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PROGRESS IN IDENTIFYING SOURCES OF ANIMAL VARIATION IN SUSCEPTIBILITY TO BLOAT

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SUMMARY

In animals of differing but defined susceptibility to bloat, comparative studies are being made of gastric anatomy and motility, salivary composition, total salivation rate (with/without pilocarpine), and the occurrence of "yellow bubbles" in the rumen contents. Possible differences correlating with susceptibility have been found for each parameter. Best correlations have been with "band 4" protein in saliva, and yellow bubbles (both high in highly susceptible animals). The least susceptible animal had the highest total salivation rates.

INTRODUCTION

Common pasture bloat in ruminants is caused by persistent foaming of their stomach contents. It occurs most often when succulent red or white clover or lucerne is eaten and is more prevalent in cattle than sheep. A recent comprehensive review is that of Clarke and Reid (1974).

Susceptibility to bloat varies widely amongst individuals, some exhibiting high susceptibility (HS animals) and others, at the opposite end of the range, low susceptibility (LS animals) (Clarke and Reid, 1970; Reid *et al.*, 1972). This paper reports briefly on progress in the search for causes of differences in susceptibility. The work is part of a collaborative project of DSIR, MAF and NZ Dairy Board, investigating the possibility of breeding less susceptible animals. Work on other aspects of the project will be described in the papers that follow (physiological genetics, Cockrem, 1975; salivary composition, McIntosh, 1975).

Since the bloat foam occurs in the stomach during the early digestion of the feed, the number of possible sources of animal variation appears limited. Included are factors such as the extent of chewing, the amount and quality of the saliva secreted, the anatomy and motility of the reticulo-rumen, the permeability of the stomach walls, the nature of the contents of the stomach before feeding, and its microbial population (Mendel and Boda, 1961; Clarke and Reid, 1974; Reid *et al.*, 1975). Comparative studies of anatomy and motility of the stomach, the composition and flow of saliva, and the nature of the rumen contents in a small group of animals of differing but defined susceptilibity, are described here.

EXPERIMENTAL AND RESULTS

The susceptibility of the experimental animals was first established at Ruakura under the conditions of a grazing dairy herd. The cows were then brought to Palmerston North, where the present studies were carried out. In most of the work, one member from each of 4 sets of identical twins has been used, assuming that two of the selected animals would be HS and two LS. All animals have rumen fistulae, are non-lactating and, during experiments, are stall-fed.

1. Anatomy and Motility of the Reticulum and Rumen

The dysfunction in bloat is interference with the expulsion of gas from the reticulo-rumen. Persistent foaming represents a longer retention time of gas in the digesta mass, which increases in volume as a result. These changes reduce the effectiveness of eructation, the main pathway of gas expulsion (Clarke and Reid, 1974).

Differences in anatomy or motility that affect the efficiency of movement of gas within or out of the reticulo-rumen could be a source of variation in susceptibility to bloat. Eructation is normally associated with B sequences of rumen contractions (those independent of the reticulum — Reid, 1963), but may also occur during A sequences (those associated with reticulum contractions).

A study of the relative levels of structures in the reticulo-rumen at rest, and their vertical displacement during A and B sequences is being undertaken, using an adaptation of the method of Reid and Cornwall (1959). The most obvious differences so far found between animals of higher and lower susceptibilities relate to the positions and excursions of the cranial and caudal pillars. The vertical component of pillar movements is significantly greater in the animals of higher susceptibility.

We do not know the meaning of these results. Other observations suggest that gastric structure and motility are not major determinants of susceptibility. Thus LS animals can be induced to bloat merely by exchanging rumen contents with HS animals before feeding (Clarke and Reid, 1970). Also rumen motility can persist until advanced stages of bloating. Indeed, bloating animals, whether HS or LS, characteristically continue to attempt to eructate in association with B sequences, with the result that increasing amounts of foaming digesta are delivered to the oesophagus, only to be re-swallowed, a form of gastric refluxing. Changes in motility do occur when bloating becomes more severe. Observations through perspex windows in rumen cannulae show that the effectiveness of contractions in moving the foaming contents progressively diminishes. The excursions of the cranial pillar also diminish in amplitude, especially during B sequences.

2. Saliva

Involvement of saliva in bloat has long been suspected but the full nature of the involvement remains uncertain. Various possibilities include dilution, effects on pH of the digesta, gas production by acidification of bicarbonate and the provision of mucoprotein (Clarke and Reid, 1974). Research in this area is not easy. First, there are several sources of saliva, the secretions from each of which have different properties. Second, both secretion rate and composition vary in relation to factors such as feeding, mechanical and chemical stimuli arising in the mouth, tactile and stretch stimuli arising in the oesophagus and stomach, hormone levels in the blood, sodium status, blood osmolality, and drugs. Salivation at any given time will represent a summation of the effects of all factors, a balance that is readily upset by experimental procedures. Lastly, there are practical problems of collection. Individual secretions, other than those of the parotid glands, are difficult if not impossible to obtain in the conscious cow: total secretions can be collected reliably only from the oesophagus, using either a surgical fistula or intubation, each with its disadvantages.

In the present studies, swallowed saliva has been collected from the lower oesophagus, using a modification of the oesophageal intubation method of Mendel (Mendel and Boda, 1961). The rumen contents are removed through a rumen fistula and a cuffed, wide-bore tube inserted into the terminal oesophagus and held there by hand, saliva being aspirated by suction. This procedure introduces abnormalities in that it removes stimuli provided by the rumen contents and introduces others, stretch and tactile stimulation of the cardia.

(a) *Composition*

Macromolecules in bovine saliva fall into three groups: (1) Salivary mucoprotein, a high MW sialoprotein originating in the submaxillary glands and associated with stabilization of leaf protein foams (Jones, 1971; Jones and Lyttleton, 1973); (2) Salivary mucin, a high MW carbohydrate; and (3) Low MW proteins, small enough to enter a 7½% acrylamide gel during electrophoresis. The small protein fraction can be separated by electrophoresis into some 10 "bands", and the concentration of one of these, band 4, appears to correlate with susceptibility to bloat and has been suggested for use as a possible genetic marker (Clarke *et al.*, 1974). Band 4 concentration was found to be less than 2% of the total small protein fraction in LS animals and 17 to 18% in HS.

In Fig. 1, band 4 concentration has been plotted against susceptibility, as determined by stall-feeding fresh red clover (*Tri-*

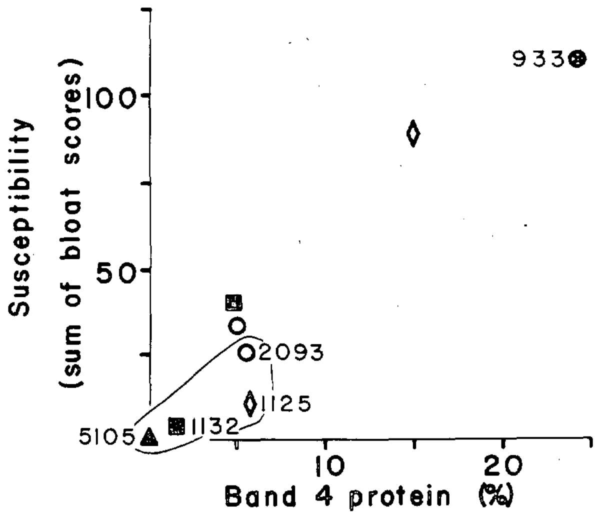

FIG. 1: *Band 4 concentration in the small MW protein fraction of swallowed saliva and susceptibility to bloat of the experimental cows, their identical twin sisters, and a known HS indicator cow, 933. Band 4 concentration determined by method of Jones (1971); susceptibility assessed by summing maximum daily bloat scores during 28 days of stall-feeding fresh red clover for two 2 hr periods each day. Solid symbols, reputed LS animals; open symbols, reputed HS animals. Twin sets indicated by using same symbol for each member; the twins of 5105 and 933 were not available. Envelope encloses the 4 animals intensively studied; cows 1125, 2093 were thought at the time of selection to be HS.*

folium pratense), for the present group of animals, their twin sisters, and a known HS animal (933) included as an "indicator" cow. It will be seen that there is a good overall correlation between band 4 and susceptibility. Secondly, in two cases members of a twin set differ in susceptibility and this is associated with differences in band 4 concentration. Lastly, of the four animals selected for intensive study — the points within the envelope two are clearly LS (5105–0% band 4; 1132–1.5%), but neither of the other two is HS.

(b) *Total Salivary Flow*

Several investigations have suggested that non-bloaters or LS animals secrete more saliva than do bloaters or HS animals (Mendel and Boda, 1961; Clarke and Reid, 1974).

One of the present studies is aimed at determining whether there are differences in total salivary secretion and/or in the response to doses of a salivation-stimulating drug, pilocarpine, that correlate with susceptibility. Two sets of experiments have been carried out so far.

As expected, the introduction of the tube into the cardia initially caused a marked stimulation of flow which then declined over a period of 10 to 20 min. Pre-dosing recovery periods of 12 or 24 min were therefore allowed. Treatments included the intramuscular injection of physiological saline, or 1, 2, 3 or 4 mg/

TABLE 1: TOTAL SALIVA SECRETION FOLLOWING DOSING WITH PILOCARPINE
(Experiment 1. Feed: lucerne chaff)

	Cow			
	2093	*1125*	*1132*	*5105*
Peak flow (g/min/kg B.W.)*:				
Control†	0.27	0.28	0.28	0.41
Pilocarpine‡	0.51	0.35	0.36	0.53
Total flow (g in 16 min/kg B.W.):				
Control	2.68	3.49	2.26	4.95
Pilocarpine	6.04	3.95	3.73	6.99

*Collected by cardiac intubation. All figures are the average of 3 dosings.
†Physiological saline, intramuscularly.
‡2 mg/kg B.W. pilocarpine nitrate in saline, intramuscularly. Higher doses were inhibitory.

kg of pilocarpine in saline. The observations made have included the weights of saliva collected over timed intervals and the protein and other materials contained in the saliva. The best LS animal had the highest secretion rate whether or not pilocarpine was given; but when the results from all animals are considered, no clear relationship between secretion and susceptibility has been immediately evident (Table 1). Chemical analyses of samples are proceeding.

3. The Nature of the Rumen Contents

The experiments in which susceptibility was transferred by exchanging rumen contents between HS and LS animals suggested that an immediate determinant of susceptibility was present in the contents. Its identity is not yet known. The change of susceptibility induced was only temporary, lasting approximately 24 h (Clarke and Reid, 1970).

Earlier observations (Mendel and Boda, 1961) suggested that there was a difference in the dry matter percentage of the rumen contents (DM%) of HS and LS animals. This observation appeared to be confirmed by casual sampling from the cows in the present trials. However, when examined more closely it was found that DM%, dry matter content, and parameters such as rumination times were all strongly dependent on feed intake level; if intake was considered, residual differences between HS and LS animals appeared small (Reid *et al.*, 1972).

Laby and Weenink (1966) have reported the presence of more "yellow bubbles" in the rumen contents of animals prior to bloating. The origin and nature of these lipid-rich bodies remain uncertain. In a brief examination of our cows, we appear to have confirmed Laby and Weenink's observations. Sampling was carried out before the morning feed and was by total removal of the rumen contents and sampling outside the animal (Reid, 1965). The quantity of yellow bubbles varied from animal to animal, and in the same animal from day to day. There was a good, positive correlation between the quantity of yellow bubbles assessed visually and the subsequent degree of bloat.

In a further experiment rumen contents were exchanged between the best LS animal (5105) and the best HS animal (933) and then the quantities of yellow bubbles found on the three subsequent mornings observed. There had not been a complete return to the pre-exchange pattern when observations were stopped. Unfortunately, the clover at this stage was rapidly declining in potency and reliable observations on bloating behaviour were not possible.

DISCUSSION

This paper presents first results from work in progress. Any conclusions are accordingly only tentative. The limitations of the work is also recognized. The number of animals being used is small, and clearly some of higher susceptibility are needed to compare with the LS animals. Further, much of the work has been carried out with dry feed: it well may be that important differences emerge only under green feeding conditions.

There are many factors other than those currently being studied that could affect susceptibility to bloat — some were listed in the Introduction. It is possible that an animal is LS for more than one reason and that different animals may be LS for different reasons. The subject has been discussed in detail elsewhere (Mendel and Boda, 1961; Clarke and Reid, 1970, 1974; Reid *et al.*, 1975).

It is logical to consider specifically those factors which could affect foam persistence. In this regard, apart from consolidating present work, other studies about to be commenced at DSIR include quantitative studies of the dynamics in rumen contents of solid particles, surfactants and salivary proteins. Each of these factors could have a marked effect on foam persistence. However, the writers place particular emphasis on the dynamic aspect: susceptibility seems most likely to be the resultant of opposing and continuously changing forces.

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Note added to proof: Further work using more animals, all of known susceptibility, has shown: *pillar-movements* — within-animal variability is such that the significant differences between HS and LS cows reported here is likely to have been a chance event; *saliva flow* — LS cows have a greater response to pilocarpine than have HS cows. The results will be published in full elsewhere.