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PHYSIOLOGICAL GENETICS AS A BASIS FOR THE STUDY OF SUSCEPTIBILITY TO BLOAT IN COWS

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SUMMARY

The basis of a physiological genetic approach to animal breeding problems is described and the type of problem to which it could be applicable is discussed.

The inheritance of bloat susceptibility is given as an example of a problem and a description is given of the general hypothesis under investigation.

The experimental approach is described to show how different facets of work by Applied Biochemistry Division, DSIR, Ruakura Research Centre and the New Zealand Dairy Board are being linked together.

INTRODUCTION

The relevance of knowledge of gene structure and action to animal breeding has been presented in a previous paper to this society (Cockrem, 1962). Since then there have been considerable advances in both molecular biology and in biochemical techniques. However, while many investigations have been reported on biochemical variants in the body fluids of domestic animals, few, if any, of these have been extended to practical animal breeding.

In this paper the relevance of recent knowledge in genetics to practical animal breeding is described and it is illustrated by the application of a physiological genetic approach to the problem of bloat.

The approach described is a collaborative one between Applied Biochemistry Division, DSIR, and Ruakura Animal Research Station. Inter-relationships involved are described in a context of animal and genetic variability.

GENE ACTION AND ANIMAL BREEDING

Davidson and Britten (1973) review animal gene regulation and action in relation to the animal genome. At the molecular level the subject is now very complex but the main advances concern the control of gene action and the organization of DNA sequences. It appears that a considerable part of the genome is

concerned with regulation of those structural genes which determine proteins and enzymes. Thus, although the main concern is with the latter, the intra- and extra-cellular environment and hence the environment of the animal must also be considered as part of the complex. In other words, the product of the gene must be studied under the conditions in which it is produced.

The basic elements relevant to animal breeding are illustrated in Fig. 1.

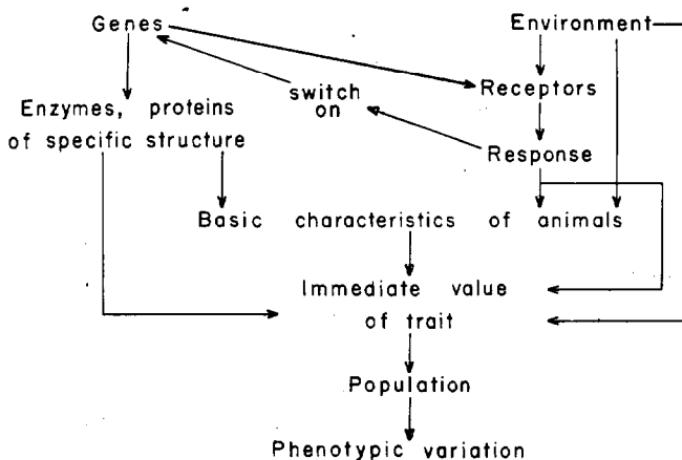


FIG. 1: *Aspects of gene action relative to animal breeding.*

The structural (producer) genes will determine the structure of enzymes and proteins, but the production by a specific cell at a particular time will depend on other controlling genes or induction by other compounds, such as hormones ("Switch" — see Fig. 1). Some of these compounds will depend on their production as a response to receptors (*e.g.*, light and pineal gland) and these will also be under genetic control (*e.g.*, melanocyte stimulating hormone). Genetically controlled variation could be introduced at any of these stages so that a quantitative variation in the final production of single genes might be expected except under very standardized conditions.

The structural differences of proteins controlled by a single locus can cover a wide range of possible amino acid substitutions associated with single codon changes. Thus the occurrence of

multiple alleles and polymorphism is common. Lewontin (1973) has reviewed the possible relevance of this to evolution and hence selection, but it is not yet clear whether many of the variants have similar actions or whether they contribute to variation which is available for selection. However, this is another way in which quantitative variation could occur in a protein controlled by a single locus.

The remainder of the figure illustrates two main points:

- (1) Values of a trait measured at a point in time may depend on the previous environment and genetic control as well as on that operating at the moment.
- (2) The datum which can be measured in the field is the phenotypic variation of the trait. It is this that the animal breeder studies in order to deduce if selection is worth while and, if so, the optimum methods to use.

In general the biochemical genetics approach has been to study variants of particular proteins or enzymes, usually from body fluids which are easily obtained and open to large-scale analyses. The results are then, hopefully, related back to traits of economic importance. Such information is likely to be useful in the long run, both in the detection of "marker" genes and in contributing to understanding of physiological mechanisms. It is not likely to offer a direct solution to faster and more accurate selection for a particular trait unless the substances measured are known to be pertinent to the problem.

For this purpose some knowledge of the physiology of the trait is required as a lead to the investigation of the biochemical and hence genetic basis. Initially data are also required of the variation under field conditions so as to determine the relative importance of genetic and environmental effects, although the accuracy required will not be as exact as that for actual breeding plan predictions. However, clearly there must be evidence of a genetic component.

A HYPOTHESIS INCLUDING GENETICALLY CONTROLLED ANIMAL FACTORS IN BLOAT

Reid *et al.* (1972) discussed evidence for an inherited animal factor in the bloat syndrome. New Zealand Dairy Board data suggest an heritability of 0.6 (P. Shannon, pers. comm.) while analysis of identical twin data in the present project suggests a maximum figure of about 0.6. However, results depend on the

environment as the variation between animals depends on the "potency" of the pasture (Reid *et al.*, 1974) and there could be a high heritability under severe testing if this were practicable.

Thus a heritable basis for bloat susceptibility seems likely and previous work, reviewed by Clarke and Reid (1974), gives some leads on a possible physiological basis.

The outline of such a hypothesis is shown in Fig. 2.

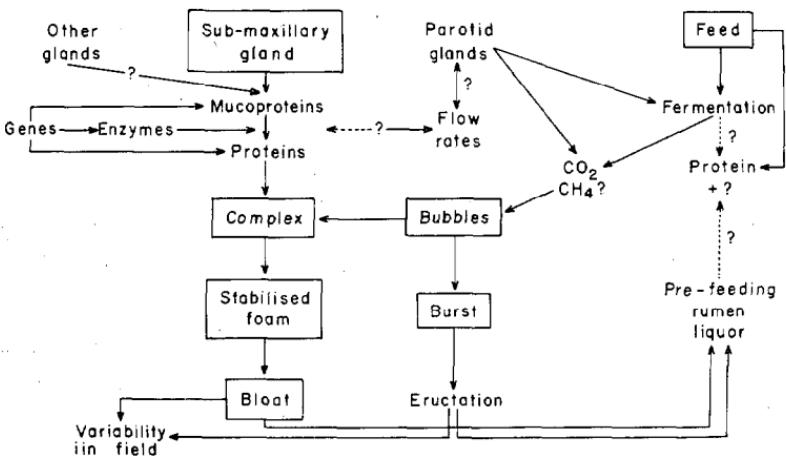


FIG. 2: *Bloat hypothesis and possible gene action.*

The figure excludes some possibilities which at present appear to be peripheral to the problem and also a number of theories which have been inadequately tested.

It is clear from field observations that the problem is one of a plant/animal interaction with a considerable range of variation. Thus a highly susceptible cow (HS) will bloat on pasture which has little effect on the rest of the herd, while a low susceptible (LS) cow will show only mild bloat when the majority of the herd are bloating badly. Thus, there is a pasture constituent(s), probably protein (Mangan, 1959), which can lead to a stable foam under the conditions of rumen pH at which bloat occurs. Jones and Lyttleton (1972a, b) confirmed this result and also found that salivary mucoprotein but not mucin produces persistent foams. W. T. Jones (pers. comm.) has studied an *in vitro* system of clover protein and salivary mucoprotein which have a synergistic effect from the formation of a complex

which results in a very stable foam. While this has yet to be tested on the actual bloat condition, it could provide the core of the plant/animal interaction.

The role of the parotid gland in bloat is not clear. The carbonate could have effects by lowering pH or by gas formation as carbon dioxide. Mendel and Boda (1961) compared fistulated cows selected on susceptibility but the only clear-cut result was the greater total saliva production of the LS cows. Collections were made at the cardia. Although a high gas prduction would appear to be a requirement for bloat, these authors did not find differences between LS and HS cows.

Present results (McIntosh, 1975; Reid *et al.*, 1974) show that sub-maxillary (possibly with sub-lingual) flow rates are lower in HS cows and related both to bloat grades (negatively) and to the proportions of some electrophoretic bands. On evidence at the start of the present work and on the preliminary evidence so far (McIntosh, *op. cit.*) the genetic control of salivary gland mucoproteins and other proteins seems the most promising source of the genetic component of bloat susceptibility. This does not exclude the possibility that the genetic component lies in the nervous or hormonal control of these glands and their secretions, but experimental tactics favour the investigation of the former hypothesis first.

Another aspect shown in Fig. 2 is that of the rumen liquor prior to feeding after an animal has been on a bloat-producing diet. A number of authors (*e.g.*, Laby and Weenink, 1966; Mendel and Boda, 1961) have observed differences in this liquor. Clarke and Reid (1970) have shown that exchange of these contents can temporarily exchange susceptibility. Thus if saliva contributes active agents then these may accumulate in the rumen.

Two further points not illustrated in Fig. 2 are, first, that the type of feed may affect both salivary flow and the constituents of the saliva — *i.e.*, the genes may be switched on only in certain circumstances, and, secondly, the previous feed and the salivary constituents may affect the microbial population differentially between LS and HS cows. Fina *et al.* (1961) give some evidence for this occurring. If this is so, then there could be some significance in saliva differences in rumen digestion quite apart from the bloat problem.

EXPERIMENTAL APPROACH

The general experimental approach and the co-operative nature of the project are illustrated in Fig. 3.

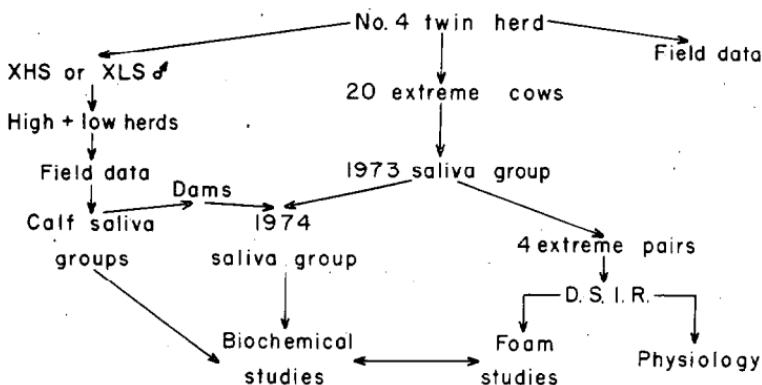


FIG. 3: *The overall experimental plan.*

All the animals from an identical twin herd at No. 4 Dairy, Ruakura, have been graded for bloat and used for:

- (1) Studies of the sources of variation of bloat grade.
- (2) Drenching trials of prophylactic agents .
- (3) Test cows for pasture analyses.
- (4) Testing the animal repeatability on different feeds such as clover, lucerne and artificial feeds.

From this herd, 5 HS pairs and 5 LS pairs were selected for preliminary saliva sampling in 1973 (Saliva group 1 — McIntosh, 1975). After a series of samplings the two extreme pairs of HS and LS cows were then sent to DSIR at Palmerston North, to be used for detailed physiological work by Dr Reid and his group.

In 1972 the HS animals were mated to an HS and the LS to an LS, Jersey bull. The bulls were selected by the Dairy Board on a progeny test basis. The HS/LS distinction (50% of each) may be an artificial division of a continuous variation and genuine LS cows are probably only about 15% of the herd.

The first calf crop were all sampled for saliva at birth and 90 days of age. After a first grade for bloat at 6 months of age, 6 HS and 6 LS heifers (progeny group) were selected for regular saliva sampling, selection being on bloat grade within their genetic groups. These animals have also been used to determine the effects of variables such as feed type and time of sampling on saliva flow and constituents.

The parent cows of this heifer group were used to make up a second cow group for saliva samples in 1974 (Saliva group 2), both to check on the preliminary group results and to get an indication of parent/offspring relationships. With the number of animals used, indications rather than firm parameters are the initial objective.

Analyses of data from the saliva samples have included:

- (1) Analyses of variance to study LS and HS group differences and sampling date differences and interactions.
- (2) Simple regressions to study overall relationships and the consistency of relationships within HS, LS and sampling date groupings. This has been done for relationships with bloat grade and between band proportions.
- (3) Stepwise multiple regressions have been used to study the complex inter-relationships indicated by (2). The limitations of this technique and the use of proportions at this stage, together with small numbers and a lack of knowledge of cause and effect, mean that it is used for defining hypotheses rather than testing them.

In general this initial work and its analyses have had two purposes: The first is to determine whether the hypothesis shown in Fig. 2 was worth continuing with and whether selection on salivary constituents seemed a possible objective. The evidence so far is that the prospects are good. The second purpose is to determine hypotheses for the band relationships and provide some leads for the biochemical and physiological work on saliva. This is discussed by McIntosh (1975).

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