents to trap the gas and a standard amount of substrate (e.g., sucrose) to produce it, might result in measurable differences in distension. The basic foaming agents may be present in rumen fluid for a large part of spring and autumn and this could be made use of.
4. Measurement of variation in volumes of rumen liquor by weighing after feeding and again after 12 hours starvation (Tulloh *et al.*, 1965). Preliminary trials indicate that this might work but the exact conditions could be difficult to define.
5. Estimation of the level by defining the interface between rumen liquor and gas after starvation. X-rays are being tried but there is some problem in always obtaining a clear interface, possibly due to some foaming. The alternative of using ultrasonic reflections has been tried with commercial apparatus but the frequencies available do not distinguish

between rumen fluid and gas. We can detect levels in a plastic bag of water placed in the rumen. Apparatus which would produce a range of frequencies might enable suitable ones to be found.

6. Given the right conditions relative to feeding then blood volume differences as haematocrits or plasma osmolality variations might lead to the detection of susceptibility from blood samples. It also is possible under this model that differences occur in blood pressure and that this could be measured.

All these approaches depend on the assumption that the level differences observed in the 4 cows are generally applicable to our HS/LS herds. They are also most likely to succeed if and when we know more about the causes of the variations in the system which we have outlined.

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