PHYSIOLOGICAL PREDICTORS OF GENETIC MERIT

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INTRODUCTION

In an attempt to genetically improve the efficiency of livestock production, breeders have for many years selected, as replacement breeding stock, the highest performing animals in production traits such as milkfat yield or fleeceweight. However, selection based on these commercially important traits seldom results in annual rates of genetic gain greater than 3% of the mean (Smith 1984). The employment of techniques such as artificial insemination, multiple ovulation with embryo transfer and progeny testing in the breeding programme may enhance annual genetic gains, but it is likely that the annual rate of genetic gain will still be less than 8% of the mean (Land and Hill 1976; Nicholas and Smith 1983; Rae 1984). These relatively slow rates of change reduce the flexibility of livestock producers who are trying to meet the demands of constantly changing markets. In the last decade there has been a considerable research effort expended in attempting to identify indirect predictors of genetic merit that might enhance the rate at which genetic progress can be made.

POTENTIAL BENEFITS OF INDIRECT PREDICTORS

The potential benefits of indirect predictors of genetic merit for production traits can all be related to the components of the equation for predicting genetic gain per year:

$$G/\text{year} = (r_{TI} i \sigma)/L$$

where: G/year = genetic gain in the desired trait(s) (T) per year
r_{TI} = correlation between the desired trait(s) and trait(s) used as predictor(s), also known as the accuracy of selection
i = standardised selection differential
σ = genetic standard deviation of the desired trait(s)
L = generation interval.

The major benefits can be listed as:

(i) Selection at a younger age Virtually all research in the area of non-traditional predictors has concentrated on identifying traits that could be measured prior to puberty. This would then enable the generation interval (L) to be reduced to the minimum defined by the age at which animals can be first mated. This objective will become even more important when the time taken for animals to reach puberty can be artificially reduced. There has recently been substantial attention given to the increased genetic gains that can be made via juvenile MOET (multiple ovulation and embryo transfer) schemes (eg Nicholas and Smith 1983). However, such a programme is dependent on being able to identify breeding replacements at a young age.

An associated benefit from selecting at a younger age is that the selection differential (i) is also likely to be increased. In most industries, it is not practical or economically feasible to carry all potential replacements to the age at which assessment traditionally occurs. With traits measurable soon after birth, all animals become potential replacements. This benefit could be sufficient reason for pursuing traits measurable at an early age without necessarily attempting to decrease the generation interval. For example, in the dairy industry, progeny testing might still be used, but selection of bulls for progeny testing could be based on pedigree plus physiological information.

An economic advantage of early selection, particularly in pastoral-based systems, would be the sale of unwanted stock prior to their first winter. These could be used either as breeding stock on other farming units or as animals for meat production.

(ii) Indirect selection for sex-limited traits Several traits of major importance in the livestock industries are expressed only in the female (eg milk production, weight of lamb weaned). As a consequence, selection of males is typically based on information from female relatives (eg dam, daughters or half-sisters). Because both sexes carry essentially the same genetic information, except for that on the Y chromosome which normally exists only in males, it may be possible to identify physiological traits in males that are associated with the level of production in sex-limited traits in females. An example of this is where testicle size in rams has been suggested as an indirect predictor of reproductive rate in female offspring (Land 1973).

The genetic impact of indirect selection for sex-limited traits would be to decrease the generation interval (L), increase the selection differential (i) and/or increase the accuracy of selection (r_{TI}). If selection is based on half-sister or ancestry information alone, the maximum accuracy is .25 and .50, respectively. With indirect selection, it may be possible to exceed these values, thereby increasing the rate of genetic gain per year. The utilisation of indirect physiological predictors in males would help maximise the number of animals eligible for selection, so increasing the selection differential. Finally, with indirect physiological predictors, it would not be necessary to wait for information from female progeny or half-sisters to become available. Therefore the generation interval could be reduced.

(iii) Increased accuracy of selection A limitation in selecting for several economically important traits is that they have only low to moderate heritabilities (and hence low to moderate accuracies). If traits could be found that had moderate to high heritabilities and large genetic correlations (positive or negative) with the production trait of interest, then it is likely that the accuracy of selection could be increased. This is equivalent to producing a more accurate ranking of replacement stock based on their true genetic worth. As a consequence, the rate of genetic gain per year would increase.

MARKERS VERSUS LINKAGE

A number of different terms have been used in the literature to describe non-traditional selection criteria. However, these criteria typically fall into two broad categories:

(i) Physiological or metabolic traits These are sometimes called physiological or metabolic markers. It seems unlikely that any one physiological trait will result in superior genetic gains compared with selecting directly for the desired trait. Thus an index of physiological (and possibly production) traits will be necessary to enhance the rate of genetic gain. There is still some discussion as to whether selection for an index of physiological traits will be more effective than selecting for the desired trait itself. This argument will be resolved only upon the application of physiological indices in research flocks or herds.

The animal breeding methodology to enable selection for a variety of physiological traits as indicators of some desired trait(s) has yet to be fully developed. However, it is likely to require the solving of a new series of problems relating, for example, to negative feedback systems, time-dependent variation in concentrations of hormone/metabolites and inherited environmental variation. Furthermore, it may require a distinction to be made between physiological traits that are components of a pathway resulting in the desired trait (e.g. the involvement of the hormone insulin-like growth factor-1 (IGF-1) in growth, Blair et al. 1989) versus those traits that are merely correlated with the desired trait (as blood urea levels are correlated with genetic merit for wool growth, McCutcheon et al. 1987).

(ii) Genetic linkage This occurs when two or more traits are completely or partially determined by genes that are located near each other on a chromosome. If a trait of interest in a selection programme is difficult or expensive to measure, has a low heritability, cannot be measured until after the age of selection or is sex-limited, it may be possible to use levels of a linked trait to assist with selection. Few examples of the use of linkage in selection plans exist. However, this is likely to change in the future when the use of Restriction Fragment Length Polymorphisms (RFLPs) becomes more routine. One proposed use of this technology is to find RFLP variants that are associated with differences in levels of economically important traits (for example, Hallerman et al., 1988). Having identified the RFLP associated with the improved performance, selection could be based on the RFLP, obviously at a very young age. A problem with the genetic linkage approach is that linkage groups can be broken during meiosis. By ensuring close linkage, this problem can be reduced so that the chances of an erroneous selection decision are rare, but the problem can never be entirely eliminated.

REQUIREMENTS FOR SUCCESSFUL INDICATOR TRAITS

For physiological indicator traits to be useful in selection programmes, they will have to meet a number of criteria:

(i) Ease of sampling In many situations, it is unlikely that the personnel undertaking the sampling would be highly trained technicians. As a consequence, sampling would likely be limited to milk, fibre, blood, semen, skin and possibly subcutaneous tissues. Clearly, sampling procedures will have to meet increasingly strict ethical requirements. Apart from trying to avoid the need for highly trained personnel, simple sampling procedures are required to ensure that large numbers of animals can be sampled quickly, to enable the selection differential to be maximised. Where a smaller number of animals are to be sampled, for example bulls in a progeny test scheme, it may be feasible to employ more highly trained personnel to undertake complicated sampling procedures.

(ii) Assay expense To make the use of physiological indicator traits attractive to sire breeders, it will be necessary to keep assay costs to a minimum. There will be a trade-off between assay costs and selection differential. This situation already exists in the wool industry where samples from only the best rams, rather than those from all rams and ewes, are sent to centralised laboratories for fibre testing. Furthermore, it is unlikely that the required assays will be carried out by individual breeders. Therefore, the development of centralised laboratories will be necessary.

(iii) Sampling of young stock Although measurement of physiological indicator traits at an early age is not a prerequisite for improved rates of genetic gain, it would certainly be desirable.

(iv) Sampling of both sexes To enable the maximum benefit from physiological indicator traits, it would be desirable to be able to assess both sexes.

(v) Moderate to high accuracy of selection This requires first, that a large proportion of the observed variation in the physiological indicator trait be genetic in origin, and second that it show a strong genetic relationship with the desired trait. Implicit in these requirements is that the environmental impact on the physiological indicator trait is minimal or can be readily controlled.

APPROACHES TO FINDING USEFUL PHYSIOLOGICAL INDICATOR TRAITS

Animals With respect to the identification of indicator traits, it is useful to divide the population of potential research animals into 4 groups. In doing so, it must be remembered that the major purpose of undertaking these studies is to find a genetic link between some physiological trait(s) and the desired trait. This requires at least two properties from the potential research animals. First, there is a need to uncouple the normally confounded effects of genotype and environment, with respect to the physiological trait and the desired trait. Second, to enhance the chances of finding a relationship between the physiological and desired traits, it would be helpful to have animals divergent in either the desired trait or the physiological trait, or both. With these criteria in mind, the available animals could be grouped as:

(i) Stock from selection experiments Research institutions around the world have generated a variety of selection lines in production animals. Some examples are:
sheep: Pattie et al. (1965); Turner et al. (1970); Blair et al. (1985),
dairy cattle: Hickman (1971); Yerex et al. (1988),
beef cattle: Bailey et al. (1971); Koch et al. (1974), and
pigs: Hetzer and Harvey (1967); Vangen (1979).
Often laboratory animals have been used to obtain a result more quickly, due to their rapid generation turnover. For example, Falconer (1953); Baker et al. (1984); Eisen (1987); Schuler (1987).

Until the present time, most selection experiments have used production traits as the selection criterion. However, a few have used physiological traits, for example: thyroid iodine release rate, Chai and Melloh (1972); thyroxine levels, Tilakaratne et al. (1981); enzymes involved in fat synthesis, Rogdakis (1982); IGF-1 levels, Blair et al. (1989).

The main advantage of using animals from selection experiments in the search for physiological indicator traits is that the difference between the selection lines in mean level of production is genetic in origin. Furthermore, the difference is likely to be greater than those that could be found in the population at large, unless expensive screening programmes were embarked upon.

The use of selection lines does not guarantee success in the search for physiological indicator traits. It may be that the genetic change in the desired trait has been brought about by a plethora of physiological changes. In that case, even a large difference between the selection lines (say 30% - which represents 15 to 30 years of selection in production animals) may not result in a detectable physiological change in any one trait. Where a large physiological change does result, it may be that a variant allele has resulted that removes a limiting step in some physiological pathway. In that situation, it is likely that the animal breeder will have already noted the existence of some major gene (Bradford and Famula 1984) and, as a consequence, the need for a search is obviated. A further problem is that unless the genetic correlation is near unity, there is no guarantee that selection pressure on the indicator trait will generate any change in the desired trait. In fact, at the physiological level the traditional concept of genetic correlation may require re-evaluation. When dealing with traits that are the culmination of many physiological processes, the concept of an average relationship may be adequate. However, when dealing with physiological traits that result from only a small number of loci, a different approach may be necessary. Finally, it should be emphasised that any physiological trait implicated as a potential indirect predictor of a desired trait (from studies of selection experiments), must itself be tested for effectiveness in inducing changes in the desired trait. Furthermore, in the situation of incomplete knowledge of relationships between physiological processes, it is possible that selection on the implicated trait could have undesirable effects on other economically important traits.

(ii) Stock from different breeds As with the use of selection lines, it is often possible to choose breeds that are genetically divergent in some production trait. These may originate because of different selection objectives (for example dairy cattle versus beef cattle) or be intensely selected stock (for example terminal-sire sheep) versus wild-type stock (for example Soay or St Kilda sheep). The major danger in using such animals is that they do not have a recent common genetic base (as occurs in most selection experiments). Under these circumstances, the breeds may be different in a variety of physiological traits and it is impossible to say which one (or combination) is responsible for the difference in the desired trait.

(iii) Progeny testing This approach has been used for decades to detect significant sire effects on production traits and to give some indication of the heritability. The approach needs little modification to assist in the search for useful physiological indicator traits, save the requirement of greater sire numbers to enable the detection of genetic covariation. Thus, physiological traits and the desired trait(s) would be measured in either the sires or the offspring or in both (to enable the environment and the genotype to be uncoupled). Compared with using animals from selection experiments, this approach suffers two disadvantages. First, there is a need to measure more animals to detect a relationship of interest. Second, it may take several years to collect the required information on the progeny. However, progeny testing provides two advantages. The total cost is likely to be substantially less than that associated with generating and maintaining selection lines, and it is possible to test for all traits of interest rather than being limited to the selection line or between breed difference.

(iv) Remaining stock Although there will be other useful animals in the population (eg, dam/offspring relationships, mutants (such as lustre sheep; Blair 1989)) the majority of stock are not useful in the search for physiological indicator traits. In the future, transgenic animals may provide another source of stock but they will be little different to current mutant/major gene stock or selection line animals.

PHYSIOLOGICAL CHARACTERISTICS AS POTENTIAL INDICATOR TRAITS

When sampling blood or body tissues from animals, it will be necessary to specify the environmental conditions under which the samples are taken (eg field conditions vs controlled environments; basal conditions or responses to metabolic challenge) because, as will be shown, these conditions can markedly affect results. Futhermore, a decision must be made whether to measure hormones, metabolites, enzymes or a combination of these as the potentially useful indicator trait.

Four examples from the above possibilities will now be discussed:

(i) basal metabolite concentrations
(ii) basal hormone concentrations
(iii) metabolic challenges
(iv) in vitro metabolic activity of specific tissues.

Basal metabolite concentrations Basal metabolite concentrations in plasma have appeal as potential physiological indicator traits because plasma can be readily sampled (by venipuncture or via indwelling catheters) and because modern automated assay systems permit rapid sample analysis with high levels of precision. However, circulating metabolite levels represent the balance between entry rates into plasma, rates of uptake or excretion by body tissues/organs, and the size of the plasma pool into which the metabolite distributes. Thus measurements of plasma concentrations represent the net effect of many dynamic and interrelated processes.

The role of each metabolite in relation to physiological function must also be considered. Some metabolites, such as glucose, have a key role in energy metabolism of many tissues and highly specific roles in certain tissues (eg in the case of glucose as the major energy substrate in the central nervous system). Accordingly, their concentration in plasma is subjected to strong homeostatic regulation with the result that variation between and within animals tends to be low. This may mean that little genetic variation is likely to be expressed in levels of these metabolites even though variation may be apparent in the mechanisms which maintain their levels within narrow homeostatic limits.

Other metabolites have a non-essential role in physiological function, eg because they are the byproducts of metabolism. These metabolites may be subject to less rigorous homeostatic control (unless they are highly toxic) with the result that genetic variation may be more readily expressed. One [redacted]

Low circulating urea concentrations have been reported in cattle of superior genetic merit for milk/milkfat production (Tilakaratne et al. 1980; Sejrsen et al. 1984; Sinnett-Smith et al. 1987), sheep of superior merit for greasy fleece weight (McCutcheon et al. 1987; Clark et al. 1989) and sheep or pigs of superior merit for lean tissue growth (Mersmann et al. 1984; Bremmers et al. 1988; Carter et al. 1989; Van Maanen et al. 1989). While these results are encouraging in that they suggest a common genetic association between protein deposition and plasma urea, other studies have failed to find the same relationship (Mackenzie et al. 1988). Plasma urea concentration is strongly influenced by nutritional status (via reciprocal effects on entry rate of urea from rumen ammonia or deamination of amino acids) so that it could not be used to select animals in the field (McCutcheon et al. 1987). Moreover, the mechanisms by which genetic differences in plasma urea concentration occur appear to vary between selection lines. Thus in some lines differences in plasma urea appear to reflect differences in the use of amino acids for protein synthesis vs energy (Tilakaratne et al. 1980; Mersmann et al. 1984) while in others they may be a function of genetic effects on clearance by the kidney (McCutcheon et al. 1987; Clark et al. 1989; Van Maanen et al. 1989). If so, the apparently consistent relationship between plasma urea and genetic merit may have occurred purely by chance. Alternatively, there may be common processes contributing to the urea difference, the importance of each process varying in different selection lines. These will be difficult to detect if, as seems likely, their individual contributions are very small. In an attempt to resolve some of these issues, we have recently established replicated mouse selection lines using high or low plasma concentrations of urea as the selection criterion.

Basal hormone concentrations The investigation of hormone levels as potential physiological indicator traits is obviously appealing because many hormones are known to have a central role in the regulation of animal metabolism. Hormones of the somatotrophic axis demand particular attention in this regard because, at least in dairy cows, the mechanisms by which animals of high genetic merit achieve their superior levels and efficiency of production are similar to those mechanisms stimulated by exogenous somatotropin (Bauman et al. 1985). Attempts have therefore been made to identify between-selection line differences in basal somatotropin (ST) levels. While genetically superior dairy cows (Barnes et al. 1985; Kazmer et al. 1986), dairy calves (Mackenzie et al. 1988; Xing et al. 1988) and lean sheep or pigs (Wangsness et al. 1977; Carter et al. 1989) have been shown to exhibit higher ST levels than inferior animals, results have not always been consistent. Basal ST levels are also difficult to measure accurately because they are strongly influenced by nutrition (an important confounding effect when animals are compared at difference energy balances) and the hormone is secreted in a pulsatile manner necessitating very frequent sampling.

The insulin-like growth factors (IGF's) are hormones secreted into the circulation (primarily by liver), and produced locally in many tissues, under the influence of ST. IGF-1 is of particular interest as a potential physiological indicator trait because it is secreted in a non-pulsatile manner and appears to be involved in the genetic regulation of growth. We have recently established that plasma IGF-1 is heritable in mice and have developed lines of mice divergently selected on the basis of plasma IGF-1. Responses to this selection process included: increased body weight and weight gain; changes in the allometric growth of specific organs but little effect on fat and protein content of the body (at equal body weights); increased litter size; enhanced fetal weight (despite the increased litter size); and increased mammary gland weights in the females (Blair et al. 1989; Kroonsberg et al. 1989; Siddiqui et al. 1989). Plasma IGF-1 levels have also been shown to be positively associated with genetic merit for milk production in progeny-tested bulls (Ahlborn-Breier et al. 1987), although expression of this genetic relationship appears to depend on the nutritional status of the bulls at the time of sampling.

Insulin is another hormone which may have potential as a physiological indicator trait. Elevated insulin concentrations have been observed in cows of superior genetic merit (Flux et al. 1984; Xing et al. 1989) and in their calves (Mackenzie et al. 1988), and are also commonly observed in genetically obese lines of animals (see Carter et al. 1989).

Metabolic challenges As was noted earlier, circulating concentrations of key metabolites are generally under tight homeostatic control and little variation may be expressed between animals. Metabolic challenges, which test the responsiveness of animals to a homeostatic signal, may be used to overcome this problem. Fasting and feeding are examples of "whole-body" metabolic challenges in which the responsiveness of genetically divergent lines to a change in energy status is measured. They are easy to administer but have the disadvantage that they simultaneously influence a plethora of metabolic processes. In addition, they require that assumptions be made about the energy requirements of genetic groups (eg that two groups have equal maintenance requirements) but these assumptions are rarely tested. Nevertheless, they may be a useful technique. For example, Mackenzie et al. (1988) showed that differences between genetically distinct groups of calves in plasma levels of somatotropin, glucose and urea were enhanced by fasting and refeeding.

More specific metabolic challenges involve the administration of key hormones or metabolites, usually via the intravenous route, and measurement of the animal's response. Test animals of different genetic groups receive the same dose of the challenge (per unit liveweight) so that differences in responsiveness are readily interpreted. Commonly-used challenges include adrenaline and glucagon (to test the glycogenolytic and lipolytic sensitivity of liver and adipose tissue, respectively), insulin (testing sensitivity of peripheral tissues to insulin-mediated glucose uptake or the antilipolytic effects of insulin) and glucose or propionate (sensitivity of pancreatic insulin release). While these challenges are more specific than fasting and refeeding, they are also more difficult to administer and it cannot be assumed that they act only on one organ or tissue. Adrenaline, for example, is known to be lipolytic but also influences blood flow. Genetic differences in one effect may therefore be counterbalanced or enhanced by differences in the other. Responses to metabolic challenge are also known to be influenced by dose of challenge and energy balance so that researchers must be conscious of possible interactions between genotype and dose of the hormone/metabolite or energy status of animals under test.

Although metabolic challenges generally utilise hormones which exert acute homeostatic effects, the possibility also exists that genetically divergent animals might respond differentially to hormones which act in a homeorhetic (chronic) manner. Michel et al. (1990) have shown that the response of low genetic merit cows to ST is much greater (in both absolute and proportional terms) than that of high genetic merit cows. Thus, even if consistent differences in circulating ST cannot be identified between genetically divergent groups (as discussed earlier) it may be possible to use homeorhetic hormones such as ST to identify genetically based differences in chronic regulation of metabolism.

In vitro techniques Techniques which measure tissue metabolism in vitro have the advantage that the tissues under study are isolated from whole body effects (eg changes in blood flow) although, for obvious reasons, sampling of the tissues must be achieved without detriment to the breeding animal. A number of in vitro techniques with potential application to the search for physiological indicator traits exist, but most studies have concentrated on lipid metabolism (ie selection for low carcase fat content). It has been shown, for example, that adrenaline-stimulated lipolysis in vitro is much greater in pigs from a lean/high rate of gain line than from a line selected for fat/low rate of gain (Standal et al. 1973). Particularly interesting are the results of Strutz and Rogdakis (1979). Having observed differences between lean and fat pig lines in the activities of NADPH-generating enzymes in adipose tissue, they divergently selected pig lines for activity of these enzymes. Enzyme activity was shown to have a substantial heritable component ($h^2 = 0.53 - 0.73$) and to be genetically correlated with fatness. As a result, divergent selection on the basis of enzyme activities led to a marked divergence in body fatness of the pigs (Rothfuss et al. 1984).

SUMMARY

The rates of genetic gain that can typically be made in the livestock industries offer little assistance to the farmer who is trying to meet the demands of an often volatile consumer market. One possibility in trying to alleviate this problem is to find physiological indicator traits that can be measured at a young age, in both sexes, with reasonable ease of sampling and that are closely correlated with the economic traits of interest.

The search for physiological indicator traits is hampered by the complexity of pathways involved in producing the economic traits of interest. Furthermore, many of these pathways and their interactions are unknown or poorly understood. To overcome this problem, animals from selection experiments can be used to provide an initial indication of changes in physiological traits that have accompanied selection for an economic trait. Having found a potential candidate, it is then necessary to place the physiological trait itself under direct selection to determine whether the predicted benefits in the economic traits of interest actually accrue. One possibility as a physiological indicator trait is IGF-1, which may be useful as a component in a selection index to improve the efficiency of lean tissue production in meat animals. Other candidates have been implicated in milk-and fibre-producing animals but, as yet, none of these traits has undergone the ultimate test of being selected for directly.

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