

THE USE OF GENOMICS IN GENETIC SELECTION PROGRAMS FOR ENVIRONMENTAL STRESS TOLERANCE IN DOMESTIC ANIMALS

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ABSTRACT

Acclimation of domestic animals to high environmental temperature typically results in lower production as animals lower their metabolic rate and feed intake to accommodate the increased heat load, (1). Ideally, one would like to simultaneously select for increased production and thermal resistance to increased thermal load. This will require simultaneous identification and selection for improved heat dissipation and production mechanisms. Rate of genetic progress in domestic animal breeding programs is driven by 3 factors; accuracy of markers for phenotypic traits, intensity of marker use and generation interval. Improved tools in molecular analysis of gene expression will permit improvement in the accuracy and intensity of use of genetic markers. New techniques in reproductive biology will permit reduction of generation interval. Collectively, these new techniques offer real opportunity to improve tolerance for environmental heat stress in domestic animals. Presently, genetic markers for phenotypic traits do not generally correspond to the coding regions for the genes of interest. They are located close to loci of traits of interest but lose their value over time due to genetic recombination. Ideally, genetic markers should identify the exact location of the gene of interest. Thus, identifying these genes is a major objective of any breeding program. Combining use of consomic lines of rats with single gene deletions and gene expression microarrays that permit evaluation of expression levels of thousands of genes at a time will rapidly increase knowledge of genes associated with tolerance or sensitivity to environmental heat stress. Targets of potential genetic manipulation would include increased efficiency and capacity of thermal effectors and delayed onset of temperature threshold for thermal injury. For example, constitutive elevation of heat shock protein gene expression has been shown to be cytoprotective against thermal injury in rats. This approach has not yet been tested in domestic animals. Likewise, treatment of lactating dairy cows with bovine somatotropin has been shown to increase evaporative heat loss capacity. Understanding the molecular basis for the increased evaporative heat loss capability offers new opportunities for increasing thermal tolerance of cattle in warm climates. Finally, unraveling molecular changes associated with seasonal acclimation will offer new insights into selecting domestic animals for thermal tolerance

INTRODUCTION

Selection for tolerance to environmental stress has traditionally been counterproductive in domestic animal production. Generally, as animals acclimatize to environmental stressors they reduce or divert metabolizable energy from production to balance heat gain and loss (1). Thus, it has generally been faster and easier to obtain production increases by altering the environment around animals. However, environmental modification

comes at a high cost and in many cases these costs cannot be economically justified. Thus, in the beef industry where profit margins are very thin there is little economic advantage to capital investment for production gains. In contrast, large capital investments are made in the dairy and poultry industries to modify environments in order to maximize profitability. However, new genomics technologies offer the possibility of improving resistance of domestic animals to environmental stress while not interfering with their productive capability. This is due to new technologies that permit identification of specific genes that are associated with sensitivity to or resistance to stress and modify these genes alone. The net effect is to confer improved adaptation to specific environmental stressors. The degree to which cross-adaptation to other stressors may occur is not yet known. This opportunity exists for all animals regardless of their level of present environmental protection. Purpose of this overview is to outline the technologies and the probable direction of best opportunity for their use in improving environmental adaptation in domestic animals.

Acclimation, Acclimatization, Adaptation And Preconditioning

Physiological acclimation of animals to their environments is a process that occurs within the lifetime of the animal in response to a single environmental variable such as temperature, (2). Acclimatization is the physiological acclimation to a natural environment that contains several variables. In both acclimation and acclimatization the body's response to the environment is coordinated over a several week period at the structural, organ, cellular and molecular level to respond the stress or stressors with alterations in the organisms capacity to tolerate the stress. These changes include alteration in gene expression, enzyme activity, body organ size, fat deposition, energy consumption and a wide variety of other possible effector mechanisms depending on the stressors, (3). These responses, listed in Table 1 are lost if the stress is removed. In contrast, genetic adaptation is the process by which acclimatory responses are "fixed" through natural selection to permanently alter the genetic makeup of the animal. During one specific part of the lifecycle the application of a stress can permanently alter an individual animals response to stress over its lifetime but does not impact the gene pool, (4). Thus, the ability of broilers to resist heat stress is improved if they were exposed to high environmental temperature during the neonatal period, (5). This effect is not passed on to their offspring. Thermal preconditioning has not yet been demonstrated in domestic mammals primarily because it has not yet been tested. However, other forms of preconditioning stress have been demonstrated to permanently alter the hypothalamic-pituitary-adrenal axis in mammals, (4).

Work on the exact genetic and molecular mechanisms of acclimation, acclimatization, adaptation and preconditioning to thermal stress has only recently begun. As shown in Table 1, a wide variety of changes take place at the cellular and systemic level as animals acclimate to thermal stress. All of these involve alteration in expression of many thousands of genes. These responses are generally not homeostatic in nature as they require days or weeks to occur. They also require a high degree of coordination among cell types and tissues. This type of coordinated response to support a physiological change is referred to as homeorhesis (11).

Table 1: Partial list of molecular and systemic changes associated with acclimation to thermal stress

species	cell type or tissue	response	reference
cattle	sweat gland	increased sweating rate	8
rat	Hypothalamus	altered heat dissipation threshold	3
rat	cardiac	increased heat shock protein synthesis	3
rat	thyroid	depressed thyroid function	3
rat	cardiac	reduced heart rate	3
mouse	brain-medulla	improved synaptic transmission	9
rat	vascular system	increased vascular reserve	10
rat	muscle	switch from myosin isozyme V1 to V3	11

Thermal homeorhesis, acclimation and acclimatization are essentially the same phenomena differing only in the number of stressors involved. A key component of this process is the coordination of the responses that have received little attention to date. As reviewed by Bauman (12), homeorhesis is “the orchestrated or coordinated changes in metabolism of body tissues necessary to support a physiological state”. In this case, the physiological state is acclimation or acclimatization. As reviewed by Horowitz (3), acclimation involves changes in all levels of body organization including the reprogramming of gene expression. Thus, there is a large body of work to be done in the area of change in gene expression associated with acclimation as well as the homeorhetic control mechanisms involved in acclimation and acclimatization. One example of induced acclimation involves the role of somatotropin in lactating dairy cows. When lactating dairy cows are given exogenous somatotropin during lactation they increase milk yield, feed intake and heat output (1,8). One might predict that they would become more susceptible to heat stress given the additional increase in heat output. However, as shown in Table 2, the increase in heat production was associated with a remarkable increase in evaporative heat loss that allowed the cows to maintain normal body temperature. Thus, somatotropin coordinated the metabolism of the cows to permit the increased milk synthesis while dissipating the extra heat load. This clearly indicates that there is additional opportunity to improve thermal tolerance of cattle if appropriate regulatory control systems can be identified.

Table 2: Effect of Heat Stress on Water Turnover in Cows Treated with Bovine Somatotropin (bST)^{1,2}

H ₂ O Source	Thermoneutral		Heat		SEM	Main Effect ³	
	Control	bST	Control	bST		B	T
			<u>Kg/Day</u>				
Intake	73.5	112.2	94.5	110.2	12.1	0.04	0.4
Food ⁴	12.2	14.1	9.6	1.4	0.5	0.003	0.0001
Metabolic ⁵	3.8	4.5	3.7	4.7	0.1	0.0001	0.4
Total Intake	89.5	130.8	107.8	126.3	12.2	0.02	0.6
Skin Vaporization	12.9	17.6	19.3	24.5	1.4	0.002	0.001
Resp. Vaporization	3.1	3.7	4.3	5.0	0.2	0.002	0.001

1. Manulu et al. 1991 J. Nutr. 121:2006-2019

2. Data represents 6 cows for each treatment in switchback design

3. Abbreviations used B= bST effect, T= temperature effect

4. Calculated from moisture content of diet

Estimated using 1.23 kg water produced per 41.84 MJ (10 Mcal) heat production

Another example of improving thermal tolerance at the cellular level lies in the studies carried out with targeted disruption of heat shock protein gene expression (13) or increased constitutive expression of heat shock proteins (13,14). Disruption of heat shock protein 70 gene expression in mice reduced thermal tolerance and increased apoptosis of cells (13). Stable overexpression of heat shock proteins protects cells from thermal shock and increases thermotolerance (14). However, enhancing heat shock protein synthesis alone is not going to alter an animal's ability to dissipate heat. One may need to look higher in the regulatory control system to determine if the increase in heat shock protein synthesis can be coordinated with other changes supporting improved thermotolerance. In support of this, Horowitz, (3) reported that increased heat shock protein synthesis depends on sympathetic system excitability.

Collectively, results to date support the concept that acclimation is a coordinated process involving reprogramming of expression of many genes. The next stage of research will involve the identification of these genes and their control systems.

Gene Discovery and Mapping

One obvious approach of identifying genes associated with acclimation to thermal stress is to utilize gene expression microarrays in models of thermal acclimation to identify changes in gene expression during acute and chronic thermal stress. Currently, several laboratories are in the process of producing cattle, pig, horse and poultry arrays. These arrays will produce a wide variety of information useful in determining key metabolic pathways involved in acclimation. Another approach will be to use consomic lines of

rats

with single gene deletions exposed to a defined thermal environment. This permits the identification of those genes that are involved in key regulatory pathways for thermal resistance and thermal sensitivity. Finally, gene knockout models in single cells will also allow better delineation of the cellular metabolic machinery required to acclimate to thermal stress. Those genes identified as key to the process of thermal acclimation will then need to be mapped to their chromosomal location and the sequences of these genes will need to be determined in order to see if there are single nucleotide polymorphisms (SNPs) that are associated with changes in the coding for gene expression or protein function. Identification of SNP's that are associated with variation in animal resistance or sensitivity to thermal stress will permit screening of animals presence or absence of desirable or undesirable alleles. These "markers" can also be utilized in breeding programs. Thus, utilization of new reproductive technologies will be required to maximize genetics of the most desirable animals and to insert new traits into animals.

Reproductive Tools

Rate of genetic progress for a given breed of domestic animal can be depicted by the equation shown in Figure 1.

$$\text{Rate of genetic improvement} = \frac{\text{Accuracy of marker} \times \text{intensity of marker use} \times \text{population variance}}{\text{generation interval}}$$

Figure 1: **Genetic Progress**

The accuracy of a given genetic marker improves as it approaches the location of the specific gene of interest. Identification of the specific gene equates to 100% accuracy of the marker. If we assume that this marker is a SNP that results in offspring with improved expression of heat shock proteins in every cell and we use that marker to screen every animal in the herd we have 100% intensity of marker use. If it turns out that the occurrence of the SNP of interest is highly variable then we have an excellent opportunity to increase genetic gain. If it turns out the SNP is already represented in 90% of animals we have little opportunity for genetic gain. The final component of the equation is the denominator. Presently, the generation interval in cattle is 2 years. If we can reduce that to zero by using the marker to identify superior embryo's and then clone those embryo's to put into every available female we have maximized the rate of genetic progress.

Breakthroughs in reproductive technologies will improve rate of genetic progress in the area of thermal tolerance once specific genes have been identified (15). The first stage will be utilization of SNP's as markers for specific genes that will permit identification of superior animals and embryo's. These markers will be used in "nucleus herds" to permit a high intensity of marker use. Essentially, every animal in the nucleus herd will be screened using the markers. In the case of the dairy industry where females are more desirable, gender selection will also occur to maximize the number of females and this

will be done by sperm sorting or gender testing of embryo's. Highly valued animals will be used for Multiple Ovulation Embryo Transfer (MOET) schemes that permit maximizing the best genetics. Eventually, as we understand more concerning the regulatory genes involved in acclimation the opportunity to produce transgenic animals will become a reality. These transgenes will likely also be clones that incorporate enhanced or repressed bovine genes involved in resistance or sensitivity to thermal stress.

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