EFFECTS OF POLYMORPHIC GENES OF THE BOVINE LYMPHOCYTE ANTIGEN COMPLEX (BoLA) AND MILK PROTEIN VARIANTS ON REPRODUCTION AND GROWTH TRAITS IN NORWEGIAN CATTLE

BY

FERNANDO GRIGNOLA

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

Department of Animal Science

1993

ABSTRACT

EFFECTS OF POLYMORPHIC GENES OF THE BOVINE LYMPHOCYTE ANTIGEN COMPLEX (BoLA) AND MILK PROTEIN VARIANTS ON REPRODUCTION AND GROWTH TRAITS IN NORWEGIAN CATTLE

BY

FERNANDO GRIGNOLA

Relationships of polymorphic genes of the BoLA-A complex and milk protein genetic variants to performance traits of Norwegian bulls were studied. Conformation, testicular size, semen traits and growth records were analyzed. In most cases, it is unlikely that these polymorphic loci are themselves quantitative trait loci (QTL), but they may be markers linked to loci with an important effect on the quantitative trait.

The direct effects of marker gene substitutions were analyzed in multiple regression models, whereas the genotypic effects were analyzed in fixed classification models. In both cases, the models included corrections for non-marker genes (polygene effects) and non-genetic factors influencing the traits.

Significant marker gene effects at the BoLA-A locus were found on semen density and growth rate. However, no effect on other semen traits and body conformation were found. Milk protein genotypes did not significantly affect weight gain, testicular size and semen quality. Results for the BoLA-A locus suggest that this locus could be a marker itself or, more likely, a starting point for the search of other markers more closely linked to the QTL controlling the production traits studied.

ACKNOWLEDGMENTS

I thank Drs. R. Erickson and M. Yokoyama for their support during two long years previous to my coming to Michigan State University.

My sincere appreciation to Dr. Ivan Mao, my academic advisor, for his guidance, encouragement and financial support during my graduate program.

I extend my appreciation to Drs. D. Banks, R. Erickson and P. Coussens, members of my graduate committee, for their contribution to my graduate program. Special thanks go to Dr. J. Gill, also member of my committee, for his continuous and unselfish statistical and personal support during my academic program.

A special thank to Dr. K. Chou for giving me the opportunity to work in her laboratory and for her friendship and support.

I would like to acknowledge the Agricultural University and the Veterinary College of Norway for supplying the data for this research.

Many thanks to my friends P. Saama, F. Ngwerume, G. Ferreira and D. Krogmeier. They have been very kind to me during difficult times. They also have contributed to the knowledge and skills I have acquired during my stay at M.S.U.

My sincere thanks to Dr. Ricardo Cardellino and his family for their friendship and continuous encouragement.

A special thank to my parents, brothers and sisters in Uruguay for their love and understanding.

TABLE OF CONTENTS

LIST OF TABLES

1. INTRODUCTION

Conventional progeny testing schemes, and more recently, multiple ovulation and embryo transfer (MOET) breeding schemes are being used to genetically improve production in dairy cattle. Ranking of animals in these schemes is based on the prediction of breeding values from phenotypic records.

New techniques for the identification and characterization of genes controlling quantitative traits have been developed, making possible identification of genetic differences directly at the DNA level. Restriction fragment length polymorphism (RFLP) (Botstein et al., 1980), variable number of tandem repeats (VNTR) (Jeffreys, Wilson and Thein, 1985; Nakamura et al., 1987) and polymerase chain reaction (PCR) (Medrano and Aguilar-Cordova, 1990) are some of these techniques.

Once relationships between marker genes or genes themselves and quantitative traits have been detected, marker assisted selection (MAS) or direct selection on individual genotypes could be applied. This would be of particular value regarding traits that are not expressed in all animals (e.g.: sex-limited traits), lowly heritable traits (e.g.: reproductive traits), and traits expressed late in life. In addition, by direct selection on the gene itself, effects of Mendelian segregation could be disregarded (Dekkers and Dentine, 1991), and conclusions could be drawn on the combination of

gene effects based on a metric character (Gelderman, 1975).

Identification of allelic variants of genes that affect quantitative traits, either positively or negatively, with a net effect of practical importance over and beyond the polygene effect may be used to more accurately assess genetic potential in livestock species. Therefore, the specific aims of this study were:

1. To evaluate the effects of marker gene substitutions (MHC class I antigens) on conformation, semen volume, density and quality, and growth rate in Norwegian bulls. The influence of homozygosity at the BoLA-a locus on the traits analyzed is also of interest.

2. To estimate associations between genotypes of κ-casein and β-lactoglobulin and weight gain, testicular size and semen quality in performance test Norwegian bulls.

2. REVIEW OF LITERATURE

2.1 Genetic Markers in Animal Breeding

A genetic marker is a polymorphic gene, or a DNA segment whose allelic variants are inherited in a Mendelian fashion and are associated with genetic variation in a trait of interest (Lundén, 1991).

In the field of animal breeding, genetic markers can be used to identify quantitative trait loci (QTL) controlling economically important traits. This information is valuable for several reasons. Firstly, knowing the number of QTL and the magnitude of the genetic effects influencing the traits, makes possible more realistic models describing the sources of the phenotypic variation that influence the traits and facilitates effective response to selection. Secondly, it will provide fundamental knowledge about gene actions and interactions (Haley et al., 1991).

In a more general prospective, the relevance of research in the area of genetic markers is that it may permit acceleration of rate of genetic improvement via marker-assisted selection (MAS). Marker assisted selection permits animals to be selected partially on their genotype in addition to their phenotypic data. Moreover, marker information can be used for identification, and possibly for introgression of genes of interest in foreign populations.

A marker system should have the following desirable characteristics for application (Lundén, 1991):

1. Marker polymorphism is associated with variation in an economically important trait.
2. The marker does not show negative effects with other traits of interest.
3. Screening for the genetic marker should be fast and inexpensive.

After these requirements are met, it is possible to use MAS to increase the rate of selection response. There are three ways in which markers could increase accuracy of selection (Dentine, 1990):

1. Marker information on parents could supplement incomplete offspring phenotypic records to give higher accuracy to estimates of their breeding values.
2. Marker information on offspring could be used to trace a portion of the Mendelian sampling variance.
3. Markers could be used to predict dominant and epistatic effects.

Two types of marker effects can be distinguished. A direct effect of the marker gene on the trait(s); an effect through linkage (equilibrium or disequilibrium) to a QTL. Either way, the marker can be used across the population. If recombination occurs frequently, selection on the marker can be then useful within families (Lundén, 1991). If linkage disequilibrium starts diminishing gradually due to exploitation or genetic recombination, Lande and Thompson (1990) suggested that the disequilibrium can be regenerated by hybridization of differentiated selection lines.

The literature shows that MAS could be useful for some traits under specific breeding schemes (Dentine, 1990, Dentine and Dekkers, 1991, Kashi et al., 1990,

Zhang and Smith, 1992). Currently, very few marker genes controlling quantitative traits of economic importance like growth, milk yield, reproduction and health, are available. The more markers available, the more efficient the MAS program is, but the faster the genetic variation in the polygene trait of interest will be exploited. This will lead to a change in gene frequencies and to an increase in genotyping costs due to necessary continuous evaluation of the marker associated effect(s). The economic value of the increase in accuracy from the use of molecular information will depend on when these data will be used and whether alternative procedures will be used to increase accuracy (Dentine, 1990).

Incorporation of marker information in the evaluation of lowly heritable traits like disease resistance, will be especially beneficial. Other traits such as milk protein variants can only be evaluated by molecular testing.

2.2 The Major Histocompatibility Complex (MHC)

2.2.1 Genetic Structure

The major histocompatibility complex (MHC) is a chromosomal region consisting of a series of closely linked loci or so-called gene families that has been maintained as a conserved linkage group. It has been found in all mammalian species (Newman and Antczak, 1983). The human MHC (HLA) is located on the short arm of chromosome 6, the murine MHC (H-2 complex) is located on chromosome 17, and the bovine MHC (BoLA) is located on the short arm of chromosome 23.

The genes of the MHC are grouped into three classes based on the structural

and/or functional characteristics of their gene products. The class I genes encode class I molecules which are made up of two polypeptides. The polymorphic α chain consists of a membrane-bound glycoprotein heavy chain, with molecular weight of 40 to 50,000 daltons, and an invariant protein, the β_2-microglobulin. This protein is a non-membrane-bound light chain, with molecular weight of 12,000 daltons, and is encoded in a different chromosome. The two chains (α and β_2) are non-covalently bonded. The class II or Ir genes, encode class II molecules which are made up of two polymorphic membrane-bound glycoproteins, the α chain (34,000 daltons) and the β-chain (28,000 daltons). These two chains are non-covalently associated. The class III genes encode for the serum complement components C2, C4 and factor B. The relationship between class III molecules and class I and II is not clear, and class III loci are not considered to be a part of the MHC proper by some authors (Klein and Figueroa, 1981).

Genetic variation in the genes of the MHC is one of its most remarkable features. More than 20 alleles have been identified at the principal class I locus of the mouse (Klein and Figueroa, 1981), and 50 specificities have been internationally recognized in cattle (Bernoco et al., 1991). As a consequence of the large number of MHC alleles in different species, few individuals in a population will carry identical MHC genes (Newman and Antczak, 1983). This high polymorphism likely is maintained by heterozygous advantage (Klein and Figueroa, 1986). With a large number of MHC alleles at the population level, it is likely that only a small proportion of individuals would be susceptible to a given pathogen as a result of Ir-

gene deficits. At the individual level, that would decrease the chance of an animal being a non-responder to an important pathogen. Thus, heterozygous animals likely would be favored (Newman and Antczak, 1983).

2.2.2 Immunological Functions

The class I molecules are expressed at all nucleated cells. The primary immunological function is to serve as restriction elements for $CD8^+$ cytotoxic T lymphocytes. The cell receptor recognizes both foreign antigen and self class I MHC antigen, forming a complex that is specifically recognized by T cells, which respond by synthesizing and secreting enzymes that bore holes into the infected cell.

Class II molecules function primarily as restriction elements for $CD4^+$ helper/inducer T-cells. Exogenous peptides are presented to $CD4^+$ T cells in association with class II molecules. The T cells synthesize and secrete lymphokines that cause antibody producing B cells to divide and differentiate into plasma cells, which in turn secrete antibodies.

Thus, the specificity of receptors of the two principal classes of lymphocytes (cytotoxic and helper) appears to be controlled by the two principal classes of MHC molecules.

2.2.3 Characterization of BoLA Genes

Products of the BoLA-A (class I) locus have been extensively characterized using serologically approaches (Anonymous, 1982; Stear et al., 1989; Bernoco et al.,

1991). Alloantisera that distinguish BoLA-A allomorphs are obtained from two different sources, (1) production by planned immunization with skin grafts, leukocytes, or purified lymphocytes and (2) the sera of parous cattle that have been immunized during pregnancy or at parturition with paternally inherited fetal antigens (Newman and Antczak, 1983). The most recent international BoLA workshop (Bernoco et al., 1991) involved the exchange of 1139 serological reagents between 15 laboratories in 9 countries which were tested over 54 animals of various breeds. This workshop expanded the number of internationally recognized BoLA specificities to 50.

For a long time, the BoLA class I region has been characterized as having one locus, the BoLA-A locus. New techniques, such as selective immunoprecipitation and molecular characterization of the products, permit answering the question of whether there is more than one locus expressed. Joosten et al. (1992), using one- and two-dimensional isoelectric focusing (1D/2D-IEF), supported the hypothesis of a second putative BoLA locus. Additional evidence also has been produced by Bensaid et al. (1991) and Ennis et al. (1988). These results provide a possible explanation of why it has been so difficult to detect expression of a second locus at the BoLA complex by using alloantisera (Lewin, 1989).

The genetic polymorphism of class II genes has been studied by serology, isoelectric focusing and restriction-fragment-length-polymorphism (RFLP) analyses (Davies and Antczak, 1991; Joosten et al., 1989; Sigurdardóttir et al., 1991). The class II region is divided into two subregions, DQ-DR and DO-DY, separated by a

recombination frequency of about 17% (Andersson, 1988). In a recent international BoLA workshop (Bernoco et al., 1991) several new DRB and DQA (isoelectric focusing) and DQB (RFLP) patterns were identified. In 46 animals that were typed for BoLA-DR and DQ genes by RFLP analysis, 47 BoLA haplotypes were tentatively defined.

2.2.4 MHC and Fertility Traits

Associations between the MHC genes or closely linked genes and reproductive performance are important because they could offer the possibility of improving reproductive efficiency, by selective breeding, and a better understanding of the biological mechanisms underlying these associations. The influence of the MHC on reproduction is largely unknown (Hunziker and Wegmann, 1986). However, several reports in mice (Yamasaki et al., 1976), rats (Gill and Kunz, 1979; Kunz et al., 1980) and pigs (Mallard et al., 1987; Rothchild et al., 1984) suggest that MHC genes are associated with reproductive performance.

Data of MHC and reproduction on the same animals are scanty. Stear et al. (1989a) found no statistical evidence for reduced pregnancy rate in matings where parents shared one or two BoLA-A locus alleles. Østergård and Dam (1987), working with Red Danish dairy cattle, reported no differences in insemination success between matings where zero and one allele (or antigen) were shared between the parents. In the same study, homozygous embryos showed no decrease in fetal viability, indicating that fetuses with MHC antigens foreign to the mother have no advantage in

implantation and embryonic growth. In contrast, indications of decreased fetal viability of homozygous individuals have been reported in several studies (Joosten et al., 1991; Gautschi et al., 1987; Rothchild et al., 1987). Joosten et al. (1991) studied the possibility that compatibility of the MHC products between calf and cow might negatively influence the placental maturation and expulsion, and therefore increase the risk of retained placenta in healthy, normally calving cattle. The MHC class I antigens were identified by 1D-IEF, and compatibility between calf and dam was established by comparing IEF banding patterns. Analysis revealed that MHC class I compatibility between dam and calf increased the risk of retained placenta, and the authors suggested that retention of the placenta is, at least in part, the result of the lack of alloreactive mechanisms.

Testicular size, which is an indicator of early puberty and increased fertility in young bulls, was reported to have large and significant association with some allele of the BoLA-A locus (Stear et al., 1989a). The same trait also has been associated with genes of the MHC in rats (Gill and Kunz, 1979) and pigs (Rothchild et al., 1986). However, it is important to mention that, to date, no association between the genes of the BoLA-A locus and semen characteristics has been reported. Research has been concentrated on testing the presence of MHC class I antigens in sperm cells (Fellows and Dauset, 1970 -in humans; Vaiman et al., 1978 -in boars). In cattle, absence of BoLA-A antigens in spermatozoa has been reported (Matoušek et al., 1989; Folger and Hines, 1976). According to Matoušek et al. (1989), it is possible to assume that the BoLA antibodies present in the ovarian follicle fluid of some cows cannot bind to

spermatozoa and do not influence bovine fertility.

2.2.5 MHC and Growth Traits

If genes within or closely linked to the bovine MHC influence growth rate, then isolation, cloning and study of the function of these genes could improve understanding some of the physiological mechanisms controlling variation in growth rate.

Associations between the pig MHC (SLA complex) and growth and carcass traits (Capy et al., 1981; Jung et al., 1989; Kristensen et al., 1982) and birth and weaning weights (Mallard et al., 1991; Rothchild et al., 1986) have been reported. Studies on experimental animals suggest that the effects of the MHC on growth and development are due to genes closely linked to the class I loci. In rats a group of genes linked to the MHC, the growth and reproduction complex (Grc), influence body size, and an analogous region in the mouse (the T/t complex) governs developmental defects (Gill and Kunz, 1979).

In beef cattle, Stear et al. (1989b) reported significant gene-substitution effects of some BoLA-A antigens on birth weight, preweaning weight gain and postweaning weight gain. Nevertheless, no consistency of effects of class I antigens across the nine breeds studied was found. Batra et al. (1989), in a study involving 179 Canadian Holstein cows, showed significant gene substitution effects associated with increased weight at 350 days of age and weight at first calving. However the authors did not consider these findings conclusive. A significant association between MHC class I and

a carcass trait has been reported. Beever et al. (1990), in a paternal half-sib family of Angus cattle, found significant differences in rib-eye areas (4.1 cm^2) between animals receiving two different BoLA-A haplotypes. These findings may suggest that the BoLA-A complex or closely linked genes can be used as markers in searching for associations with growth and carcass traits.

Previous evidence show that some genes may have pleiotropic effects antagonistic to improving both production traits and disease resistance. In poultry, Han and Smyth (1972) reported that selection for higher growth rate resulted in increased susceptibility to Marek's disease. In addition, chickens resistant to Marek's disease had lower adult body weight and produced smaller eggs (Gavora et al. cited by Warner et al., 1987). One possible explanation might be that modern management techniques (vaccination, preventive medication) masked the genetic capacity of the animals to resist disease. Thus, if the MHC prove to be a useful marker for growth rate, in the future it will be very important to obtain information on genetic correlations among disease resistance and production traits before selective breeding can be applied.

2.3 Milk Protein Genetic Variants

2.3.1 Introduction

Six proteins, α_{s1}, α_{s2}, β and κ-caseins and the two major whey proteins, αlactalbumin and β-lactoglobulin, compose 95% of the total protein in bovine milk. They are known to occur in the form of products that reflect the action of autosomal

genes transmitted from parents to offspring by simple Mendelian inheritance (Aschaffenburg, 1968).

2.3.2 Genetic Structure

Aschaffenburg and Drewry (1955) were the first to assess the polymorphic nature of the gene encoding for β-lactoglobulin: "we found that individual cows produce either a mixture of two electrophoretically distinct β-lactoglobulins or only one or the other of these. The two single components will be termed β_1 and β_2-lactoglobulin ...". Later on, as new laboratory techniques developed, polymorphism of other milk protein genes were discovered.

Milk protein genes can be subject to duplication, deletion or insertion, which in some situations change the amino acid composition of the protein resulting in a new genetic variant. Therefore, the gene frequencies vary from breed to breed, and while some of the variants are universal, others are restricted in occurrence.

Bovine casein genes reside in a region of less than 200kb on chromosome 6 in the following order: α_{s1}-casein - β-casein - α_{s2}-casein - κ-casein (Threadgill and Womack, 1990) and a low recombination rate is thus expected. This is in agreement with studies using classical linkage analysis (Aleandri et al., 1990; Hines et al., 1981), and those using DNA amplification from single sperm cells (Sigbjorn et al., 1992). These studies strongly argue against recombination "hot spots" in the region encoding casein genes. The gene for α-Lactalbumin has been assigned to the bovine syntenic group U-3, which has been assigned to the bovine chromosome 5. The β-

lactoglobulin gene was located on syntenic group U16, but the chromosome carrying the syntenic group has not yet been identified (Threadgill and Womack, 1990).

2.3.3 Biochemical Characteristics of Milk Protein Genetic Variants

It is known that all major protein fractions are genetically polymorphic (Ng-Kwai-Hang, 1984). Table 1 shows the differences in amino acid composition between the main milk protein variants. Separation of milk protein genetic variants has traditionally been performed by applying electrophoresis techniques (Bovenhuis, 1992). Because these methods are based on variations in electric charge, only those amino acid substitutions resulting in a net change of electric charge will be detected. Some mutations at the DNA level can lead to the production of another protein that some times will not be detected by electrophoresis (Grosclaude, 1988). However, the development of new techniques, e.g., isoelectric focusing (Seibert et al., 1985), has increased resolution and speed in detection of milk protein variants. This is very important, because accurate genotyping is needed if additional information is going to be incorporated in selecting bulls to be used in artificial insemination (AI) or in selecting and breeding cows for specific milk protein variants.

2.3.4 Milk Protein Genetic Variants and Production Traits

Several workers have reported that milk protein variants are associated with milk yield, milk composition and processing properties of the milk (Aleandri et al., 1990; Baldwin et al., 1986; Bech and Kristiansen, 1990; Bovenhuis, 1992; Mao et

al., 1992; McLean et al., 1984; McLean and Schaar, 1989; Marziali and Ng-Kwai-Hang, 1986; Ng-Kwai-Hang et al., 1984; Schaar et al., 1985). Various strong associations have been found, and recommendations about including particular genotypes in animal

Table 1. Total number of amino acids and amino acid substitutions of the main genetic variants of milk proteins (Bovenhuis, 1992).

Milk Protein	Number of amino acid residues	Comparison of genetic variants	Change in amino acid composition
α_{s1}-Casein	199	C ---> B	192*:Gly --> Glu
α_{s2}-Casein	207		
β-Casein	209	A^2 ---> A^1	67 :Pro --> His
		A^2 ---> B	67 :Pro --> His
			122 :Ser --> Arg
		A^2 ---> A^3	106 :His --> Gln
κ-Casein	169	A ---> B	136 :Thr --> Ile
β-Lactoglobulin	162	B ---> A	64 :Gly --> Asp
			118 :Ala --> Val
α-Lactalbumin	123		

* number of amino acid that has been substituted

breeding programs have been made. However, some authors (Schaar et al., 1985) have suggested further research on the genetic associations between milk protein variants and other traits to ensure that cow performance and health are not adversely affected by selection for a specific genotype. Studies to date have revealed no association with reproductive performance (Hargrove et al., 1980), but no one has published a study of possible associations with animal growth.

2.3.5 Milk Protein Genetic Variants and Performance Traits

The main method of cumulative genetic improvement in economic performance of livestock has been selection on performance traits. If the traits are heritable, then there must be quantitative trait loci (QTL) affecting them, and any information (direct or indirect) on these QTL and their effects are theoretically useful (Smith and Simpson, 1985).

In most testing schemes for bull performance, strong emphasis is put on growth rate, conformation, testicular size, semen quality traits and health, before a bull is considered for progeny testing. The bulls are selected on genetic values estimated from phenotypic measures, but the question of interest is how the variation for these traits is controlled at the genetic level. If MAS is going to be applied, genes or markers of genes affecting economically important quantitative traits have to be identified. In addition, with the advent of transgenic techniques, molecular biologists will need to know what genes other than growth hormone to transfer. Two approaches have been suggested to detect differences in specific genes (Dentine, 1990). Indirect observation from a difference in the gene product includes electrophoretic variants (milk protein polymorphisms), antigen variants (major histocompatibility complex, blood types) and differences in enzyme levels (DUMPS syndrome). Direct observation in the DNA structure is possible with the development of newer molecular techniques such as amplification by polymerase chain reaction, restriction enzymes digestion (restriction fragment length polymorphism), nucleotide sequencing, and variable number of tandem repeats.

To date, no researchers have reported possible associations between milk protein genetic variants and testicular size, semen quality and growth rate. Athough it is unlikely that genes controlling milk protein variants have a direct effect on performance traits, they might well be markers linked to the QTL controlling them. Evidence for a major gene in mice, inherited as an autosomal recessive, which increases postweaning growth rate and mature size by as much as 50% has been reported (Bradford and Famula, 1984). However, the authors noted that the gene went undetected for some time because it was not expected, nor were tests made to detect its presence. In addition, most quantitative traits are likely to be influenced by many genes that have small individual effects but large aggregate effects (Kennedy et al., 1982). Therefore, milk protein genetic variants should not only be seen as affecting milk and whey proteins, but also as possible markers for other traits as well.

In this context, possible associations between different milk protein genetic variants and growth rate, testicular size and semen quality in young bulls are of research interest.

2.4 NRF-Norwegian Cattle

2.4.1 A Description of the Breed

The NRF-breed is a population of high-milking, dual-purpose cattle, developed by using modern breeding techniques and strategy. The NRF is not a cattle breed in the traditional sense. It is a synthetic breed with an open structure. This means that alongside effective selection programs within breed, excellent material from other

breeds has been introduced into the population. Semen from bull sires of NRF, SRB (Swedish Red) and Finnish Ayrshire, and both Swedish Friesian and Holstein-Friesian from the USA and Canada have been introduced into the population.

Some characteristics of the NRF-breed are high milk and butterfat yield (6,363 and 253 kg/cow a year), high growth rate, well shaped udder and teats, good body conformation and strong legs, good temperament, low frequency of still-born calves and calving difficulties, and good fertility and health.

2.4.2 Cow Index

A cow index system has been used in Norway for more than 20 years. The cow index is an estimate of the cow's breeding value for milk yield and it is estimated for all recorded cows. The cow index is based on the following elements: the cow's own milk yield, herd average for milk yield, average genetic value of the herd, and breeding value of the cow's pedigree. The cow index is the basis for selecting dams of bulls.

2.4.3 Performance Test of Young Bulls

All potential AI bulls go through an individual test at performance test stations. Annually, 400 bulls are selected for this test. All are sons of elite sires and dams. The testing period is from 90 to 330 days of age. The feeding regime is based on concentrates,by age, and silage/hay ad libitum.

Primary selection criteria are daily gain and conformation. Emphasis also is put

on semen quality (volume, motility and viability). Following the test, about a third of the bulls are selected for use in AI. Current avera9ge daily gain at the stations is between 1250 and 1300 g/day. For bulls selected at the end, daily gain is about 100 g higher.

As an example, the following breeding calendar for bull calves born in 1991 is presented:

1991 Selection of 400 bull calves from elite sires and dams. Performance test at stations (3-11 months)

1992 Selection: 125 bulls for A.I.- 250 bulls slaughtered. Sampling 2500 doses of semen from each of the 125 bulls for immediate use, to produce heifers for progeny testing.

1993 Sampling about 40,000 doses of semen for storage until end of progeny test. All bulls are slaughtered.

1994-1995 Waiting for progeny test results

1996 The progeny test is available:

The 5 best bulls: bull sires, used for high index cows. (Bull dams with breeding value more than 7. About 7% of the cows)

The 15 next good bulls, for commercial use. For the rest, about 100 bulls, semen is discarded.

3. Association of Class I Bovine Lymphocyte Antigen Complex Alleles (BoLA-A) with Growth and Semen Characteristics in Norwegian Young Bulls

3.1 ABSTRACT

Relationships between the bovine major histocompatibility complex (BoLA-A) and performance test results of bulls were investigated. A total of 279 Norwegian bulls in performance test between 1988 and 1989 were typed for BoLA-A. A single trait animal model was used to estimate gene substitution effects of BoLA-A on conformation, semen volume, density and quality. A fixed linear model was applied to analyze the breeding values of growth rate for the same purpose.

Allelic frequencies ranged from .2 to 28%, with alleles w16 (28%), A2 (15%), A10(w50) (12%) and A8 (10%) being the most frequent. Effects of several BoLA-A alleles appeared to be significant ($P<.10$) on conformation (A10(w50), w25), semen density (w16) and semen quality (A10(w50), A11), but none of those had perceptible effect on semen volume. However, associations were significant between allele A12(A30) and semen volume ($P<.05$) and between alleles A9 and A12(A30) and growth rate ($P<.002$). Heterozygosity at the BoLA-A locus did not show significant advantage in any of the traits.

The BoLA-A genes could be potential markers for the quantitative trait loci (QTL) controlling semen density and growth rate. Because of limited data, further research is necessary to confirm these findings.

3.2 INTRODUCTION

The bovine major histocompatibility complex or bovine lymphocyte antigen

complex (BoLA) consists of three highly polymorphic types of cell-surface glycoproteins involved in the regulation of immune response. These are denoted as BoLA class I, II and III antigens (Klein, 1979) and are encoded on the short arm of bovine chromosome 23 (Fries et al., 1989). Neither the exact order of the genes on the complex nor the size of the BoLA region is known but, as in other species, genes of the bovine MHC have been maintained as a conserved linkage group (Klein, 1986). Despite the fact that most of the serologically MHC class I molecules are encoded by the BoLA-A locus, there is evidence for a second class I locus (Bensaid et al., 1991; Ennis et al., 1988; Stear et al., 1982). Class I genes encode the classic transplantation antigens that reside on all cells, and act as restricting elements in T-cell recognition of virally infected cells (Newman et al., 1983).

Most genetic polymorphisms consist of a major allele in high frequency and one or a few variant alleles that differ from the main one by only one or two amino acid substitutions (e.g.: κ-casein alleles). However, the MHC complex presents extreme polymorphism. In the recent International BoLA Workshop (Bernoco et al., 1991), 50 BoLA specificities were recognized, and it is likely that this polymorphism is maintained by heterozygous advantage (Antczak, 1982; Klein and Figueroa, 1986). Another characteristic of the MHC antigens is that they are expressed codominantly and inherited in a Mendelian fashion (Oliver et al., 1981).

Immune related response, high genetic polymorphism, and Mendelian inheritance of the allelic variants qualify the MHC as a potential marker for disease resistance.

The majority of studies have concentrated on the role of MHC in regulating

immune responses and disease resistance. In cattle, enzootic bovine leukosis, ocular carcinoma, mastitis, tick resistance and intestinal parasites have been shown to be associated with different BoLA antigens (Østegård et al., 1989). A considerable amount of information suggests that the genetic control of a wide variety of nonimmunological related traits like growth rate, birth and weaning weights (Batra et al., 1989; Mallard et al., 1991; Stear et al., 1989a; Stear et al., 1989b), carcass traits (Beever et al., 1990), and fertility traits (Batra et al., 1989; Joosten et al., 1991; Mallard et al., 1987; Østegård and Dam, 1987; Rothschild et al., 1984; Stear et al., 1989a) in swine and cattle, may be associated to genes within or closely linked to the MHC. Results from those studies have shown inconsistency regarding the effect of specific BoLA alleles. On the other hand, the existence of a chromosomal region, linked to the MHC, that influences developmental traits (body and testicular size, among others) in rats and mice, suggests that this may be a general phenomenon in mammals (Gill and Kunz, 1979). This fact has stimulated the extensive research that is being carried out on different production traits and different species.

One of the most difficult problems in detecting quantitative trait loci (QTL) in cattle is the small number of available polymorphic genetic markers. If relationships between the MHC genes and production or reproduction traits are found to be significant, they can be used in searching for other markers which are more informative and more closely linked to the QTL controlling the trait of interest.

The objectives of this study were to evaluate the substitution effects of MHC class I genes on conformation, semen volume, semen density, semen quality and

growth rate in Norwegian young bulls. The influence of homozygosity at the BoLA-A locus on these traits also was investigated.

3.3 MATERIALS AND METHODS

3.3.1 Data

The data included 279 young Norwegian bulls born between 1988 and 1989. They were evaluated under the Norwegian scheme of performance testing, in which they are housed in three separate test stations, under standardized conditions, from 2 to 12 mo of age. They had free access to roughage and were fed fixed amounts of concentrates according to age. The graduates or selected group (25-25%) go through progeny test, and the top 12-15% of those become AI bulls.

Growth rate was measured from 3 to 11 month of age. A relative growth index was used to evaluate the breeding value for growth (1 to 10, including half points). Conformation was scored linearly (1 to 10, including half points) combining information on feet, legs and body conformation. Testicular size was measured as the scrotal circumference at 12 month of age. An overall semen quality score (0 to 5) was given, with semen characteristics consisting of semen volume, density, morphology, motility and viability. Table 2 shows the mean, standard deviation and coefficient of variation for these traits (statistics for testicular size, semen morphology, motility and viability are not included since in previous analyses convergency of the estimation procedure was not reached). Eventually, the young bulls were selected if their index

score on growth rate exceeded 5, conformation score exceeded 5 and semen quality score exceeded 3.

Typing for BoLA class I antigens was performed by a standard microlymphocytotoxicity test (Bull et al., 1989). A total of 120 alloantisera were used and 16 antigens were identified (Table 3). The nomenclature followed that of Bernoco et al. (1991).

3.3.2 Methods

The BoLA allele frequencies were calculated by direct counting. Undetected alleles and alleles with frequencies below 1% were not included in the analysis (Table 3).

Single-gene associated effects on quantitative traits often may be confounded with non-random genetic effects due to relationships existing between individuals sharing a particular allele of the trait being analyzed (Bentsen and Klemetsdal, 1991). Therefore, to estimate the direct effect of the marker genes on conformation and semen traits a single trait animal model (Henderson, 1988) was used. The model was:

$$\mathbf{y} = \mathbf{Xb} + \mathbf{Mm} + \mathbf{Zu} + \mathbf{e} \qquad (1)$$

where **y** was the vector of records of young bulls; **b** contained fixed effects of two groups (selected, unselected) and three test stations; **m** contained the fixed gene-substitution effects of different BoLA-A alleles; **u** was an animal vector of bulls, sires and maternal grandsires; **X** was the incidence matrix for **b**; **M** was the incidence matrix, with elements representing the number of copies (0,1 or 2) a bull had of a particular allele; **z** was the incidence matrix for **u**; and **e** represented the vector with

random residuals. The (co)variance matrix of the random vectors of the model was:

$$V\begin{bmatrix}\mathbf{u}\\ \mathbf{e}\end{bmatrix} = \begin{bmatrix}\mathbf{A}\sigma_a^2 & \mathbf{0}\\ \mathbf{0} & \mathbf{I}\sigma_e^2\end{bmatrix}$$

where **A** represented the matrix of additive genetic relationships between the bulls and their sires and maternal grandsires. The relationship matrix was included to account for the non-random distribution of the bulls over marker genotypes. Additive allelic effects were simultaneously analyzed as multiple regression coefficients on variables that define the number of copies of each allele in the bull's genotype. Dependencies within a marker locus were removed according to Østegård et al. (1989). This is done by applying the constraint that the sum of all regression coefficients has to be equal to zero.

Gene-substitution effects and variance components for animals and residual effects were estimated by using the computer algorithm of derivative-free restricted maximum likelihood (DFREML) developed by Meyer (1991). The convergence criterion was the variance of the function values, Var(-2logL), which was chosen to be 10^{-8}, where L is the likelihood function.

Gene-substitution effect is the effect of substituting one copy of a particular BoLA-A allele for a copy of a hypothetical allele, constructed from the mean of other BoLA-A alleles present in the population and weighted by their respective frequencies (Falconer, 1981). Direct gene-substitution effects may not be distinguished from linkage effects, indicating that the polymorphic gene may function as a marker gene.

Therefore, the net additive (or substitution) effect estimated for each marker gene, can be separated neither from the additive and dominance effects taking place between the two gene regions marked for these genes, nor from the average epistatic effects between these two gene regions and the rest of the genotype (Bentsen and Klemetsdal, 1991). In addition, if gametic imprinting effects are real and important, yet to be established, these effects also could not be detected (Kennedy et al., 1990). Direct marker gene-substitution effects then, reflect the linear (or additive) effect associated with the different allelic gene regions.

Indexed breeding values for growth rate (g_k) were analyzed by using a model fitting only the mean and the marker gene effects:

$$g_k = \mu + \sum_r b_r X_k + e_k \qquad (2)$$

which was computed by the SAS GLM procedure (SAS, 1985).

The effect of homozygosity at the BoLA-A locus on conformation, semen volume, semen density and semen quality were analyzed according to (1) and (2), by replacing the marker gene-substitution effects by the fixed effect of homozygosity. According to the number of copies of the same allele, heterozygous and homozygous bulls were assigned a value of 1 and 2 respectively. Since not all the animals had records for all the traits, each single trait analysis included different number of animals and alleles.

3.4 RESULTS AND DISCUSSION

A summary of the basic statistics for the traits under evaluation is presented in

Table 2. Not all bulls had records for all the traits. On average, most bulls exceeded the minimum score for conformation and growth rate established for selection; however, this was not the case for semen quality. In addition, all the traits had considerable variation (CV > 20%).

Table 2. Mean ($\bar{X}$), standard deviation (SD), and coefficient of variation (CV) for conformation, semen volume, density and quality, and growth rate

Trait	No. Bulls	$\bar{X}$	SD	CV(%)
Conformation (1-10)	262	5.3	1.1	20.8
Semen Volume (ml)	153	4.1	.92	21.9
Semen Density (10^6/ml)	153	911.6	233.4	25.6
Semen Quality (0-5)	153	3.5	.82	22.9
Growth Rate (1-10)	262	5.2	2.3	44.2

3.4.1 Gene Frequencies

Allelic frequencies ranged from .2% to 28%, with w16 having the highest frequency (Table 3). Alleles os2 and 25c represented local specificities. Significant differences were reported in antigen frequency between breeds (Amorena and Stone, 1978; Nonnecke et al., 1989; Stear et al., 1982; Oliver et al., 1981). The Third International BoLA Workshop (Bull et al., 1989) reported that w8 may be the most frequent BoLA-A antigen in Holstein cattle (44.1%). Allele w8 is identical to A8 under the new nomenclature (Bernoco et al., 1991). Natural selection, linkage disequilibrium and genetic drift may explain some of the differences in allelic frequencies between breeds. Significant differences in allelic frequencies between

sexes within a breed also have been reported (Oliver et al., 1981).

Table 3. BoLA-A gene frequencies

Allele	Frequency (%)
A2	15.1
A5	4.5
A6	7.2
A8	9.9
A9	.9
A10(w50)	11.8
A11	5.9
A12(A30)	8.8
A13	1.8
w16	27.5
w25	2.9
A3	.2
A20	.2
A21	.2
os2	.4
25c	.9
Undetected	1.8

3.4.2 Conformation Score

In Table 4, marker gene-substitution effects for conformation score are presented. Alleles A2 and A10(w50) decrease conformation score whereas w25 increases conformation score ($P<.10$). This parameter, as a consequence of its complexity, may not be the optimal choice when searching for associations. However, from these results and those from Batra et al. (1989) it seems that most of the significant associations between the BoLA-A system and conformation are negative.

Table 4. Gene-substitution effects for conformation score

Allele	No. bulls Homozygous/ heterozygous	Conformation score Estimate	SE
A2	13/56	- .16 (P<.10)	.1
A5	3/18	- .05	.2
A6	5/28	- .03	.1
A8	3/49	.03	.1
A9	1/3	.12	.3
A10(w50)	9/46	- .16 (P<.10)	.1
A11	5/23	.03	.1
A12(A30)	6/36	- .06	.1
w16	31/87	.02	.1
w25	2/12	.29 (P<.10)	.2

3.4.3 Semen Traits

Results on relationships between semen traits and BoLA-A marker alleles are in Table 5. There were no significant associations between BoLA-A alleles and semen volume. Semen density was significantly decreased (P<.05) by allele A12(A30) while it was increased (P<.10) by allele w16. Neither A12(A30) nor w16 had a significant effect on the overall semen quality score, which seems to be slightly affected by alleles A10(w50) and A11 (P<.10). Since overall semen quality score combines information on semen motility, morphology and viability, it is likely that genes affecting some of these traits also affected the overall index.

3.4.4 Growth Rate

The marker gene effect of some BoLA antigens on growth rate was significant

Table 5. Gene-substitution effects for semen volume (ml), semen density (10^6/ml) and semen quality (score)

Allele	No. bulls Homozygous/ heterozygous	Semen Volume		Semen Density		Semen Quality	
		Estimate	SE	Estimate	SE	Estimate	SE
A2	8/33	-1.9	1.2	5.3	31.1	-.06	.1
A5	1/9	-2.8	2.6	-74.4	63.2	-.21	.2
A6	4/19	- .6	1.5	1.6	37.8	.08	.1
A8	2/31	2.3	1.5	-38.4	38.3	-.19	.1
A9	1/2	2.7	3.3	60.8	83.2	-.04	.3
A10(w50)	5/33	- .6	1.4	55.8	34.5	-.19 (P<.10)	.1
A11	1/17	- .2	2.0	19.9	49.9	.29 (P<.10)	.2
A12(A30)	2/20	.8	1.8	-95.8 (P<.05)	44.3	-.19	.2
w16	14/46	-1.2	1.1	44.7 (P<.10)	27.0	.10	.1
w25	1/10	1.6	2.3	20.6	56.2	.03	.2

(Table 6). Alleles A9 and A12(A30) showed highly significant associations with growth rate ($P < .002$). However, for allele A9, only four animals were positive and the only homozygous bull had a growth index score of 10. On the other hand, the more abundant information for allele A12(A30) suggests more strongly an effect on growth rate. This may not be surprising, because growth reportedly is influenced by the MHC complex in swine (Mallard et al., 1991), cattle (Stear et al., 1989b) and mice (Simpson et al., 1982).

Table 6. Gene-substitution effects for growth rate index

Allele	No. bulls Homozygous/ heterozygous	Growth rate index Estimate	SE
A2	13/56	- .27	.2
A5	3/18	- .07	.4
A6	5/28	.20	.3
A8	3/49	- .40	.3
A9	1/3	2.39 ($P < .002$)	.8
A10(w50)	9/46	- .30	.3
A11	5/23	- .25	.3
A12(A30)	6/36	- .97 ($P < .002$)	.3
w16	31/87	- .28	.2
w25	2/12	- .05	.5

Homozygosity at the BoLA-A locus did not significantly affect conformation score, semen-related traits and growth rate (Table 7). This is in agreement with previous results in pigs (Mallard et al., 1991) and cattle (Stear et al., 1989a). However, except for growth rate, heterozygous bulls appeared to perform slightly better.

Table 7. Effect of homozygosity at the BoLA-A locus

Trait	No. bulls Homozygous/ heterozygous	Homozygous vs heterozygous Contrast	SE
Conformation	78/184	-.1	1.0
Semen Volume	39/144	-2.3	1.6
Semen Density	39/144	-19.9	41.0
Semen Quality	41/112	-.1	.1
Growth Rate	79/183	2.2	3.0

3.5 CONCLUSIONS

The significant effects of allele A12(A30) on semen density and A9 and A12(A30) on growth rate, provide clues for future research. The gene-substitution effects observed suggest that these marker genes or (linked genes) may have some potential use as markers for production and reproduction traits. Differences between homozygous and heterozygous bulls were not significant.

Our results, as well as those reported in the literature, were not consistent regarding the effect of specific alleles on conformation and growth rate. Some possible explanations are: different models, randomness of the samples and sample size are common limitations, and associations may have been due to chance or caused by MHC-linked genes for which linkage phase differs among populations and breeds.

4. Effects of polymorphic milk protein genes on weight gain, testicular size and semen quality in Norwegian Young Bulls

4.1 ABSTRACT

Associations between milk-protein genotypes and weight gain, testicular size and semen quality, were estimated in young Norwegian bulls. Exact tests of hypotheses and unbiased estimates of genotype effects were obtained using an animal model. Effects κ-Casein and β-lactoglobulin genotypes were estimated by using a model in which each milk-protein variant was analyzed alone (single-gene analysis) and a model in which both were analyzed simultaneously (multigene analysis). Additive genetic and phenotypic standard deviations (SD) and heritability estimates were obtained from each model.

The results for the two models were similar, indicating that the effects of these two protein variants are independent. Genotype effects of neither variant significantly affected weight gain, testicular size or semen quality. In addition, κ-Casein and β-lactoglobulin did not account for a significant portion of the additive genetic variance of these traits. Although the data are limited the κ-casein and β-lactoglobulin genetic variants apparently do not affect weight gain, testicular size and semen quality in young bulls.

4.2 INTRODUCTION

Major milk-protein variants in the bovine are polymorphic (Aschaffenburg, 1968; Eigel et al., 1984). Associations between milk-protein polymorphisms (MiPPo) and milk components (Aleandri et al., 1990; Gonyon et al., 1987; Mao et al., 1992;

Ng-Kwai-Hang et al., 1984), milk processing properties, including cheese yield (Aleandri et al., 1990; Schaar et al., 1985; Baldwin et al., 1986; Marzialy and Ng-Kwai-Hang, 1986) and milk yield (Aleandri et al., 1990; Mao et al., 1992; McLean et al., 1984; Ng-Kwai-Hang et al., 1984) have been reported. Two milk-protein genes, κ-casein and β-lactoglobulin, have been intensively studied. Genes encoding κ-casein as well as α_{s1}-casein, α_{s2}-casein and β-casein, the major-milk proteins, have been assigned to bovine chromosome 6. By using classical linkage analysis, investigators have shown that the casein genes are closely linked (Hines et al., 1981). One of the two major whey proteins, β-lactoglobulin, is located on syntenic group U16, but the chromosome carrying the syntenic group has not been identified (Threadgill and Womack, 1990).

In general, β-lactoglobulin BB genotype has been reported to increase fat percentage, while the AA genotype decreases protein yield and protein percentage. On the other hand, κ-casein BB genotype has been shown to be positively associated with protein yield and protein percentage. Results from most studies differ with respect to the size and significance of the genotype effects. A possible explanation might be that these observed effects are not only caused by the polymorphic loci themselves, but also by linked genes for which the effects are different in magnitude and sign across populations (Gelderman et al., 1985; Cowan et al., 1992). Even if the same genes were linked, linkage phase most likely was different. Another reason could be the statistical models used to analyze different data. When estimating single-gene effects, even in the absence of linkage, it is important to separate the confounding effect that

background genes might have on the trait being analyzed. However, some investigators (Ng-Kwai-Hang et al., 1984) used ordinary least squares procedures which, in non-random mating and/or selected populations, tends to bias estimates of the genotype effects upwardly (Kennedy et al., 1992). In contrast, mixed-model procedures for an animal model with fixed genotype effects give unbiased estimates of the single genotypic effects and exact tests of associated hypotheses (Kennedy et al., 1992).

In recent years, increasing protein yield through breeding has been of interest. However, before recommendations can be made regarding animal breeding programs, genetic associations between the caseins and β-lactoglobulin loci and other production traits should be investigated to ensure that traits like health, reproduction and growth are not adversely affected by selection on particular genotypes. Previous research has revealed no associations between milk genetic polymorphisms and reproduction traits in Holstein cattle (Hargrove et al., 1980); however, associations with performance traits in bulls (e.g. growth, reproductive traits) have not been investigated.

The specific aims of this study were to estimate associations between κ-casein and β-lactoglobulin genotypes and weight gain, testicular size and semen quality in Norwegian bulls, and to examine their overall and net additive effects on those traits.

4.3 MATERIALS AND METHODS

4.3.1 Data

The analysis included 274 young Norwegian bulls. The bulls were born

between 1983 and 1985 and they were sampled from bulls evaluated annually under the Norwegian scheme of performance testing. The calves were housed under standardized conditions in two test stations between 2 and 12 month of age. Growth rate was evaluated from 90 to 360 days of age, and weight gain was computed as the difference in body weight at those two ages. Testicular size was measured by scrotal circumference at 12 month of age. Semen quality was scored subjectively (range 0 to 5). Data on semen volume, density, motility and viability also were available , but they were not considered because, in previous analyses, convergency of estimation was not reached. About 60% of the bulls were born between August and October ("in season"), the remaining 40% from November to July ("out of season").

Typing for κ-casein (κ-CN) and β-lactoglobulin (β-LG) was performed. Genotypes AA, AB, and BB for κ-casein and β-lactoglobulin were identified for 194 bulls (Table 8).

4.3.2 Methods

The following animal model (Henderson, 1988) was used to estimate the independent effects of κ-casein (κ-CN) and β-lactoglobulin (β-LG) on body weight gain:

$$y_{ijklm} = \mu + Y_i + St_j + S_k + b(WGT90)_{ijklm} + MP_l + a_{ijklm} + e_{ijklm} \quad (1)$$

where:

y_{ijklm} weight gain of the *ijklm*th young bull;

μ overall constant;

Y_i year of birth (i=1,2,3);

St_j test station (j=1,2,3);

S_k season of birth (k=1,2);

b linear regression coefficient for weight at 90 days;

$WGT90_{ijklm}$ weight at 90 days;

MP_l fixed effect of the lth milk-protein genotype (κ-CN or β-LG);

a_{ijklm} random animal effects;

e_{ijklm} random residual effects.

The random animal effect, a, was defined as a vector of bulls and their sires, and e represented the random residual effects. The (co)variance matrix of the random vectors of the model was:

$$V \begin{bmatrix} \mathbf{a} \\ \mathbf{e} \end{bmatrix} = \begin{bmatrix} \mathbf{A}\sigma_a^2 & \mathbf{0} \\ \mathbf{0} & \mathbf{I}\sigma_e^2 \end{bmatrix}$$

The relationship matrix **A** was based on 24 paternal halfsib groups. Only the additive portion of polygene effect was considered. Different numbers of young bulls and ancestors were involved in the analysis for the two milk-protein variants, because not all bulls were genotyped for both milk-proteins. Fixed effects, other than the milk-protein genotypes, were not factors of main interest but were included as correction terms.

To permit estimation of the combined effect of the two milk-protein variants genotypes on weight gain, a second model (2) was used:

$$y_{ijklmn} = \mu + Y_i + St_j + S_k + b(WGT90)_{ijklm} + (\kappa\text{-CN})_l + (\beta\text{-LG})_m + a_{ijklmn} + e_{ijklmn} \qquad (2)$$

where:

κ-CN$_l$ fixed effect of the lth κ-CN genotype (l= AA, AB, BB);

β-LG$_m$ fixed effect of the mth β-LG genotype (m= AA, AB, BB).

Models (1) and (2) were also used to evaluate testicular size and semen quality, and are referred to as single gene and multigene models, respectively.

Using the derivative free algorithm (DFREML) described by Meyer (1991) additive genetic standard deviations, phenotypic standard deviations and heritability estimates, adjusted for the effects of the protein genotypes, were obtained from models 1 and 2. The convergence criterion of 10^{-8} was the variance of the function values, i.e., Var(-2Log L), where L is the likelihood function. Starting values for iteration were taken from the literature.

4.4 RESULTS AND DISCUSSION

The distribution of the observed genotypes of κ-casein and β-lactoglobulin in the present study is shown in Table 8. Gene frequencies, calculated assuming Hardy-

Table 8. Genotypic frequencies for κ-casein (κ-CN) and β-lactoglobulin (β-LG)

Genotype		No. of bulls	Frequency
κ-CN	AA	143	73.7
	AB	46	23.7
	BB	5	2.6
β-LG	AA	6	3.1
	AB	78	40.2
	BB	110	56.7

Weinberg equilibrium from Table 8, are in Table 9 along with estimates for some other breeds. The κ-casein A allele and the β-lactoglobulin B allele tended to be the

Table 9. Gene frequencies for κ-casein (κ-CN) and β-lactoglobulin (β-LG) in different breeds

Milk protein		Jersey[1]	Jersey[2]	White Danish Dairy Cattle[1]	Friesian[2]	Holstein[3]	Norwegian Cattle[4]
κ-CN	A	.31	.23	.85	.67	.77	.86
	B	.69	.77	.15	.33	.23	.14
β-LG	A	.31	.33	.54	.39	.41	.33
	B	.68	.56	.46	.61	.59	.77
	C	.01	.11	0	0	0	0

1 Bech and Kristiansen, 1990
2 McLean et al., 1984
3 Mao et al., 1992
4 Present study

most common in the majority. Exceptions were the κ-casein B allele in Jersey and the β-lactoglobulin A allele in the black and white danish dairy cattle. For the breeds compared, the β-lactoglobulin C allele appeared only in the Jersey breed.

A summary of the basic statistics for weight gain, testicular size and semen quality over κ-casein and β-lactoglobulin genotypes is presented in Table 10. For each trait, the coefficients of variation within protein variant were small and the means showed little variation across genotypes.

Table 10. Descriptive statistics for weight gain, testicular size and semen quality over genotypes of κ-casein (κ-CN) and β-lactoglobulin (β-LG)

Protein	Genotype								
	AA			AB			BB		
	$\bar{x}$	SD	CV	$\bar{x}$	SD	CV	$\bar{x}$	SD	CV
	Weight gain (wgt90d-wgt360)								
κ-CN	336.9	23.5	7.0	338.1	24.4	7.8	331.8	17.9	5.4
β-LG	342.5	42.6	12.4	334.2	22.9	6.8	338.8	23.6	7.0
	Testicular Size (cm)								
κ-CN	34.3	1.6	4.6	34.7	1.8	5.3	34.3	1.5	4.5
β-LG	36.3	2.1	5.7	34.5	1.6	4.8	34.2	1.6	4.7
	Semen Quality (0-5 score)								
κ-CN	3.4	.5	15.4	3.7	.6	17.2	4.0	.0	.0
β-LG	3.5	.6	16.5	3.5	.6	15.8	3.5	.6	16.2

Table 11. Estimates of protein genotype effects on weight gain, testicular size and semen quality for a single gene and a multigene analysis

	Genotype	No. Obs.	Single gene analysis		Multi-gene analysis	
			Est.	SE	Est.	SE
			Weight gain (wgt90-wgt360)			
κ-CN	AA	143	.0		.0	
	AB	46	2.58	4.10	2.02	4.13
	BB	5	-7.16	10.50	-6.97	10.52
β-LG	AA	6	.0		.0	
	AB	78	-11.69	9.78	-11.08	9.84
	BB	110	-8.58	9.73	-8.13	9.77
			Testicular size (cm)			
κ-CN	AA	99	.0		.0	
	AB	35	.37	.31	.34	.31
	BB	3	.70	.89	.73	.89
β-LG	AA	3	.0		.0	
	AB	50	-.99	.91	-.84	.92
	BB	84	-1.11	.90	-.99	.91
			Semen Quality (0-5 score)			
κ-CN	AA	124	.0		.0	
	AB	38	.22	.10	.22	.11
	BB	4	.56	.28	.55	.28
β-LG	AA	4	.0		.0	
	AB	67	-.09	.29	-.02	.29
	BB	95	-.12	.29	-.07	.29

None of the milk-protein genotypes exhibited any indication of being significant in influencing weight gain, testicular size and semen quality, as shown in Table 11. Results from fitting κ-CN and β-LG genotypes individually (single gene analysis) were not different from those fitting both protein variants simultaneously (multigene analysis). This means that κ-casein genotype effects were not affected by correcting the data for β-lactoglobulin genotype effects and vice versa. These results were expected, because β-lactoglobulin is not linked to the casein genes. Although sample size was small, there was no indication that these two milk-protein genetic variants have any effect on weight gain, testicular size and semen quality in growing young bulls.

The estimates of additive genetic and phenotypic standard deviations (SD), and heritabilities of the performance traits analyzed after removing effects of one or both milk-protein genes are shown in Table 12. The same parameters were estimated with a model in which milk-protein effects were ignored. Based on the assumption of complete additivity of the models, additive genetic SD would be expected to be greater when milk-protein variant effects were not accounted.

Genetic SD estimates for weight gain were of similar magnitude among models, indicating that neither κ-CN nor β-LG contributed much to the genetic variation of this trait. For testicular size, genetic SD estimates for the single gene model including β-LG and the multigene model were the same. On the other hand, the single gene model including κ-CN and the model ignoring milk-protein genetic variants gave also the same estimates. This may indicate that the observed reduction

Table 12. Additive genetic and phenotypic standard deviation and heritability estimates for weight gain, testicular size and semen quality using different models

Model	Weight gain (wgt90-wgt360)			Testicular size (cm)			Semen quality (0-5 score)		
	Genetic SD	Phenotypic SD	Heritability	Genetic SD	Phenotypic SD	Heritability	Genetic SD	Phenotypic SD	Heritability
No MiPPo	13.28	22.03	.33±.24	.92	1.51	.37±.28	.17	.55	.10±.23
Only κ-CN	13.06	23.07	.32±.24	.92	1.51	.38±.28	.15	.54	.08±.21
Only β-LG	13.23	23.03	.33±.24	.85	1.51	.32±.28	.18	.56	.11±.22
κ-CN and β-LG	13.06	23.09	.32±.24	.85	1.51	.32±.28	.15	.55	.08±.22

in genetic SD for testicular size could be associated with the β-LG variant. For semen quality, it was the κ-CN genetic variant which seemed to be associated with a reduction in the genetic SD for this trait. However, the large standard errors of the heritability estimates indicated that there were not meaningful differences between models regarding the possible contribution of milk-protein genetic variants to the additive variation of the performance traits analyzed.

4.3 CONCLUSIONS

The milk protein genetic variants, κ-casein and β-lactoglobulin, were not associated with weight gain, testicular size and semen quality in Norwegian young bulls. The lack of significant association between κ-casein and these traits also indicates that it is very unlikely that other casein variants can affect weight gain because of the linkage existing among casein genes. Neither did the same genes contribute significantly to the additive genetic variation in weight gain, testicular size or semen quality.

5. SUMMARY

5. SUMMARY

The possibilities to study and locate genes with an effect on quantitative traits is largely dependent on the availability of genetic markers (or polymorphic genes) with demonstrable variation. Quantitative markers can be associated with quantitative traits through linkage disequilibrium or pleiotropic effects. It is not likely that most genetic markers found are quantitative trait loci (QTL) themselves, but one may expect polymorphic marker loci to be linked to QTL with effects on quantitative traits. In this context, markers for candidate genes like the major histocompatibility complex and milk-protein genetic variants, were tested for association with related production traits of bulls with the objective of identifying loci with significant effects on traits of economical importance in animal breeding.

5.1 Major Histocompatibility Complex (MHC) and Performance Traits

Relationships between the bovine MHC class I polymorphism (BoLA-A) and performance traits were studied. A group of 279 young Norwegian bulls in performance test between 1988 and 1989 was serologically typed for BoLA-A genes. The average gene-substitution effects were estimated for the 10 most frequent A haplotypes. The performance traits included conformation (overall linear score including feet, legs, and body conformation), semen volume (ml), semen density (10^6 sperm/ml), semen quality (total score including semen volume, density, morphology, motility and viability), and growth rate (index given from breeding values).

Gene-substitution effects on conformation and semen-related traits were

analyzed using a single trait animal model, whereas, growth rate was analyzed using a fixed linear model. Heterozygosity at the BoLA-A locus also was examined.

Sixteen different BoLA-A alleles were identified in this population of young bulls, w16 being the most frequent (28%). Some alleles showed significant effects ($P < .10$) on conformation, semen density and semen quality, but none on semen volume. Statistically significant associations were detected with semen volume ($P < .05$) and growth rate ($P < .002$). Heterozygous bulls at the BoLA-A locus did not perform better than homozygous ones ($P > .05$).

The results suggest that some genes of the BoLA-A system might have some potential use as markers for production and reproduction traits. Given the small sample analyzed, further research is necessary to confirm these results.

5.2 Milk-Protein Genetic Variants and Performance Traits

The relationships between milk-protein genotypes and weight gain, testicular size and semen quality were studied. The analysis included records from 274 performance tested young Norwegian bulls. The bulls were born between 1983 and 1985 and were genotyped for κ-casein and β-lactoglobulin.

Weight gain, computed as the difference in body weight between 60 and 360 days of age; testicular size, as the scrotal circumference measured in cm; and semen quality, scored subjectively, were analyzed. Genotypes AA, AB and BB for κ-casein and β-lactoglobulin were identified.

Milk-protein genotype effects were estimated using a model in which each protein was analyzed separately (single trait model) and simultaneously (multigene

model). Additive genetic and phenotypic standard deviations and heritabilities were estimated from the same models.

Gene frequencies in this population indicated that the κ-casein A allele and β-lactoglobulin B allele were the most frequent. The results of the two models were not different, indicating that adjustments for κ-casein did not affect the genotypic effect of β-lactoglobulin, and vice versa. No significant effects of κ-casein or β-lactoglobulin were detected on weight gain, testicular size and semen quality. This was confirmed by the fact that both milk-protein genetic variants did not account for a significant fraction of the additive genetic variation in weight gain. Although the sample was small, these results strongly suggest that variations in the performance traits studied in this population of young bulls were not affected by κ-casein and β-lactoglobulin genetic variants.

6. LIST OF REFERENCES

6. LIST OF REFERENCES

Aleandri, R., L.G. Buttazzoni, J.C. Schneider, A. Caroli, and R. Davoli. 1990. The effects of milk protein polymorphisms on milk components and cheese-producing ability. J. Dairy Sci. 73:241.

Amorena, B., and W. H. Stone. 1978. Serologically defined (SD) locus in cattle. J. Dairy Sci. 201:159.

Andersson, L., A. Lundén, S. Sigurdardóttir, C. J. Davies, and L. Rask. 1988. Linkage relationships in the bovine MHC region. High recombination frequency between class II subregions. Immunogenetics 27:273.

Anonymous. 1982. Proceedings of the second international bovine lymphocyte antigens workshop. Anim. Blood Groups Biochem. Genet. 13:33.

Antczak, D. F. 1982. Structure and function of the major histocompatibility complex in domestic animals. J. Amer. Vet. Med. Assoc. 181:1030.

Aschaffenburg, R. 1968. Genetic variants of milk proteins: their breed distribution. J. Dairy Res. 35:447.

Aschaffenburg, R., and J. Drewry. 1955. Occurrence of different beta-lactoglobulins in cow's milk. Nature 176:218.

Baldwin, K. A., R. J. Baer, J. G. Parsons, S. W. Seas. K. R. Spurgeon, and G. S. Torrey. 1986. Evaluation of yield and quality of cheddar cheese manufactured from milk with added whey protein concentrate. J. Dairy Sci. 69:2543.

Batra, T. R., A. J. Lee, J. S. Gavora, and M. J. Stear. 1989. Class I alleles of the bovine major histocompatibility system and their association with economic traits. J. Dairy Sci. 72:2115.

Bech, A. -M., and K. R. Kristiansen. 1990. Milk protein polymorphism in Danish dairy cattle and the influence of genetic variants on milk yield. J. Dairy Res. 57:53.

Beever, J. E., P. D. George, R. L. Fernando, C. J. Stormont, and H. A. Lewin. 1990. Associations between genetic markers and growth and carcass traits in a paternal half-sib family on Angus cattle. J. Anim. Sci. 68:001.

Bennett, D. 1975. The T-locus of the mouse. Review. Cell 6:441.

Bensaid, A., A. Kaushal, C. Baldwin, H. Clevers, J. R. Young, S. J. Kemp, N. D. McHugh, P. G. Toye, and A. J. Teale. 1991. Identification of expressed bovine class I MHC genes at two loci and demonstration of physical linkage. Immunogenetics 33(4):247.

Bentsen, H. B., and G. Klemetsdal. 1991. The use of fixed effect models and mixed models to estimate single gene associated effects on polygenic traits. Genet. Sel. Evol. 23:407.

Bernoco, D., H. A. Lewin, L. Andersson, M. A. Arriens, S. Cwik, C. J. Davies, H. C. Hines, W. Leibold, Ø. Lie, D. Meggiolaro, H. Østergård, R. L. Spooner, A. J. Teale, J. W. Templeton, and M. Zanotti. 1991. Joint report of the fourth international Bovine Lymphocyte Antigen (BoLA) workshop. 1991. East Lansing, Michigan, USA, August 25, 1990.

Botstein, D., R. L. White, M. Skolnik, and R. W. Davies. 1980. Construction of a genetic linkage map using restriction fragment length polymorphisms. Am. J. Hum. Genet. 32:314.

Bovenhuis, H. 1992. The relevance of milk protein polymorphisms for dairy cattle breeding. Doctoral thesis, Department of Animal Breeding, Wageningen Agricultural University, P.O. Box 338, 6700 AH Wageningen, The Netherlands.

Bradford, G. E., and T. R. Famula. 1984. Evidence for a major gene for rapid postweaning growth in mice. Genet. Res. 44:293.

Bull, R. W., H. A. Lewin, M. C. Wu, K. Peterbaugh, D. Antczak, D. Bernoco, S. Cwik, L. Dam, C. Davies, R. L. Dawkins, J. H. Dufty, J. Gerlach, H. C. Hines, S. Lazary, W. Leibold, H. Leveziel, Ø. Lie, P. G. Lindberg, D. Meggiolaro, E. Meyer, R. Oliver, M. Ross, M. Simon, R. L. Spooner, M. J. Stear, A. J. Teale, and J. W. Templeton. 1989. Joint report of the third international bovine lymphocyte antigen (BoLA) workshop, Helsinki, Finland, 27 july 1986. Anim. Genet. 20:109.

Capy, P., C. Renard, P. Sellier, and M. Vaiman. 1981. Étude préliminaire des relations entre le complexe majeur d'histocompatibilité (SLA) et des caractères de production chez le pork. Ann. Genet. Sel. Anim. 13:441.

Cowan, C. M., M. R. Dentine, and T. Coyle. 1992. Chromosome substitution effects associated with κ-Casein and β-lactoglobulin in Holstein cattle. J. Dairy Sci. 75:1097.

Dentine, M. R. 1990. Using molecular biotechnology to improve the accuracy of selection. 4th World Congress on Genetics Applied to Livestock Production. July 1990. Edinburgh, Scotland. XIV:35-44.

Davies, C.J., and D. F. Antczak. 1991. Production and characterization of alloantisera specific for bovine class II major histocompatibility complex antigens. Anim. Genet. 22:417.

Dekkers, J. C. M., and M. R. Dentine. 1991. Variance associated with genetic marker in population. Theor. Appl. Genet. 81:212.

Eigel, W. N., J. E. Butler, C. A. Ernstrom, H. M. Farrel Jr., V. R. Harwalker, R. Jenness, and R. Mcl. Whitney. 1984. Nomenclature of proteins of cow's milk: fifth revision. J. Dairy Sci. 67:1599.

Ennis, P. D., A. P. Jackson, and P. Parham. 1988. Molecular cloning of bovine class I MHC cDNA. J. Immunol. 141:642.

Falconer, D. S. 1981. Introduction to quantitative genetics. 2nd edition. Longman, London.

Fellows, M., and J. Dausset. 1970. Probable haploid expression of HLA antigens on human spermatozoa. Nature 225:191.

Fernando, R. L., and M. Grossman. 1989. Marker assisted selection using best linear unbiased prediction. Genet. Sel. Evol. 21:467.

Folger, R. L., and H. C. Hines. 1976. Bovine lymphocyte antigens: serological relationships with erythrocyte and spermatozoan antigens. Anim. Blood Groups Biochem. Genet. 7:137.

Fries, R., J. S. Beckmann, M. Georges, M. Soller, and J. Womak. 1989. The bovine gene map. Anim. Genet. 20:3.

Gautschi, C., C. Gaillard, B. Schwander, and S. Lazary. 1987. Study on possible associations between the MHC and reproduction traits in swine. Anim. Genet. 18(Suppl. 1):26.

Gelderman, H. 1975. Investigations on inheritance of quantitative characters in animals by gene marker. Theor. Appl. Genet. 46:319.

Gelderman, H., V. Piper, and Roth, B. 1985. Effects of marked chromosome sections on milk performance in cattle. Theor. Appl. Genet. 70:138.

Gill, T. J. III., and H. W. Kunz. 1979. Gene complex controlling growth and fertility linked to the major histocompatibility complex in the rat. Am. J. Pathol. 96:185.

Gonyon, D. S., R. E. Mather, H. C. Hines, G. F. W. Haenlein, C. W. Arave, and S. N. Gaunt. 1987. Association of bovine blood and milk polymorphisms with lactation traits: Holsteins. J. Dairy Sci. 70:2585.

Grosclaude, F. 1988. Le polymorphisme génétique des principales lactoprotéines bovines. INRA Prod. Anim. 1:5.

Haley, C. S., Knott, S. A., and R. Thompson. 1991. Mapping quantitative trait loci in farm animals. 42nd Ann. Meeting of the EAAP, Comm: Animal Genetics, Session II, Berlin, pp. 1-8.

Han, P. F. S., and J. R. Smyth. 1972. The influence of growth rate on the development of Marek's disease in chickens. Poul. Sci. 51:975.

Hargrove, G. L., C. A. Kiddy, C. W. Young, A. G. Hunter, G. W. Trimberger, and R. E. Mather. 1980. Genetic polymorphisms of blood and milk and reproduction in Holstein cattle. J. Dairy Sci. 63:1156.

Henderson, C. R. 1988. Theoretical basis and computational methods for a number of different animal models. J. Dairy Sci. 71(Suppl. 2):1.

Hines, H. C., F. R. Allaire, and M. M. Michalak. 1986. Association of bovine lymphocyte antigens with milk fat percentage differences. J. Dairy Sci. 69:3148.

Hunziker, R. D., and T. G. Wegmann. 1986. Placental immunoregulation. CRC Crit. Rev. Immunol. 6:245.

Jeffreys, A. J., V. Wilson, and S. L. Thein. 1985. Hypervariable "minisatellite" regions in human DNA. Nature 314:67.

Joosten, I., M. F. Sanders, and E. J. Hensen. 1991. Involvement of major histocompatibility complex class I and compatibility between dam and calf in the aetiology of bovine retained placenta. Anim. Genet. 22(6):455.

Joosten, I., A. J. Teale, A. vander Poel, and E. J. Hensen. 1992. Biochemical evidence of the expression of two major histocompatibility complex class I genes on bovine peripheral blood mononuclear cells. Anim. Genet. 23(2):113.

Joosten, I., M. F. Sanders, A. vander Poel, J. L. Williams, B. G. Hepkema, and E. J. Hensen. 1989. Biochemically defined polymorphism of bovine MHC class antigens. Immunogenet. 29:213.

Jung, Y. C., M. F. Rothchild, M. P. Flanagan, L. L. Christian, and C. M. Warner. 1989. Association between restriction fragment length polymorphisms of swine leukocyte antigen class I genes with production traits of Duroc and Hampshire boars. Anim. Genet. 20:79.

Kashi, Y., E. Hallerman and M. Soller. 1990. Marker-assisted selection of candidate bulls for progeny testing programmes. Anim. Prod. 51:63.

Kennedy, B. W., M. Quinton, and J. A. M van Arendonk. 1992. Estimation of effects of single genes on quantitative traits. J. Anim. Sci. 70:2000.

Kennedy, B. W., A. M. Verrinder Gibbins, J. P. Gibson. and C. Smith. 1990. Coalescence of molecular and quantitative genetics for livestock improvement. J. Dairy Sci. 73:2619.

Klein, J. The major histocompatibility complex of the mouse. 1979. Science 203:516.

Klein, J. 1986. Natural history of the major histocompatibility complex. John Wiley & Sons Inc., New York, NY, 1986.

Klein, J., and F. Figueroa. 1981. Polymorphisms of the mouse H-2 loci. Immunol. Rev. 60:23.

Klein, J., and F. Figueroa. 1986. Evolution of the major histocompatibility complex. CRC Crit. Rev. Immunol. 6:165.

Kristensen, B., P. Wafler, and A. L. de Weck. 1982. Histocompatibility antigens (SLA) in swine. Possible correlation between SLA haplotypes and performance in piglets. Anim. Blood Groups Biochem. Genet. 13:109.

Kunz, H. W., T. J. III. Gill, B. D. Dixon, F. H. Taylor, and D. L. Greiner. 1980. Growth and reproduction complex in the rat. J. Exp. Med. 152:1506.

Lande, R., and R. Thompson. 1990. Efficiency in marker-assisted selection in the improvement of quantitative traits. Genetics 124:743.

Lundén, A. 1991. Marker genes and production traits in domestic animals. Doctoral thesis, Swedish University of Agricultural Sciences. Department of Animal Breeding and Genetics. PO Box 7023-s-750 07 UPPSALA. Sweden.

Lewin, H. A. 1989. Disease resistance and immune response genes in cattle: Strategies for their detection and evidence of their existence. J. Dairy Sci. 72:1334.

Mallard, B. A., B. W. Kennedy, and B. N. Wilkie. 1991. The effect of swine leukocyte antigen haplotype on birth and weaning weights in miniature pigs and the role of statistical analysis in this estimation. J. Anim. Sci. 69:559.

Mallard, B. A., B. N. Wilkie, B. A. Croy, B. W. Kennedy, and R. Friendship. 1987. The influence of the swine major histocompatibility complex (SLA) on reproductive traits in miniature swine. J. Reprod. Immunol. 12:201.

Mao, I. L., Buttazzoni, L. G., and R. Aleandri. 1992. Effect of polymorphic milk protein genes on milk yield and composition traits in Holstein cattle. Acta Agric. Scand., Sect. A, Animal Sci. 42:1.

Marziali, A. S., and K. F. Ng-Kwai-Hang. 1986. Relationships between milk protein polymorphisms and cheese yielding capacity. J. Dairy Sci. 69:1193.

Matoušek, J., V. Sršeň, M. Simon, P. Hořín, and F. Louda. 1989. Absence of BoLA class I antigens on bovine spermatozoa. Anim. Genet. 20:65.

McLean, D. M., E. R. B. Graham, R. W. Ponzoni, and H. A. McKenzie. 1984. Effects of milk protein genetic variants on milk yield composition. J. Dairy Res. 51:531.

McLean, D. M., and J. Schaar. 1989. Effects of β-lactoglobulin and κ-casein genetic variants and concentrations of syneresis of gels from renneted heated milk. J. Dairy Res. 56:297.

Medrano, J. F., and E. Aguilar-Cordova. 1990. Polymerase chain reaction amplification of bovine β-lactoglobulin genomic sequences and identification of genetic variants by RFLP analysis. Anim. Biotech. 1:73.

Meyer, K. 1991. Programs to estimate variance components by restricted maximum likelihood using a derivative-free algorithm. User Notes, Version 2.0, Edinburg Univ., Scotland.

Nakamura, Y., M. Leppert, P. O. O'Connell, R. Wolf, T. Holm, M. Culver, C. Martin, E. Fujimoto, M. Hoff, E. Kumlin, and R. White. 1987. Variable number of tandem repeat (VNTR) markers for human gene mapping. Science 235:1616.

Ng-Kwai-Hang, K. F., J. F. Hayes, J. E. Moxley, and H. G. Monardes. 1984. Association of genetic variants of casein and milk serum proteins with milk, fat and protein production by dairy cattle. J. Dairy Sci. 67:835.

Newman, M. J., and D. F. Antczak. 1983. Histocompatibility polymorphisms of domestic animals. Adv. Vet. Sci. Comp. Med. 27:1.

Nonneke, B. J., H. A. Lewin, and L. L. Timms. 1989. Preliminary report on BoLA polymorphisms in Guernsey cattle. J. Dairy Sci. 72:3255.

Oliver, R. A., C. M. McCoubrey, P. Millar, A. L. G. Morgan, and R. L. Spooner. 1981. A genetic study of Bovine Lymphocyte Antigens (BoLA) and their frequency in several breeds. Immunogenetics 13:127.

Østergård, H., and L. Dam. 1987. Investigation on selection forces acting on MHC in cattle during reproduction. Anim. Genet. (Suppl. 1):20.

Østergård, H., B. Kristensen, and S. Andersen. 1989. Investigation in farm animals of associations between the MHC system and disease resistance and fertility. Livestock Prod. Sci. 22:49.

Rothchild, M. F., Ch. Renard, G. Bolet, P. Dando, and M. Vaiman. 1986. Effect of SLA haplotypes on birth and weaning weights in pigs. Anim. Genet. 17:267.

Rothchild, M. F., Ch. Renard, C. Legault, and M. Vaiman. 1987. Effect of SLA haplotypes on birth and weaning weight and on deficit of homozygotes in Meishan pigs. Anim. Genet. 18(suppl. 1):33.

Rothchild, M. F., D. R. Zimmerman, R. K. Johnson, L. Vernier, and C. M. Warner. 1984. SLA haplotype differences in lines of pigs which differ in ovulation rate. Anim. Blood. Groups Biochem. Genet. 15:155.

SAS® User's Guide: Statistics, Version 5 Edition. 1985. SAS Inst., Inc., Cary, NC.

Seibert, B., G. Erhardt, and B. Senft. 1985. Procedure for simultaneous phenotyping of genetic variants in cow's milk by isoelectric focusing. Anim. Blood. Groups and Biochem. Genetics 16:183.

Sigbjørn, L., S. Kaminski, P. Aleströn, and S. Rogne. 1992. Conference on milk protein variants. 2-4 September, 1992. Hankø Fjordhotell, Norway.

Schaar, J., B. Hansson, and H. -E. Pettersson. 1985. Effects of genetic variants of κ-Casein and β-lactoglobulin on cheese making. J. Dairy Res. 52:429.

Sigurdardóttir, S., P. Mariani, M. A. M. Groenen, J. J. vander Poel, and L. Andersson. 1991. The organization and polymorphism of bovine MHC class II genes as revealed by genomic hybridization with bovine probes. Anim. Genet. 22:465.

Simpson, E., G. Bluefield, M. Brenan, W. Fizpatrick, C. Hetherington, and A. Blann. 1982. H-2 associated differences in replicated strains of mice divergently selected for body weight. Immunogenetics 15:63.

Spooner, R. L., H. Leveziel, H., F. Grosclaude, R. A. Oliver, and M. Vaiman. 1978. J. Immunogenet. 5:335.

Stear, M. J., B. A. Mallard, M. J. Newman, and B. N. Wilkie. 1989. The current status of the major histocompatibility system: Definition in cattle, goats, horses, pigs, and sheep. Int. J. Anim. Sci. 4:32.

Stear, M. J., M. J. Newman, and F. W. Nicholas. 1982. Two closely linked loci and one apparently independent locus code for bovine lymphocyte antigens. Tissue Antigens 20:289.

Stear, M. J., T. S. Pokorny, S. E. Echternkamp, and D. O. Lunstra. 1989a. The influence of the BoLA-A locus on reproduction traits in cattle. J. Immunogenet. 16:77.

Stear, M. J., T. S. Pokorny, N. E. Muggli, and R. T. Stone. 1989b. The relationships of birth weight, preweaning gain and postweaning gain with the bovine major histocompatibility system. J. Anim. Sci. 67: 641.

Vaiman, M., M. Fellows, J. Wiels, C. Renard, J. Lecointre, F. du Mesnil du Buisson, and J. Dusset. 1978. Presence of SLA and Ia-like antigen on boar spermatozoa. J. Immunogenet. 5:135.

Threadgill, D. W., and J. E. Womack. 1990. Genomic analysis of the major bovine milk protein genes. Nucleic Acid Res. 18:6935.

Warner, C. M., D. L. Meeker, and M. F. Rothschild. 1987. Genetic control of immune responsiveness: A review of its use as a tool for selection for disease resistance. J. Anim. Sci. 64:394.

Weigel, K. A., A. E. Freeman, M. E. Kehrli Jr., M. J. Stear, and D. H. Kelley. 1990.Association of class I bovine lymphocyte antigen complex alleles with health and production traits in dairy cattle. J. Dairy Sci. 73:2538.

Yamazaki, K., E. A. Boyse, V. Miké, H. T. Thailer, B. J. Mathieson, J. Abbott, J. Boyse, Z. A. Zayas, and L. Thomas. 1976. Control of mating preferences in mice by genes in the major histocompatibility complex. J. Exp. Med. 144:1324.

Zhang, W. and C. Smith. 1992. Computer simulation of marker-assisted selection utilizing linkage disequilibrium. Theor. Appl. Genet. 83:813.