THE COMPONENTS OF BOVINE SALIVA IN RELATION TO BLOAT

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SUMMARY

Preliminary results are presented from a physiological genetics approach to the study of bloat. Several protein band proportions separated by electrophoresis are shown to be correlated with bloat. Results from immunochemical techniques are outlined and a hypothetical explanation of the animal variation based on enzyme induction is mooted.

INTRODUCTION

Saliva is a heterogeneous mixture of proteins and electrolytes produced by a number of distinct glands. These glands can be broadly classified as either serous or mucous, the latter type producing most of the protein found in saliva.

Several workers have studied the physiology of the parotid gland in the cow (Ash and Kay, 1959; Bailey and Belch, 1961) but only a very limited amount of work has been carried out on the biochemistry of secretions from the submaxillary gland and other salivary glands in this animal. The first attempt to look at specific salivary proteins in relation to bloat was carried out by Lyttleton (1960) and later by Jones (1971); and pers. comm.) and Clarke *et al.* (1974). Jones showed on a limited number of animals that one particular protein region, designated band 4, appeared to be present in an increased quantity in HS (high susceptibility) animals.

This paper summarizes some early results on the relationship of salivary proteins to bloat. In addition, the relation of the results to the bloat problem is discussed in the context of the paper by Cockrem (1975). Details and statistical analyses will be published elsewhere.

MATERIALS AND METHODS

Saliva Collection

Cows used in this work were from the progeny group and saliva groups 1 and 2 as defined by Cockrem (1975).

The saliva was collected routinely for a 5 min period by means of a perforated bit which fitted below the tongue and immediately behind the opening of the submaxillary gland (Phillipson and Mangan, 1959).

The bit was connected to the collecting vessel which remained over ice throughout the collection, and this in turn was coupled to an electrically driven suction pump. Saliva samples were then centrifuged for 10 minutes at 1500 × g. The quantity, viscosity, amount of deposit and protein content (Lowry *et al.*, 1951) were measured on all samples.

Electrophoretic Separations

Saliva contains a number of different proteins. Therefore, to assess their relative importance in the bloat syndrome it was necessary to separate them analytically and this was achieved by polyacrylamide gel electrophoresis. The widespread use of electrophoresis may be attributed to the sensitivity of the technique but for the analysis of saliva, the main disadvantage is that the large mucoprotein molecules do not enter the gel.

The saliva was applied to the electrophoretic media and electrophoresed for just over one hour. The proteins were then fixed in trichloroacetic acid, stained with coomassie brilliant blue R, followed by electrophoretic destaining and scanning on a densitometer to determine the relative proportions of each of the electrophoretic bands (McIntosh and McLaren, in prep).

RESULTS

Saliva Flow

The volume of saliva collected per minute between samplings within cows and within twin sets showed a high repeatability (*e.g.*, the correlation between twins of a pair were 0.92 for saliva group 1 and 0.87 for saliva group 2). In most collections LS animals had a greater flow than HS animals ($P < 0.05$). Saliva flow is an example of an environmental effect in that the differences in saliva volume are affected by the time after feeding. Samples were collected from the first year progeny group (15 months of age) at 0, 3, 6 and 14 hours after feeding and the saliva volumes were recorded. The results are shown in Fig. 1 where the standard error of an individual mean was 0.75 which would give a significant difference ($P < 0.05$) at 0 and 14 hours only.

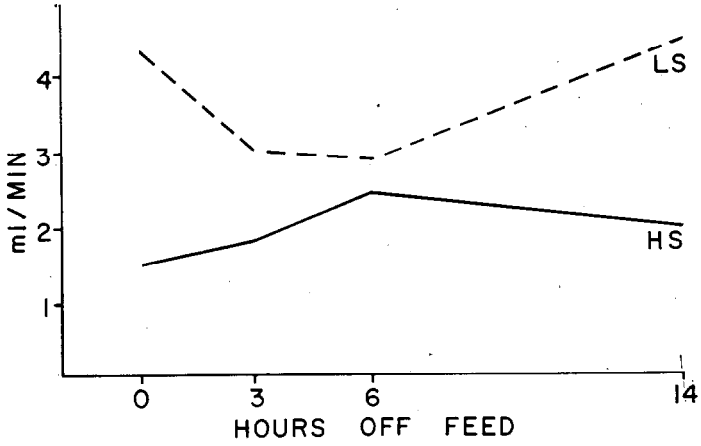

FIG. 1: *Saliva flow (ml/min) of LS and HS heifers at 0, 3, 6 and 14 h post feeding.*

PROTEIN PATTERNS

Using immunoelectrophoresis, with antisera prepared by Dr W. T. Jones (Applied Biochemistry Division, DSIR, Palmerston North) the band 4 region of Clarke *et al.* (1974) was positively identified in mouth collections on all of the cows in saliva group 1. Comparisons made between mouth and oesophageal collections from the same animals demonstrated that only about 20% of band 4 protein was collected by means of the standard mouth bit per unit time. Statistically band 4 and band 6 appear interrelated and they both seem to be associated with bloat grade but the correlations do not appear very consistent between samplings. Band 6 and probably band 4 have been shown to be negatively correlated with saliva flow and there was a significant difference between HS and LS cows for this parameter. Using quantitative immunochemical techniques it was demonstrated in adult cows and heifers that band 6 immunologically cross-reacted with bovine serum albumin antisera (McIntosh and Cockrem, unpublished data).

An interesting result was obtained when foam was collected from the rumen of a steer which was stabbed because of acute bloat. When analysed by electrophoresis, approximately 30% of the total protein entering the gel migrated in the band 6 region and furthermore it cross-reacted with bovine serum albumin antisera when examined by immunochemical techniques.

Some major points from the analyses of data from the cows are:

(1) Preliminary evidence from twin pairs indicates that all band proportions except 2 and 9 are inherited (correlation between pairs 0.7 to 0.8).

(2) Band 4 is affected by diet, being proportionally higher for both HS and LS cows when pastured on clover.

(3) There are strong and consistent relationships between certain band proportions. (Derived relationships from use of proportions have been considered.)

(4) Some band proportions are related to bloat criteria or saliva flow. Points 3 and 4 are illustrated in Fig. 2.

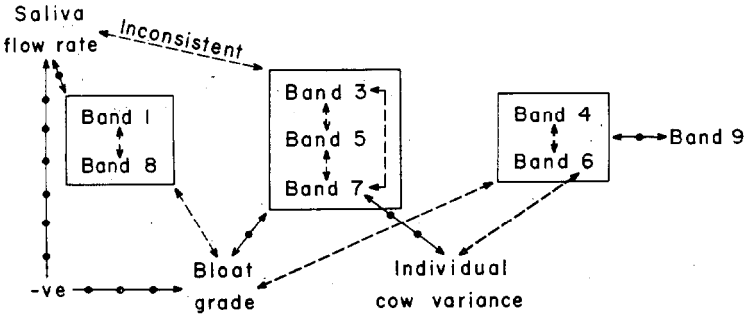

FIG. 2: *Relationships between band proportions, bloat grade, saliva flow rate and individual cow variance.*

As can be seen from Fig. 2, band proportions 1 and 8 are inter-related and are positively correlated with bloat grade and negatively correlated with saliva flow, whereas band proportions 3, 5 and 7 are themselves inter-related but negatively correlated with bloat grade and variability of bloat grade.

Results from the first calf crop are still being analysed but similar relationships are present. At six months of age there were significant differences in the bloat grade between progeny of HS and LS cows. However, during spring 1974 not only did both LS and HS progeny show a high degree of bloat, but they also showed band 1, 4 and 6 levels well above observed parental values. There is preliminary evidence of relationships between parental band proportions and progeny bloating scores so far.

DISCUSSION

The most important findings from the last 18 months' research are the good statistical relationships between both saliva flow and protein band proportions with bloat susceptibility in cows. This physiological genetics approach is new to the study of metabolic diseases in farm animals (Cockrem, 1975), and its main function is to indicate which components warrant detailed biochemical investigations and whether or not it would be possible to establish a selection index for the desired trait.

Characterizing these proteins biochemically and determining their physiological role is anticipated to be an involved task. However, it has been possible to probe some of these band relationships by immunochemical techniques, for example, the statistical relationship between bands 4 and 6. These two protein bands were shown to be immunologically distinct when tested against band 4 antisera prepared by Jones; furthermore band 6 was shown to cross-react with bovine serum albumin antisera. This would suggest that these two proteins are chemically distinct.

Statistical relationships of proteins having the same polypeptide chain can be explained physically in several ways — for instance, the same protein may exist as monomer, dimer, trimer, etc. Each different form will exhibit electrophoretic mobility differences owing to their variation in molecular size. An alternative explanation for glycoproteins could be the dissimilar number of carbohydrate or sialic acid molecules attached to the polypeptide chain. Each additional molecule added to the protein will alter the electrical charge, and hence the electrophoretic mobility. The carbohydrate and sialic acid content of these glycoproteins may to some extent be reflected by the blood group substances of the animal (Ginsburg *et al.*, 1971). Work in this laboratory is now concentrating on the purification of the salivary proteins by means of preparative electrophoresis and column chromatography.

The induction of protein synthesis is an important aspect of biochemical control of metabolism. Jacob and Monod (1961) described the mechanism in micro-organisms when they proposed the Operon Hypothesis. However, in higher animals the nervous and hormonal controls add complexities to this subject. It is certain, however, that in humans and other mammals salivary proteins can be induced by a change in diet, as illustrated by Squires (1953) who studied salivary amylase levels in different human populations.

Results from work in progress at this laboratory would suggest that certain salivary protein bands may be induced by various

stimuli such as the response to the site of food (psychic), different feeds ingested, and acid stimulation. It is tempting to suggest that this induction of protein synthesis could explain why certain pastures cause bloat more readily than others. If, for instance, there was a constituent of plants which induced synthesis and secretion of a protein involved with bloat, and cows were genetically different in their response to this induction, then their response to bloat-producing pasture would also differ. It is interesting to note that the percentages of both band 4 and band 6 were increased in cows pastured on clover as compared with results obtained when fed silage (McIntosh and Cockrem, unpublished data).

Regardless of the role of the salivary proteins in bloat, the allied projects in progress at present, including studies on rumen physiology, physico-chemical studies of protein foams and chemical analysis of the pasture, are all of equal importance if a unified explanation of the cause of bloat is to be established. However, it is possible that through the biochemical and physiological characterization of these salivary proteins a selection index for low bloating animals (using these salivary parameters) will be established and hence make possible the breeding of bloat-free animals in New Zealand.

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