The genetics of certain protein components in milk from beef cows and their effect on calf production
by Arlo Bryan Weston

A thesis submitted to the Graduate Faculty in partial fulfillment of the requirements for the degree of
DOCTOR OF PHILOSOPHY in Genetics
Montana State University

Abstract:

A study of milk protein components in 471 beef cows was made to determine the frequencies of various alleles controlling variation in caseins in the milk, to study Hardy-Weinberg equilibrium of zygotic frequencies, and to investigate the effect of milk protein genotype on weaning weight, 180-day gain and 180-day weaning weight. Milk from straightbred Hereford, inbred lines and linecross Hereford, Angus and Charolais cattle was studied along with milk from all possible crosses between these three breeds. In addition, Hereford X Brown Swiss, Angus X Brown Swiss, and Charolais X Brown Swiss crossbred cowl's milk was studied. One hundred eighty-one cows were sampled twice in different years to check casein typing techniques.

Milk casein was resolved into its component parts by starch-gel electrophoresis. Genotypic frequencies were computed from the phenotypes manifested in the gels. The αs1-casein B allele, β-casein A allele and the κ-casein A allele occurred in frequencies of 0.80-1.00; 0.72-1.00; 0.56-0.74, respectively, in the various sub-groups of cattle.

The Hardy-Weinberg zygotic equilibrium analysis of neither the αs1- nor β-casein genotypes indicated significant deviation from the expected. However, in analyses of equilibrium of the κ-casein genotypes there were significant deviations from the expected in four out of seven populations. All genotypes of κ-casein were shown to display a common pattern of deviation from the expected; the homozygotes were always less than expected and the heterozyotes always exceeded the expected. It was suggested that this was caused by greater fitness of the heterozygotes.

Although some significant effects of protein genotype on weaning traits were obtained in least squares analyses, the lack of consistent results suggest that these alleles do not contribute substantially to variation in the weaning traits studied.

THE GENETICS OF CERTAIN PROTEIN COMPONENTS IN MILK FROM BEEF COWS AND THEIR EFFECT ON CALF PRODUCTION

by

ARLO BRYAN WESTON

A thesis submitted to the Graduate Faculty in partial
fulfillment of the requirements for the degree

of

DOCTOR OF PHILOSOPHY

in

Genetics

Approved:

P D Skaar
Head, Major Department

Dr R. L. Blackwell
Chairman, Examining Committee By J. L. Van Horn

Henry L. Parsons
Graduate Dean

MONTANA STATE UNIVERSITY
Bozeman, Montana

August, 1971

ACKNOWLEDGEMENT

The writer would like to express particular appreciation to Dr. R. L. Blackwell, Dr. S. R. Chapman, and Dr. A. M. El-Negoumy for their encouragement, interest, and help during the course of this study. Special thanks are due Dr. El-Negoumy for making his laboratory and facilities available for the typing of the milk proteins. The encouragement and suggestions of the other members of the graduate committee, Professor A. E. Flower, Dr. P. D. Skaar, Dr. E. P. Smith, and Dr. R. Thibeault were greatly appreciated.

Gratitude is expressed to Dr. E. P. Smith, Roger V. Stewart, Dr. D. D. Kress and Dr. P. J. Burfening for their help and suggestions with the statistical analyses in this study.

The author expresses his gratitude to Dr. S. R. Chapman and Donald H. Fritts for help with the photography of equipment and starch-gels.

Sincere appreciation is expressed to the staff of the U. S. Range Livestock Experiment Station for their cooperation and help during the course of this study. Mr. B. W. Knapp's assistance in obtaining weaning data for the Miles City cattle was most helpful.

Special thanks is expressed to Mrs. Frankie Larson for her conscientious type of the manuscript. Also gratitude is extended to M. Richard Casper and to his wife for help with typing.

The author expresses his special appreciation to his wife, Joanne.

Her support and encouragement during the study and in preparing the manuscript has helped to keep purpose in the work and make it an enjoyable experience. Without her support and the support of the rest of the family this work would have been much more difficult.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

A study of milk protein components in 471 beef cows was made to determine the frequencies of various alleles controlling variation in caseins in the milk, to study Hardy-Weinberg equilibrium of zygotic frequencies, and to investigate the effect of milk protein genotype on weaning weight, 180-day gain and 180-day weaning weight. Milk from straightbred Hereford, inbred lines and linecross Hereford, Angus and Charolais cattle was studied along with milk from all possible crosses between these three breeds. In addition, Hereford X Brown Swiss, Angus X Brown Swiss, and Charolais X Brown Swiss crossbred cow's milk was studied. One hundred eighty-one cows were sampled twice in different years to check casein typing techniques.

Milk casein was resolved into its component parts by starch-gel electrophoresis. Genotypic frequencies were computed from the phenotypes manifested in the gels. The αs_1-casein B allele, β-casein A allele and the κ-casein A allele occurred in frequencies of 0.80-1.00; 0.72-1.00; 0.56-0.74, respectively, in the various sub-groups of cattle.

The Hardy-Weinberg zygotic equilibrium analysis of neither the αs_1- nor β-casein genotypes indicated significant deviation from the expected. However, in analyses of equilibrium of the κ-casein genotypes there were significant deviations from the expected in four out of seven populations. All genotypes of κ-casein were shown to display a common pattern of deviation from the expected; the homozygotes were always less than expected and the heterozyotes always exceeded the expected. It was suggested that this was caused by greater fitness of the heterozygotes.

Although some significant effects of protein genotype on weaning traits were obtained in least squares analyses, the lack of consistent results suggest that these alleles do not contribute substantially to variation in the weaning traits studied.

INTRODUCTION

The presence of variation in an allelic series of genes controlling certain protein components in milk from dairy cattle has been established. Improved techniques for the resolution of the milk protein genotypes have facilitated the typing of large numbers of cows and the computing of allelic frequencies.

Little genetic information is available in the literature concerning the situation with respect to milk proteins in beef cattle. The present study was undertaken with the objective of studying some genetic and physiological relationships of the milk protein alleles in certain beef breeds. Since milk is important to the beef industry as a vital source of nourishment for the young, the possibility exists of an important relationship between milk protein genotype and weaning triats of the calf.

This report deals with the zygotic frequencies of αs_1-, β-, and κ-casein alleles, with attempts to fit their frequencies to Hardy-Weinberg equilibriums to determine if any genotypes possess superiority. It is also concerned with possible effects of casein genotypes on weaning characteristics in beef breeds and breed crosses.

REVIEW OF LITERATURE

From earliest recorded history, milk has been important to man. Some have claimed milk to be "nature's most nearly perfect food", while others have merely stated that milk is "an almost complete natural food" (McKenzie, 1967). Milk has been important directly as a food source for mankind and equally as important in nourishing the young of other animals which have subsequently furnished food, energy, etc., to man. This review will attempt to bring together a part of the total story of milk -- that part related to cow's milk, variations within various milks and their effect on selected production traits. It will refer to evidence in the literature of inheritance of these variations in milk, the biochemical basis for this variation, and finally some practical applications of this knowledge in the beef cattle industry.

I. COMPOSITION OF MILK

The composition of milk can be highly variable, depending upon time of lactation, nutritional level of the mother at the time of sampling, breed sampled, and species considered. For the purposes of this review "normal" milk is considered to be that milk which can be obtained anytime during lactation after the brief colostral period following parturition. Following the colostral period, the milk continues to change somewhat in quantity during lactation, but with little change in protein content. The greatest variables are the fat and carboydrate portions of the milk.

A. General Composition

McKenzie (1967) gives the following as normal constituents of milk: Water, proteins, enzymes, lipids, carboydrates, minerals, vitamins, non-protein nitrogen, phosphate esters, and some trace elements. The average percentage composition of these components varies widely from species to species and to a lesser extent within species. The actual relative composition of milk in a species seems to bear a direct relationship to the rate of growth of the offspring. Webb and Johnson (1965) have summarized some of the relationships (Table I). The results generally indicate that the more concentrated the milk constituents, the faster the rate of growth possible.

While the variation among species has an obvious relationship to growth of the young, there is still much variation which is unaccounted for within species. The average gross composition of bovine milk according to Watt and Merrill (1963) is given as follows: Water 87%, fat 3.9%, lactose 4.9%, proteins 3.5%, and ash (minerals) 0.7%. However, the fat content varies from 3.0% to 5.5% just within the dairy breeds, and the protein content varies from 3.3 to 3.9% according to Webb and Johnson (1965).

Lush (1960) and Comberg, Groning and Meyer (1967) report that the percent composition figures are gradually changing because of selection. The dairy industry has long considered fat content of milk to be the single most economically important component. Thus selection for increased production of fat in the milk has resulted in higher amounts of fat as well as other related constiuents. Particularly, the protein content has increased with increased fat production. Von Krosigk, Young, and Richardson (1960) showed a positive genetic correlation between fat and protein content ($r=.62$) of milk when estimated among breeds. This compares with a somewhat lower genetic correlation ($r=.42-.43$) when measured without respect to breed (Legates, 1960; Lush, 1960). Therefore, selecting animals for increased fat content in milk should produce a concomitant increase in protein content. This may turn out to be very fortunate for the industry. With increased attention being focused on good sources of protein in the world today and a concurrent reduced demand for animal fat, protein may become the economic basis of value in milk (see Lebaron, 1970).

B. Composition of Milk with Respect to Proteins

Bouchard and Brisson (1969) suggest that after the first week of Lactation the components making up total protein remain fairly constant throughout the rest of the lactation period. Frandsen (1958) gives the following percentages of components of cow's milk proteins: Casein 86% and whey proteins 14% of the total protein. A further breakdown, though still somewhat general, is given by Jenness *et al*. (1956) and by Brunner *et al*. (1960), and is summarized in Table II. Since this work was done, much research has shown a further subdivision of the milk proteins. These subdivisions will be considered later in this review.

C. Influence of Various "Milks" on the Young

Ramshaw and Dunstone (1969) have commented that milk is one of the most complete protein sources available from a nutritional standpoint.

TABLE I. COMPOSITION OF MAMMALS' MILK AS RELATED TO THE RATE OF GROWTH OF THE YOUNG MAMMAL (Webb and Johnson, 1965)

Species	Days Required to Double Birth Weight	Percent of Constituent in Milk			
		Protein	Ash	Lime	Phosphoric Acid
Man	180	1.60	0.20	0.033	0.457
Horse	60	2.00	0.40	0.124	0.131
Cow	47	3.50	0.70	0.160	0.197
Goat	22	3.67	0.77	0.197	0.284
Sheep	15	4.88	0.84	0.245	2.293
Pig	14	5.21	0.81	0.249	0.308
Cat	9.5	7.00	1.02	...	...
Dog	9	7.44	1.33	0.454	0.508
Rabbit	6	10.38	2.50	0.891	0.997

TABLE II. COMPOSITION OF COW'S MILK PROTEINS (Jenness *et al*. 1956 and Brunner et al. 1960)

Protein Component	Percent of Protein in Skimmilk	Distinctive Characteristics
Casein*		
α-casein	45-63	1% Phosphorus
β-casein	19-28	0.6% Phosphorus
γ-casein	3-7	0.11% Phosphorus
Total	76-86	
Whey Proteins		
β-lactoglobulin	7-12	1.1% Cysteine
α-lactalbumin	2.5	7.0% Tryptophan
Blood serum albumins	0.7-1.3	Identical to blood albumin
Immunoglobins	1.4-3.1	Carries antibodies especially high concentration found in colostrum milk

* Precipitated from skimmilk ab pH 4.6

This is an important factor since the milk provides the main source of nourishment for the offspring of livestock. In his review of milk proteins, McKenzie (1967), suggested that the caseins in milk resemble denatured proteins with respect to their behavior in solution. This property is significant inasmuch as the disfigured or random coil configuration of the casein makes it particularly susceptible to digestion.

Some research has been reported with respect to the influence of both quantity and quality of milk on livestock young. The basic finding of Whiting, Slen, and Bezeau (1952) was that the level of protein in the mother's diet influenced both the quantity and quality of her milk. Essentially their findings showed that adequate dietary protein was needed for normal protein levels to occur in the female's milk. Coccodrilli, Chandler, and Polan (1970) investigated the relationship between quality of dietary protein and calf weight gains. Their results indicated that the quality of protein in the diet of the mothers was far more important than quantity. Butterworth _et_ _al_. (1968) reported their observations on lactation in ewes and the effect upon lambs. They studied planes of nutrition of the mother and subsequent effect upon the young. They found that there was a high correlation (r=.71-.84) between amount and quality of feed given to the mother and the growth of the offspring. Tagari (1969) was able to show some basis for differences in production efficiency in sheep when he compared two protein sources, soybean meal and alfalfa hay. He found that the alfalfa had more free amino acids which were readily usable in protein synthesis even though the total amino acid content of the two feeds was similar.

While there are no known reports in the literature on the relationship between various milks and their effect on calf gains, there has been some rsearch done relating slight changes in protein quality and their effects on calf gains. Tagari and Roy (1969) heated milk to various temperatures and then fed the treated milk to calves. They were able to show that heated milk (which contained some denatured protein), caused detrimental effects on the calves in three ways:

1. Weight gains were reduced by as much as 30% during the first three weeks of life.
2. Infection of calves increased upon exposure to disease.
3. Incidence of diarrhea and calf mortality increased once infection increased.

They were able to restore calves to normal by adding undenatured whey proteins to the calves' dietary milk. Earlier research by Kannan and Jenness (1961) had shown that when whey proteins are denatured the coagulation of casein is inhibited. It seems clear then, that there is a very sensitive relationship between the milk proteins within the milk and the value of milk as a source of nourishment for the young.

The one area which seems related but on which there is no known research reported is that of the relationship between calf production and variations which occur in nature within the milk. It would seem that one basis of variation in milk which occurs among species and among breeds might be the different needs of the young within their different natural environments. Some further review of literature relating to this line of reasoning will be presented in the next section.

II. CASEIN IN COWS' MILK

A. Historical Development

The development of knowledge of the heritable components of caseins in milk has come about almost wholly within the past 15 years. Waugh and von Hippel (1955) showed that the casein micelle could be subdivided into several components. These included β-casein, α-casein, κ-casein, (the κ-casein being the component upon which rennet acts immediately), and γ-casein. The remaining α-casein after the κ-casein had been removed soon came to be known as the calcium sensitive or α_s-casein.

Aschaffenburg and Drewry (1955) paved the way for extensive rsearch when they reported finding variation in part of the whey protein fraction, the β-lactoglobulin. Using paper electrophoresis, they were able to deomonstrate two variants which they designated β-Lg A and β-Lg B. Later, they were able to show that the β-Lg A and β-Lg B variants are controlled by two autosomal alleles

(Aschaffenburg and Drewry 1957). Many previous workers had suggested that the components of milk protein were made up of several subunits, but genetic polymorphism among milk samples was a new idea. Blumberg and Tombs (1958), found evidence in Zebu cattle for further genetic polymorphism in the variation of the second most abundant whey protein, α-lactalbumin, in an A and B form.

Aschaffenburg (1961), using paper electophoresis with urea, reported the finding of five different variants in the β-casein component. These he designated β-casein A/A, A/B, A/C, B/B, and B/C. He found no cows with type C/C. This was the first indication of individual variation in the caseins of cows' milk. His work was confirmed by Thompson _et al_. (1962), who used the technique of polyacrylamide and starch-gel electrophoresis at an alkaline pH. Not only did they confirm the work of Aschaffenburg in the variation of β-casein, but they also showed that the α_s-casein was polymorphic. They designated three forms of the component, α_s-casein A, B, and C, with accompanying heterozygote combinations being found, i.e. A/B, A/C, and B/C.

Almost immediately following the work cited above, evidence of further genetic polymorphism came from several workers independently, yet all on variation in the κ-casein component. Neelin (1964), Schmidt (1964) and Voychik (1964), all reported two variants controlled at one automosmal locus in κ-casein, A and B along with the heterozygote A/B.

Aschaffenburg (1963) followed up his previous work with an investigation of the frequency of alleles determining the β-casein system in cows' milk. He confirmed his own previous work and that of others in finding that the variants, A, B, and C always occurred singly or in pairs, but never more than two in any cow. He found the A allele to be the most common among all the five dairy breeds in his study, with the Ayrshires and Shorthorns producing no other variant. The technique he used to resolve these proteins was paper electrophoresis which gave adequate resolution of the β-casein, but was inadequate for the typing of κ-casein. Kiddy et al. (1963) provided evidence for the genetic polymorphism of the caseins in typing 958 cows for both the α_s-casein and β-casein components.

This work was followed by a series of review articles and experiments verifying and reviewing the progress to date in the genetics of some of the casein components in cow's milk. Kiddy and Johnston (1964) summarized the information to date confirming genetic control of the α_s-casein variants. Thompson et al. (1964) summarized the work on β-casein which also confirmed the prevailing theories of genetic control of the polymorphism. Thompson and Kiddy (1964) brought together the available information regarding isolation and biochemical properties of the α_s-casein variants. They also proposed at this time that the α_s-casein designation be changed to α_{s1}-casein to differentiate it from the other α_s-caseins and to specify the α_s-casein component containing the A, B, C variants, which were the only ones known at that time. This proposal was accepted and is currently used.

While, from the reviews, it may seem that a plateau had been reached, much new information was added during the next few years. Gossclaude et al. (1965) gave evidence for an additional variant in the α_{s1}-casein group. They designated this α_{s1}-casein D and it became the fourth variant of the α-casein. Their work was followed closely by the report of El-Negoumy (1967) and Groves and Kiddy (1968) of genetic variation in the γ-casein fraction. In three of the γ-casein fractions, variants designated A and B, were found while no variation was found in other fractions.

During the same period of time Kiddy, Peterson, and Kopfler, (1966) demonstrated further breakdown within the β-casein A variant. They were able to subdivide it into three fractions with electrophoresis at an acid pH. These fractions were designated β-casein A^1, A^2, and A^3. This was the first report of genetic variants found by means of anything but alkaline pH electrophoresis. Then in a report by Aschaffenburg, Sen, and Thompson (1968) two new variations of β-casein were added to the list, designated B_z and D. The β-casein B_z had a mobility similar to β-casein B and was discovered by means of "fingerprints" of chymotryptic peptides. Both new variants appear to be quite rare in occurrence.

The above information is summarized in Table III. These data

TABLE III. THE GENETIC VARIANTS OF MILK PROTEINS*

Protein Constituent	Variant	Year	Reference and Year
α_{s1}-casein	A B C	1962	Thompson *et al.* (1962)
	D	1966	Groschaude *et al.* (1966)
β-casein	A	1961	Aschaffenburg (1961) Comprising A^1, A^2 and A^3
	A^1 A^2 A^3	1966	Kiddy *et al.* (1966)
	B	1961	Aschaffenburg (1961)
	B_z	1968	Aschaffenburg *et al.* (1968)
	C	1961	Aschaffenburg (1961)
	D	1968	Aschaffenburg *et al.* (1968)
γ-casein	A B	1967	El-Negoumy (1967) Groves (1967)
κ-casein	A B	1964	Neelin (1964) Schmidt (1964) Woychik (1964)

---

* Adapted from Aschaffenburg (1968)

are not considered to be complete by any means. Since its complilation, Annan and Manson (1969) have isolated a new fraction from α_s-casein which they designated $_{so}$-casein. This appeared similar to α_{s_1}-casein. They also accomplished further fractionation of the whole α_s-casein, isolating at least three ($\alpha_{s_{2,3,4}}$-caseins) components. Further research to verify these data and clarify some questions as to whether these are true subdivisions of the protein or artifacts should be done.

It should be noted that the last two discoveries cited were made by varying the experimental methods. First in varying the pH of the starch-gel electrophoresis (Kiddy, Peterson, and Kopfler,1966), and second by means of a new technique with milk proteins, that of digestion by chymotrypsin and then peptide fingerprinting (Aschaffenburg *et al*. 1968). Aschaffenburg (1968) suggests that from this point in milk protein research progress might be slow because it will be dependent upon the perfection of new methods of looking at the milk proteins which will permit us to examine them in their natural state. Since the proteins are quite easily denatured, care must be taken to avoid studying fractions which are not true subdivisions of the component but only artifacts of the method of preparation.

B. Biochemical Basis for Variation in Casein Type

As better biochemical methods have become available, the biochemical basis for variation in the milk caseins has been determined. A brief summary of this basis, given by casein type follows:

1. α_{s_1}-Casein

In all populations studied so far, the A allele of α_{s_1}-casein seems to be far more rare than either the B or C alleles. It has been reported in only one family line of Holsteins in the United States (Kiddy and Johnston, 1964) and in one group of Danish cattle (Kiddy, Peterson, and Köpfler, 1966). This is to be expected from the experimental work of Kalan, Greenberg, and Thompson (1966), in which they did an amino acid analysis of the variants of α_{s_1}-casein. Their work indicated that the B and C alleles differ by a single amino acid - a glutamic acid residue in the B variant being replaced by a glycine residue in the C variant. However, the A type in α_{s_1}-casein seemed to be lacking one or two major peptides contained in the B and C types. Except for these missing peptides, the A amino acid sequences seemed to be identical to the B and C sequences. This work has been confirmed by Thompson and Gordon (1968). Aschaffenburg (1968) suggests that the A type may have arisen by a fairly recent mutation, judging from the limited distribution of that type.

2. β-Casein

Although some information is available on the variants which were isolated from the A type (A^1, A^2, and A^3), this paper will not attempt to summarize it. The information is still incomplete, few breeds have been typed with acid pH electrophoresis, and amino acid analysis of these variants is unfinished.

The A type has been the most frequent in occurrence in most of the breeds typed; the variation in all western breeds is predominently the A and B types. The C type is relatively rare in most breeds typed. The amino acid sequence in β-casein are unavailable in the literature, but most researchers suggest only one or two residues difference between the three types.

3. κ-Casein

From the appearances of number of types in the κ-casein, it should be the most simple of the caseins to study. However, it has proved to be the most elusive of all caseins typed to date. Part of the reason for the problem may be the fact that the κ-casein plays a complex role in stabilizing the casein micelle, and it also breaks and forms disulfide bonds easily. There are few reports of κ-casein frequencies in the literature.

In the reports, the A allele seems to predominate except in the Jersey breed where the results are conflicing. However, both the A and B variants have been present in all breeds typed. In the work of Woychik, Kalan, and Noelken (1966), and also that of Schmidt, Both and deKoning (1966), the κ-casein A type was shown to contain one more residue each of aspartic acid and threonine than the B type. The B variant contained one residue each of alanine and isoleucine more than the A variant.

III. GENE FREQUENCIES AND INHERITANCE OF CASEIN COMPONENTS

Much of the early genetic work in milk proteins developed concurrently with the basic information reviewed above. It has been established by the various workers cited that the variations in α_{s1}, β-, and κ-casein which were summarized in Table III, are each controlled by an autosomal locus with co-dominant alleles. (Aschaffenburg, 1961; Thompson *et al*. 1964; Kiddy *et al*. 1964; Kiddy *et al*.1963). The allele frequencies from the literature are summarized in Table IV.

The gene frequencies in Table IV illustrate several interesting points. One is the differences in frequency of the alleles between breeds. Some very divergent selection or random genetic drift seems to have taken place over the years to separate, for instance, the α_{s1}-casein frequences in the Hereford and Brahman cattle. There are no known reports in the literature of what contribution these different alleles might be making to fitness with respect to the environments in which they are found. The frequencies of the Santa Gertrudis alleles appear to reflect their recent origin from the Brahman and Shorthorn breeds. There are also some interesting differences between herds within a breed as evidenced by the protein typing of Woychik (1965) and Corradini (1969) in Jerseys with respect to κ-casein. However, whenever animals are maintained in separate populations the allelic frequencies may be expected to differ.

TABLE IV. GENE FREQUENCIES OF CASEINS IN SELECTED BREEDS*

		α_{s_1}-			β-			κ-		
Breed	No. Tested	A	B	C	A	B	C	A	B	Reference and Year
Jersey	67	0	0.81	0.19	0.64	0.36	0	-	-	Thompson *et al.* (1964)
	48	-	-	-	-	-	-	0.10	0.90	Woychik (1965)
	142	0	0.93	0.07	0.71	0.29	0	0.52	0.48	Corradini (1969)
Guernsey	400	0	0.70	0.30	0.98	0.01	0.02	-	-	Thompson *et al.* (1964)
	47	-	-	-	-	-	-	0.74	0.26	Woychik (1965)
Holstein	542	0.05	0.87	0.08	0.98	0.02	0	-	-	Thompson *et al.* (1964)
	138	-	-	-	-	-	-	0.85	0.15	Woychik (1965)
	173	0	0.98	0.02	0.37	0.63	0	0.79	0.21	Arave *et al.* (1971)
Shorthorn	115	0	0.99	0.01	0.97	0.03	0	-	-	Aschaffenburg (1968)
Brown Swiss	23	-	-	-	0.80	0.18	0.02	-	-	Kiddy *et al.* (1966)
	203	0	0.97	0.03	0.79	0.19	0.02	-	-	Thompson *et al.* (1964)
Hereford	48	0	0.98	0.02	0.75	0.25	0	-	-	Caldwell *et al.* (1971)
Angus	77	0	0.84	0.16	0.95	0.05	0	-	-	Caldwell *et al.* (1971)
Brahman	59	0	0.03	0.97	0.99	0.01	0	-	-	Caldwell *et al.* (1971)
Charolais	10	0	0.80	0.20	0.90	0.10	0	-	-	Caldwell *et al.* (1971)
Santa Gertrudis	24	0	0.54	0.46	1.00	0	0	-	-	Caldwell *et al.* (1971)

* Adapted from Aschaffenburg (1968), Caldwell, Weseli and Cartwright (1971), Corradini (1969) and Arave, Lamb and Hines (1971).

There is relatively little information available on frequencies of κ-casein. Many researchers have found that special care must be taken in electrophoresis to resolve the κ-casein into its component parts. Several of the reports in the literature have noted that it was intended to study κ-casein as well as the other caseins but resolution of these components was too inconsistent to report. The only report known at this time on beef cattle casein frequencies is one by Caldwell, Weseli, and Cartwright (1971). Horvath (1970) in studying casein frequencies of the Simmental breed from Europe, reported that the frequency of the β-casein C allele was 0.42. This is much higher than any other breed so far reported.

Further evidence regarding the inheritance of the variants was given by Grosclaude _et_ _al_. (1964). They typed several daughter-dam pairs and found no recombination in inheritance. Therefore, they postulated that the loci conditioning the α_{s_1}- and β-casein variants are located on the same chromosome in close proximity to each other. They (Groselaude _et_ _al_. 1965) continued their initial research with further daughter-dam comparisons which suggested that the genetic locus responsible for κ-casein variation is located on the same chromosome as the α_{s_1}- and β-casein alleles. The fact that they found no recombinants between any of these genes gave further verification to the theory that they are all placed closely together on the chromosome. Groves and Kiddy (1968) reported a close association also between the β- and γ-casein genes. In fact, recent evidence (Hines et al. 1969) indicates that at least some of the β- and γ-casein genes are common to both systems. Support for this view is the finding of Groves and Kiddy (1970) that the amino acid substitutions which differentiate the β-caseins also differentiate the β-caseins.

It would seem apparent that the story of the inheritance of milk proteins is far from complete with many exciting chapters yet to be written. However, that work which has been compiled has done much to give us a better vision of the inheritance of discrete factors influencing milk, both in the dairy and beef breeds.

IV. INFLUENCE OF MILK PROTEIN GENES ON PRODUCTION TRAITS

There have been few studies attempting to relate milk protein with production traits. Perhaps because the genetic effects are so small when compared to environmental effects in most production traits, researchers generally have ignored this area of research. There are a few exceptions, however.

Hoogendoorn et al. (1969) have reported on studies involving some genetic loci and production traits in dairy cattle. They found significant relationships between α_{s1}-casein genotypes and percent protein in Jersey milk ($P < 0.05$) between β-casein genotypes and percent lactose for Jerseys ($P < 0.05$) and between β-casein types and percent fat ($P < 0.01$) and percent protein ($P < 0.025$) in Milking Shorthorns. Their analysis of variance also showed a significant relationship between κ-casein genotype and percent protein in Jerseys ($P < 0.05$). They found no consistently superior genotype with a significant effect on percent protein over all breeds. One problem in their study may have been the small number of observations in some of their comparisons. Arave et al. (1971) studied the relationship between milk protein polymorphism and production traits. They found no significant contribution of milk protein genotype to production.

The literature shows that research on this aspect of milk proteins has been done entirely with dairy cattle and in measuring the effect of milk protein genotype on the percentage of constituents in the milk. Information is lacking on the effect of different milk protein genotypes on calf production for situations where the calf is raised on the mother's milk.

MATERIALS AND EXPERIMENTAL PROCEDURE

I. ANIMALS SAMPLED

The milk samples typed in this study were obtained from beef cows at the U. S. Range Livestock Experiment Station at Miles City, Montana. The breeds and breed crosses as well as the total number sampled in each group are given in Table V.

Of the 471 animals sampled, 181 cows were sampled in both 1966 and 1967. Also, in 1970, 97 milk samples were obtained from cows which were daughters of previously sampled cows. Seventy-three of these cows were three- and four-way line and breed crosses. A total of 649 milk samples were typed in this study.

The inbred and linecross Herefords listed in Table V are listed separately from the non-linebred Herefords. Brinks *et al*. (1967) gives the average inbreeding of these lines (designated as 1, 4, 6, 9, 10) to be 20-35% as of 1962. The linebred group of this study included the above lines as well as all possible crosses and reciprocal crosses between lines. The non-linebred Herefords came from the grade herd maintained at the U. S. Range Livestock Experiment Station at Miles City, Montana (Pahnish *et al*. 1969). These were the Herefords used in the breed crosses studied. The Angus, Charolais, and Brown Swiss cows sampled in this experiment were purchased prior to a crossbreeding study initiated in 1961 at the station. The Angus and Charolais represent rather divergent breeding. The Charolais females used in this experimental breeding herd were about three-fourths to seven-eighths

TABLE V. NUMBER OF COWS SAMPLED AND CLASSIFICATION ACCORDING TO BREED OR BREED CROSS

Breed or Cross Sire	 X	 Dam	Total Cows Sampled
Hereford	X	Hereford[a]	161
Hereford	X	Hereford	26
Angus	X	Angus	30
Charolais	X	Charolais	34
Hereford	X	Angus	19
Angus	X	Hereford	22
Angus	X	Charolais	17
Charolais	X	Angus	27
Hereford	X	Charolais	17
Charolais	X	Hereford	18
Hereford	X	Brown Swiss	10
Angus	X	Brown Swiss	9
Charolais	X	Brown Swiss	8
Three- and four-way breed crosses			73
		Total	471

[a] Inbred lines and linecrosses

Charolais with Hereford and a limited amount of Brahman making up the remainder (Pahnish et al. 1969). Additional background information on the animals in this experiment is given elsewhere: Flower et al. 1963; Brinks et al. 1967; Urick et al. 1966; and Urick, 1968.

II. SAMPLING PROCEDURES

A. Obtaining Milk Samples

Milk samples were obtained in the summer of 1966, 1967, and the spring of 1970. The first year (1966) the milk was obtained after withholding calves from the cows for a few hours before milking. Some difficulty was experienced in obtaining milk from some of the cows. In each subsequent year the milk samples were obtained following the injection of 1/2 cc of oxytocin into the jugular vein of the cow. No problems were experienced obtaining the milk after this treatment. Milk was collected in plastic bags identified with the cow number.

B. Handling of Milk Samples

Following collection, the samples were frozen either by dry ice or by being placed in a freezer at the experiment station. The samples were transported while frozen to Bozeman, Montana, and kept frozen (-10°C) until the caseins were recovered from the milk for typing in the laboratory.

III. TYPING OF MILK PROTEINS

A. Preparation of the Casein

The casein was recovered by the technique of El-Negoumy (1966). The frozen samples were thawed and then warmed in a warm water bath to 40° C. The milk sample (10 ml) was then mixed with an equal volume of pH 4.6 acetate buffer (made from an equal mixture (V/V) of sodium acetate and acetic acid, 1M) in a 25 ml centrifuge tube. The casein precipitated immediately. The mixture was then centrifuged in an International Centrifuge No. 2 at 3000 X g for 7 minutes. The casein settled to the bottom of the tube, and the whey and fat were poured off. The casein was washed twice with distilled water, adjusted to pH 4.6 with HCL, and centrifuged for 5 minutes, each time at 3000 X g. The washed casein was dissolved in solid urea and distilled water to bring the resulting mixture to a 4.6% casein solution (about 10 ml).

The solution was then absorbed on 1.5 cm wide strips of Whatman No. 3 filter paper (each identified with the cow number from which the sample had been taken) with excess solution removed from the filter paper strips by blotting. The strips were then dried and stored without significant change in electrophoretic results until they could be electrophorized (El-Negoumy, 1966).

B. Starch-Gel Preparation

The starch-gel was prepared by El-Negoumy's technique (1966) who modified the procedure of Wake and Baldwin (1961). Table VI lists the composition of the gel.

TABLE VI. COMPOSITION OF THE STARCH-UREA GEL

Gel Components	Quantities
Starch	53 gm [a]
Deionized distilled water	228 ml [b]
Tris-citrate buffer	60 ml
Urea	126 gm
2-Mercaptoethanol	1.7 ml

[a] Commercial Hydrolyzed Starch was used (Connaught Laboratories, Canada).

[b] Depending upon the starch obtained, the amount of water in the gel varied from 228 to 240 ml.

This tris-citrate buffer, water, and starch were mixed together until the starch was suspended freely in the solution. The mixture was then slowly heated to a full boil over a medium flame while being shaken to keep the mixture as homogeneous as possible. It was then removed from the heat and the urea added immediately. The entire mixture was swirled until the urea dissolved. The gel solution was then placed under a vacuum using a water pump to remove all air bubbles. The resulting semi-clear and fairly viscous mixture was cooled by immersing the container in a water bath at about 25-30° C while swirling intermittently. When the gel reached a temperature of 25-30° C, the 2-mercaptoethanol was added and completely mixed into the gel. The gel was then poured into the mold (the electrophoresis chamber). The gel was left at room temperature for at least 3 hours and then placed in a cold room for 8-12 hours at 2-3° C before using. Gel thickness which gave the best resolution was 5 mm.

C. Electrophoresis Equipment and Procedure

Electrophoresis of the casein was performed in two different migration chambers (Figure 1). The most frequently used chamber and the preferred one, was a locally-constructed unit designed by El-Negoumy and Via (1966). It yielded better resolution than the second unit which was a horizontal unit (Research Specialties Co.). However satisfactory resolution was obtained with both chambers. The advantages of the locally constructed unit are summarized in the reference just cited.

The electrode chambers of each unit were filled with appropriate amounts of sodium borate buffer which had a pH of approximately 8.9. The sample strips (cut to 0.5 cm X 1 cm) were inserted into the cooled gels, and the electrophoresis was done in a cold room (2-3° C). It was found that the electrical power which produced the most consistent results was 20-25 milliamps. Electrophoresis then required 36-48 hours to completion.

D. Staining, Washing, and Reading the Casein Types

Following electrophoresis, the gel was stained with Nigrosin dye (National Aniline), a protein dye, prepared after the manner of Smithies (1959). The dye was dissolved in methanol, water, acetic acid (50:50:10 by volume), and placed on the gel surface for 7-12 minutes.

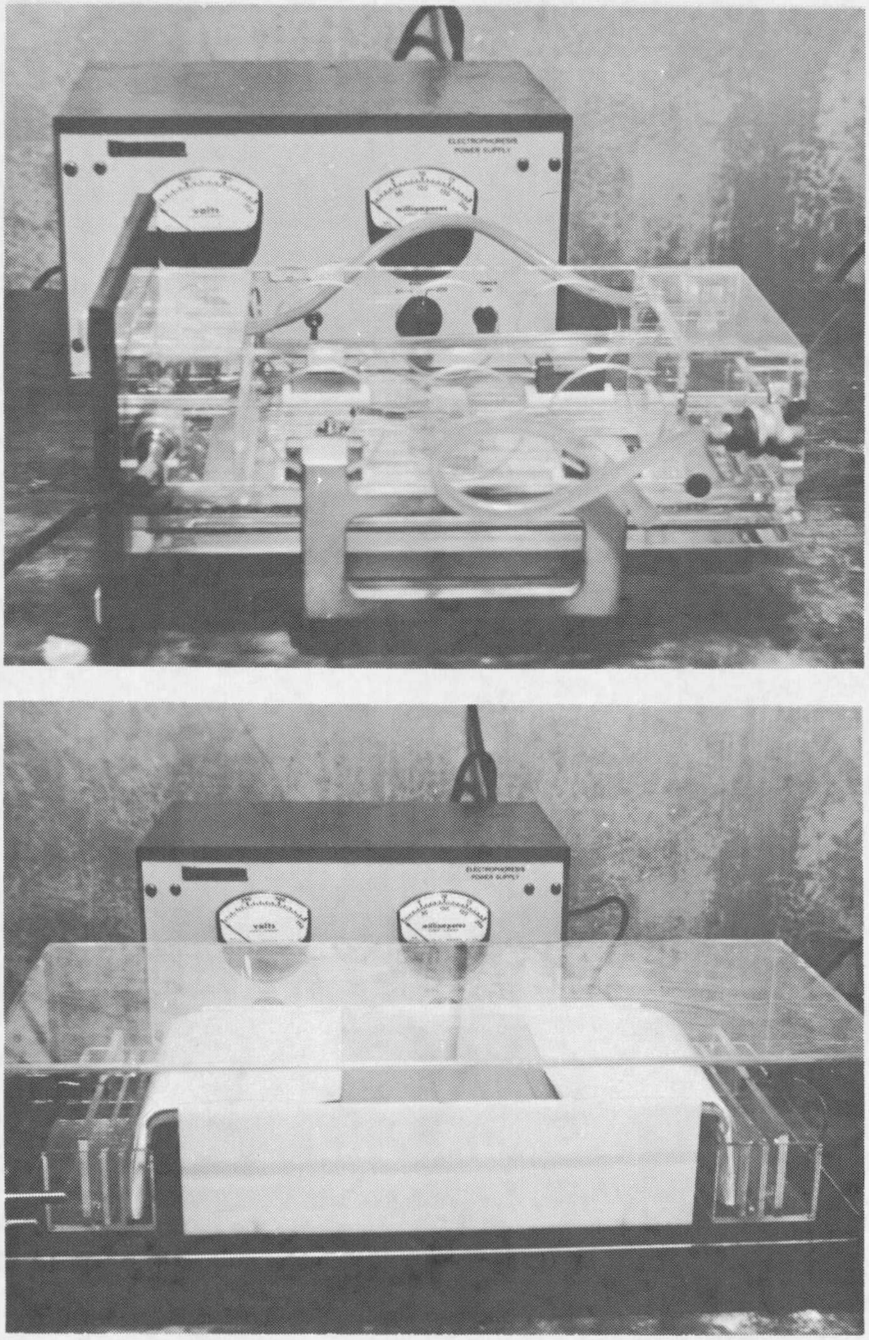

Figure 1. Electrophoresis equipment used. Top: Chamber designed and constructed by El-Negoumy and Via (1966). Bottom: Research Specialties Company migration chamber.

Following the dyeing, the gel was washed by the methanol-water-acetic acid solution until good resolution was obtained. The protein-dye complex does not wash out and in fact becomes clearer and more easily read when the gels are washed for several days and when the washing solution and gel are placed in a cold room (2-3°C) for that period of time (Figure 2). The reading of the protein types was accomplished by comparing each electrophoretic pattern with patterns of known phenotypes[1]. During the early stages of the work, the author had the advantage of conferring with Dr. A. M. El-Negoumy on the phenotyping of the gels.

IV. STATISTICAL METHODS AND ANALYSIS

Each cow typed, with the exception of some of the cows sampled in 1970, was included in the gene frequency analysis. For the gene frequency analysis, cows were classified according to the breed of their sire and dam. The balance of the 1970 data was used in the daughter-dam analysis. The gene frequencies were calculated after the manner of Stern (1960). For example the frequency of one allele (A) is calculated in the following manner:

$$\text{Frequency of allele A} = \frac{2\ (\text{No. of A/A cows}) + \text{No. of A/B cows}}{2\ \text{Total cows}}$$

---

[1] Supplied by Dr. A. M. El-Negoumy, Agriculture Products Utilization Laboratory, Montana State University, Bozeman, Montana.

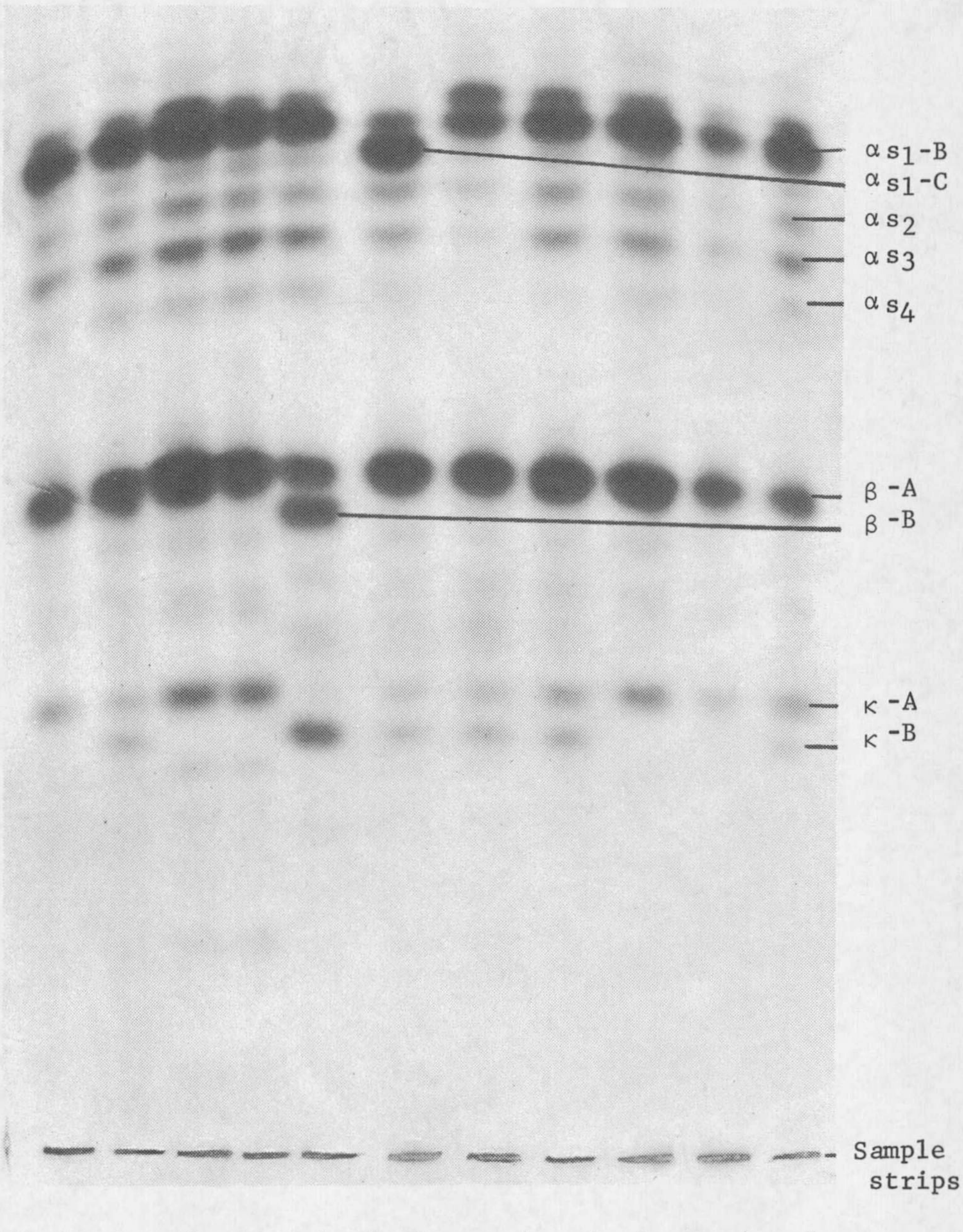

Figure 2. Photograph of electrophoreized starch-gel showing some of the variation in the α_{s1}-, β- and κ-casein components.

Zygotic frequencies were tested for conformity with the Hardy-Weinberg equilibriums by χ^2 analysis (Stern, 1960). The gene frequencies were inserted into the Hardy-Weinberg formula, $p^2 + 2p (1-p) + 1-p)^2 = 1$, to obtain expected observations of homozygotes p^2 or $(1-p)^2$ and heterozygotes $2p (1-p)$. The expected number was obtained by multiplying the frequency of the allele by the number of individuals in the analysis.

Least squares procedures were used to determine the effect of milk protein type of the cow on the weaning traits of her calves. The weaning trait data was not compiled especially for this study, but is regularly compiled on all calves at the Miles City Station. The traits measured were weaning weight, adjusted 180-day weight, and 180-day gain. The 180-day weight was obtained by computing gain/day X 180 plus birth weight. The 180-day gain was the gain/day X 180. There was a maximum of three years for which calf production data were obtained. Therefore, the number of observations per cow varied from one to three depending on the number of weaning records available. For this reason the number of production records exceeded the number of cows with milk protein classifications.

The data were analyzed separately within groups by breed of sire and breed of dam. Main effects included in the least squares model were α_{s1}-, β-, κ-casein genotypes, sex of calf (bull, steer, or heifer), age of dam (2-10 years) and year. The general model used for the separate group analysis, with appropriate notation was: $Y_{ijklmnr} = \mu + \alpha_i + \beta_j + \kappa_k + s_l + a_m + y_n + e_{ijklmnr}$. When the breeds and breed crosses were combined for an overall analysis, a main effect for breed was included in the model. One analysis was made including a β-casein type X κ-casein type interaction and a κ-casein type X sex interaction. No other interactions were included in any analysis because of missing genotypic combinations or inadequate numbers in some genotypic groups. A main effect for line of sire and line of dam was included in the analysis of inbred Hereford or linecross production data.

RESULTS

I. RELIABILITY OF MILK TYPING

Milk samples were collected from 181 of the cows, once each in 1966 and 1967. These samples were electrophorized and typed to check the reliability of the methods. All but one of the duplicate samples gave identical results. No reason could be found for the different typing in 1966 and 1967. Probably there had been an error in identification of the sample one of the years or a mistake in typing the 1966 sample. This cow and her offspring were excluded from this study. These results indicate that the typing procedures were accurate and repeatable. They also gave support for the basic premise that the protein types studied are permanent characteristics of a cow's milk and are not variable with time.

II. THE GENETIC MECHANISM CONTROLLING VARIATION IN CASEIN TYPE

Reports in the literature (Aschaffenburg, 1968, Arave *et al*. 1971, etc.) have postulated genetic control for the protein components of cow's milk. They suggest that the α_{s_1}, β-and κ-systems are each controlled at a separate autosomal locus by a series of alleles with polymorphic expression. To test this hypothesis, 97 cows which were daughters of previously sampled cows were sampled in 1970. The milk sample from each daughter was found to contain at least one of the allelic types of the dam in each casein group (α_{s_1}, β-, and κ-casein). It was not thought practical to attempt to type the sires of the cows tested in 1970 from their daughters genotype because few offspring of a sire were available. This precluded the use of these cows to study inheritance of the milk protein types.

All typing done in the present study substantiated the reports of other research concerning genetic control of the milk proteins. There were never more than two alleles observed per cow within a casein type in the milk samples. This information along with the above mentioned results from daughter-dam genotype comparisons give strong support for the concept of multiple alleles at three loci (one locus each for α_{s_1}, β-, and κ-casein) controlling variation of the casein components.

III. GENE FREQUENCIES IN BEEF BREEDS

The genotypes of a cow with respect to the casein components of her milk is inferred directly from the phenotype of the electrophoresis gel. Hence, it is possible to report allelic frequencies for the three loci from the various genotypes observed. The gene frequencies for the 13 populations into which 398 of the cows could be grouped are given in Table VII. The genotypes of the 398 cows are given in Appendix Table I. It will be observed from Table VII that not all alleles were found in the various breeds or breed crosses sampled. The α_{s_1}-casein A/A and A/C genotypes as well as the β-casein C/C and B/C genotypes were not found in the milk typed. The α_{s_1}-casein A/B and the β-casein A/C were each found only once. The inbred Hereford cow which typed α_{s_1}-casein A/B and the Angus X Brown Swiss cow which

TABLE VII. FREQUENCIES OF CASEIN ALLELES IN MILK FROM BEEF CATTLE

		α_{s1}-			β-			κ-	
BREED OR CROSS	Cows Tested	A	B	C	A	B	C	A	B
Hereford (inbreds and linecrosses)	161	0.003	0.984	0.013	0.798	0.202	0	0.742	0.257
Hereford	26	0	1.00	0	0.846	0.154	0	0.731	0.269
Angus	30	0	0.917	0.083	1.00	0	0	0.733	0.267
Charolais	34	0	0.926	0.074	0.926	0.074	0	0.721	0.279
Hereford X Angus	19	0	0.973	0.027	0.816	0.184	0	0.658	0.342
Angus X Hereford	22	0	0.977	0.023	0.932	0.068	0	0.727	0.273
Angus X Charolais	17	-	0.941	0.059	0.971	0.029	0	0.735	0.265
Charolais X Angus	27	0	0.963	0.037	0.926	0.074	0	0.704	0.396
Hereford X Charolais	17	0	0.941	0.059	0.735	0.265	0	0.647	0.353
Charolais X Hereford	18	0	0.972	0.028	0.833	0.167	0	0.611	0.389
Hereford X Brown Swiss	10	0	0.80	0.20	0.75	0.25	0	0.75	0.25
Angus X Brown Swiss	9	0	0.889	0.111	0.722	0.222	0.056	0.556	0.444
Charolais X Brown Swiss	8	0	0.938	0.062	0.813	0.187	0	0.688	0.312
Total	398								

typed β-casein B/C proved to be the only occurrence of the α_{s1}-casein A allele and the β-casein C allele.

The straightbred Herefords had only the α_{s1}-casein B/B genotype. The range of frequencies, in the populations studied, for the α_{s1}-casein B allele was 0.80 to 1.00; for the β-casein A allele, it was 0.72-1.00; for the κ-casein A allele, the frequencies ranged from 0.56 to 0.74. There is much greater variation available for selection in the κ-casein fractions than in either the α_{s1}-, or β-casein. The α_{s1}-, and β-casein allelic frequencies indicate that they are very nearly fixed.

The allelic frequencies for the α_{s1}- casein in the crossbred groups fall between the frequencies of their respective parental breeds. This is as expected. In the β-casein, the same is true for four out of the six crossbred groups. However, the κ-casein frequencies for the breed cross groups do not fall between those of their parental breeds. In most cases, the crossbreds fall below the low parent frequencies for κ-casein A. This may be chance variation due to the small sample size. However, with the exception of the Brown Swiss (which are excluded here because of not having a pure parental breed with which to compare), five out of six crossbred comparisons fall below the parental frequencies. The indication is that some type of selection is taking place in the κ-casein components.

If there were some selective advantage to some genotype, this would be most likely discerned in the κ-casein components because the A and B alleles are of such a frequency that selection can occur. In both the α_{s_1}- and β-casein, the frequencies of the B and A alleles, respectively, are so high that very little change in gene frequency can occur in one generation. As indicated in Appendix Table I, 92% of the cows typed had the α_{s_1}-casein B/B genotype, and 70% of all cows had the β-casein A/A. Conversely, only 46% of the population had the κ-casein A/A genotype and 50% had the A/B genotype, leaving a much greater opportunity for detectable selection toward a superior genotype.

Although the gene frequencies reported in this study are based upon very small populations, they agree with those reported elsewhere (caldwell, Weseli, and Cartwright, 1971). There are no known reports of κ-casein frequencies in the beef breeds. The comparisons of the frequencies for the α_{s_1}- and β-casein reported here and those reported elsewhere are given in Table VIII.

The α_{s_1}-casein frequencies are quite similar to those found in the Holstein, Brown Swiss, and Shorthorn breeds (compare Tables IV and VII). This is as expected since the frequency of the B allele is high compared to the alternative alleles. The β-casein allelic frequencies from the literature are quite divergent, so comparisons are not possible. The frequencies of κ-casein in the beef breeds were similar to the reported allelic frequencies in the Holsteins.

TABLE VIII. COMPARISON OF ALLELIC FREQUENCIES AT TWO LOCI

Breed	Study	α_{s1}-casein			β-casein		
		A	B	C	A	B	C
Hereford	(Literature[a]	0	0.98	0.02	0.75	0.25	0
	(This study	0	1.00	0	0.846	0.154	0
(Inbred & Linecrosses)	This study	0.003	0.984	0.013	0.795	0.205	0
Angus	(Literature	0	0.84	0.16	0.95	0.05	0
	(This study	0	0.917	0.083	1.00	0	0
Charolais	(Literature	0	0.80	0.20	1.90	0.10	0
	(This study	0	0.926	0.74	0.926	0.074	0

[a] Caldwell, Weseli, and Cartwright (1971).

There is little variation among the various breeds and breed crosses in gene frequency in spite of the fact that individual breeds can be considered separate populations. Most of the variation observed in the frequencies of protein types was within breeds and is found primarily in the β- and κ-casein components.

IV. GENOTYPIC FREQUENCIES AND HARDY-WEINBERG EXPECTATIONS

An analysis was made of the fit of zygotic frequencies to Hardy-Weinberg expectations using genotypic frequencies of the milk caseins. Using the technique of Stern (1960), expected frequencies of each genotype were computed from the allele frequencies obtained in this study. The differences between observed and expected numbers in each genotypic class were analyzed using χ^2. The analysis of both the

α_{s_1}- and β-casein genotypic frequencies yielded no significant deviations from the expected. Deviations from the expected numbers in each genotypic class seemed to occur at random. Table IX gives the χ^2 analysis for the Hereford breed which showed the largest deviation from the expected, yet was non-significant.

The deviations which are present in α_{s_1}- and β-casein genotypes would not be expected to be large because of the fact that the frequencies of the α_{s_1}-casein B allele and the β-casein A allele are so near to unity. This limits the opportunity for genotypic frequencies to fluctuate much from the expected. Many generations of large populations would need to be tested to detect significant deviations. It does seem appropriate to question why some α_{s_1}- and β-casein alleles have consistently attained what seems to be very high frequencies in these beef breeds. In light of the reported frequencies of the same alleles in the Brahman and some Holstein cattle, the high frequencies reported here are not a phenomenon of all cows' milk. Further research needs to be done to seek causes for these differences.

The κ-casein genotypic frequencies presented a much different picture than the α_{s_1}- and β-casein when analyzed by the above methods. The calculations are summarized in Table X. One of the breed groups (Charolais), deviated significantly ($P < .05$) while three (inbred and linecross Herefords, Angus-Charolais, and Hereford-Charlais) groups showed highly significant ($P < .01$) deviations from the expectations. In every breed group analyzed for κ-casein equilibrium, the

TABLE IX. OBSERVED AND EXPECTED FREQUENCIES OF β-CASEIN GENOTYPES IN HEREFORDS[a] BASED ON ALLETIC FREQUENCIES[b] OBTAINED IN THIS STUDY

Genotype	Observed (o)	Hardy-Weinberg Notation	Theoretically Expected Number (e)	e	$\frac{(o-e)^2}{e}$
A/A	119	p^2	$(p^2)n$	121.2	4.84/121.2 = 0.40
B/B	5	$(1-p)^2$	$(1-p)^2n$	7.1	4.41/7.1 = 0.621
A/B	63	$2p(1-p)$	$2p(1-p)n$	58.7	18.49/57.8 = 0.317
Totals	187			187	0.978[c] = ^{2}

---

[a] All Herefords combined for this analysis

[b] Computed by the following: $\frac{\text{(2(no. in A/A class) + no. in A/B class}}{\text{2(total no.)}}$ = frequency of A allele.

Frequency of A allele = 0.805; frequency of B allele = 0.195.

[c] $\chi 2$ required for significance (d.f. = 1, $P < .05$) = 3.84.

TABLE X. ANALYSIS OF THE FIT OF κ-CASEIN GENOTYPIC FREQUENCIES TO HARDY-WEINBERG EXPECTATIONS

		A/A		B/B		A/B		Total χ^2[a]
Breed	Cows typed	obs.	exp.	obs.	exp.	obs.	exp.	Calculated
Hereford (inbreds and linecrosses)	161	82	88.8	4	10.7	75	61.5	7.6794**
Hereford	26	13	13.9	1	1.9	12	10.9	0.8021
Angus	30	15	16.0	1	2.0	14	12.0	0.8958
Charolais	34	16	17.7	1	2.7	17	13.7	5.4876
Hereford-Angus[b]	41	18	19.8	2	3.8	21	17.4	1.7610
Angus-Charolais[c]	44	19	22.5	0	3.5	25	18.0	6.7666**
Hereford-Charolais[d]	35	10	13.8	1	4.8	24	16.4	7.5767**
Total	371							

[a] Significant χ^2 values with 1 df.; *, $P < .05$; **, $P < .01$

[b] Hereford-Angus reciprocal crosses combined

[c] Angus-Charolais reciprocal crosses combined

[d] Hereford-Charolais reciprocal crosses combined

homozygous genotypes (A/A or B/B) were less frequent than expected and the number with heterozygous gentoypes (A/B) exceeded the expected. The frequencies reported by Corradini (1969) showed a similar pattern of deviation. This kind of departure from Hardy-Weinberg expectation indicates that the heterozygote has a selective advantage. There are few reports in the literature dealing with κ-casein frequencies because it has been difficult to resolve the κ-casein components consistently. There are no known reports of the Hardy-Weinberg equilibrium analysis of genotypic frequencies of the milk proteins in milk from beef cows.

V. EFFECT OF MILK CASEIN GENOTYPE ON CALF PRODUCTION TRAITS

A. Overall Analysis of Effect of Protein Type on Production

In most of the analyses of the effect of milk protein genotype on weaning traits with least squares techniques, the sex, age of dam and year, main effects were significant ($P < .01$). This has been previously reported by many workers, most recently by Pahnish *et al.* (1969) on the same herd of cattle sampled in this study. In the overall analysis of milk protein type on production traits, the breed effect was also significant ($P < .01$). Table XI lists the least squares means for the analysis of the effect of milk genotype on production (weaning weight, 180-day gain, and 180-day weight) with all cows included in the analysis.

The analysis of variance of the differences in Table XI showed that the effect of κ-casein genotypes on weaning weight approached significance ($P < 0.10$) and the effect of β-casein genotypes on 180-day gain and 180-day weight approached significance ($P < 0.10$). (Appendix Table II).

B. Interactions Analyzed

In the analysis of the effect of milk protein genotype on production data with the β-casein type X κ-casein type and κ-casein type X sex interactions, the interaction effect was very small. The analysis of variance showed no significant effects for either genotype or interactions. (See Appendix Table III for the analysis of variance for 180-day gain with the above interactions.) These were the only interactions studied due to the small numbers of observations of some genotypic combinations and because many interaction comparisons were missing. The interactions were omitted from all subsequent analyses.

Data from inbred lines and linecrosses of Herefords and the combined crossbred and straightbred cattle were analyzed for milk protein effect on production traits. The results of these analyses are presented in Tables XII and XIII.

The inbred line and linecross effects from the analysis of variance were non-significant in all three traits measured. All milk protein genotypic effects were also small and non-significant. As noted in Table XII, the differences in genotype effect were generally small, but the κ-casein A/B genotype showed a superior effect on weaning weight and 180-day gain in this analysis (Table XII).

TABLE XI. OVERALL EFFECT OF CASEIN GENOTYPE OF WEANING WEIGHT, 180-DAY GAIN, AND 180-DAY ADJUSTED WEIGHT

Casein Genotype		Number of Observations	Weaning Weight (lbs.)	180-day Gain (lbs.)	180-day Weight (lbs.)
α_{s1}-	B/B	707	451.29	346.95	426.77
	C/C	3	419.17	326.95	404.18
	A/B	2	404.60	300.79	367.85
	B/C	60	440.11	338.45	415.20
β-	A/A	538	418.25	317.17	393.51
	B/B	9	423.62	325.97	401.01
	A/B	222	432.72	330.74	408.86
	A/C	3	440.57	338.66	410.63
κ-	A/A	339	428.09	327.16	402.62
	B/B	17	440.72	336.80	413.09
	A/B	416	417.56	320.45	394.80

TABLE XII. MILK PROTEIN GENOTYPE EFFECT ON PRODUCTION IN INBRED LINES AND LINECROSSES IN HEREFORDS (LEAST SQUARES MEANS)

Casein Genotype		Number of Observations	Weaning Weight (lbs.)	180-day Gain (lbs.)	180-day Weight (lbs.)
α_{s1}-	B/B	211	400.73	301.62	374.29
	A/B	1	385.75	278.23	343.78
	B/C	5	414.31	301.06	376.90
β-	A/A	128	393.52	288.91	358.95
	B/B	6	405.40	298.07	367.41
	A/B	83	401.87	293.93	368.61
κ-	A/A	110	404.16	298.09	373.41
	B/B	5	390.00	281.08	348.66
	A/B	102	406.64	301.54	372.90

TABLE XIII. MILK PROTEIN GENOTYPE EFFECT ON PRODUCTION IN STRAIGHTBREDS AND CROSSBREDS (LEAST SQUARES MEANS)

Casein Genotype		Number of Observations	Weaning Weight (lbs.)	180-day Gain (lbs.)	180-day Weight (lbs.)
α_{s1}-	B/B	436	460.36	453.81	433.73
	C/C	3	431.69	337.74	415.03
	B/C	52	447.59	345.72	422.41
β-	A/A	371	427.28	328.49	407.24
	B/B	2	465.20	360.35	437.59
	A/C	3	454.38	352.67	427.41
	A/B	115	439.31	341.52	422.65
κ-	A/A	192	440.70	338.76	416.42
	B/B	12	470.21	366.07	445.57
	A/B	287	428.72	332.43	409.18

The differences listed in Table XIII in the κ-casein genotypic effect on weaning weight were significant ($P < .05$) and approached significance ($P < .10$) for κ-casein genotype effect on 180-day gain. Neither the α_{s_1}- nor β-casein effects were significant.

C. Analysis Combining Reciprocal Breed Crosses

In the analysis combining the reciprocal breed crosses the casein genotype frequently had significant effects on weaning traits but no pattern of significance was discernible with respect to either genotype or breed. The overall analysis combining all reciprocal breed crosses showed a significant effect for casein genotype. Table XIV summarizes the significance found in the effect of milk protein types on reciprocal breed crosses.

The homozygous genotypes were superior most often (Table XIV). However, in all cases the κ-casein B/B genotype effect was based on seven or less observations so the reliability of this indication of κ-casein B/B superiority is questionable. The α_{s_1}-casein genotype B/B would appear to have some selective advantage from Table XIV, but what is essentially being measured is the α_{s_1}-casein effect overall. With a gene frequency of 0.92 to 0.977, nearly every calf obtained milk of that genotype.

TABLE XIV. SIGNIFICANT EFFECTS OF MILK PROTEIN TYPES ON PRODUCTION TRAITS IN RECIPROCAL BREED CROSSES

Analysis or Cross	Weaning Weight	180-day Gain	180-day Weight
Overall	αs_1-casein[a](B/B)[1]	-	αs_1-casein*(B/B)
	κ-casein*(B/B)	-	-
Hereford-Angus	-	β-casein*(A/B)	β-casein*(A/B)
Hereford-Charolais	αs_1-casein*(B/B)	αs_1-casein*(B/B)	αs_1-casein*(B/B)
Angus-Charolais	αs_1-casein*(B/B)	-	-
Hereford)) Angus)-Brown Swiss) Charolais)	κ-casein*(B/B)	κ-casein[a](B/B)	κ-casein[a](B/B)

[a] P< .10

* P< .05

[1] Superior genotypes determined by least squares means are given in parenthesis.

TABLE XV. SIGNIFICANT EFFECTS OF MILK PROTEIN TYPES ON PRODUCTION TRAITS OF BREED AND CROSSBRED GROUPS

Breed or Cross	Weaning Weight	180-day gain	180-day weight
Hereford	κ-casein**(A/A)^1	κ-casein**(A/A)	κ-casein**(A/A)
Angus	-	-	-
Charolais	-	κ-casein^a(A/A)	κ-casein^a(A/A)
H X A^2	(αs_1-casein*(B/C) ((β-casein**(A/B)	αs_1-casein*(B/C) β-casein**(A/B)	- β-casein**(A/B)
A X H	-	-	-
A X C^2	-	-	-
C X A	αs_1-casein**(B/B)	αs_1-casein*(B/B)	αs_1-casein*(B/B)
H X C	αs_1-casein*(B/B)	αs_1-casein^a(B/B)	αs_1-casein*(B/B)
C X H	-	-	-
H X B S^2	-	-	-
A X B S	-	-	-
C X B S	-	-	-

[a] $P < .10$

* $P < .05$

** $P < .01$

[1] Superior genotypes determined by least squares means are given in parentheses.

[2] Abbreviations H, A, C, and B S refer to Hereford, Angus, Charolais, and Brown Swiss breeds, respectively.

D. Estimation of Effect of Casein Genotype on Individual Breeds and Breed Crosses

The effects of milk protein genotype on the production traits measured in individual breeds and breed crosses seemed to be small and non-significant generally. The analysis showed no obvious patterns of significance either as to casein type overall or as to genotype within a casein type.

Table XV indicates that the homozygous genotypes seemd to be superior in most analyses. In the only instance of heterozygote superiority in Table XV there were two loci involved. In the analysis the α_{s1}-casein (B/C) and β-casein (A/B) heterozyotes showed superior effects within their respective casein types half of the time, the other half they were either in the middle or low of the genotypes. The κ-casein heterozygote (A/B) was high in three analyses and low in four. It seemed that the effects associated with casein genotypes had no clearly discernable patterns in the various analyses. The most consistent genotype from Tables XIV and XV was the α_{s1}-casein B/B. It is involved with the greatest number of significant analyses but there were some analyses in which the α_{s1}-casein(B/B effect was inferior to the effect of alternative α_{s1}-casein genotypes.

DISCUSSION

The results reported in this study support the theory of multiple allelic control of casein variation at three different loci. Daughter-dam comparisons support this by the fact that the daughter and dam were always alike in at least one allele at each locus. The data did not permit analysis for linkage relationships. The calculation of gene frequencies at each of the loci involved was possible as well as the comparison of zygotic frequencies with Hardy-Weinberg expectations.

The analysis of effect of milk protein genotype on production yielded inconsistent results for all genotypes and for all breeds and breed crosses. It appears that the milk protein genotypes do not contribute substantially to variation in the weaning traits studied.

I. EFFECTS OF MILK PROTEIN GENOTYPE ON PRODUCTION TRAITS

The lack of consistent significant effects of milk protein genotype on production traits should not be taken as an indication of no effect of milk type on production at this point. One limitation in the present study was the low number of oberservations of some genotypes and the low number of animals in some populations studied in the beef breeds.

Another limitation was in the nature of the traits studied and their actual relationship to milk obtained from the cow. The traits measured (weaning weight, 180-day gain, and adjusted 180-day weight) are all closely related. The real dependence of a calf on its mother's milk is present during the first month of life. Beyond that time, it supplements its diet with grass and other forage. Thus the traits measured constitute a somewhat insensitive test of the effect of the milk protein genotype. Indeed, the most pronounced effect of milk protein on calf production may be in survivability of the calf, resistance to disease, or efficiency of gains, none of which are measured very effectively by weaning attributes. Before the true effect of milk protein genotype can be measured, an experiment will have to be designed to include measurements of these effects.

II. α_{s_1}-CASEIN

The α_{s_1}-casein B allele had a frequency of 0.926-1.00 for the populations studied. The frequencies of the breed crosses for α_{s_1}-casein were within the range of their parental breed gene frequencies. This was as expected when two populations are combined, allowed to mate randomly, and there is no selective advantage to any genotype. However, if there were some selective advantage to the heterozygous genotypes in the α_{s_1}-casein system, it is unlikely that it could be detected when both parental breeds contain one allele at such a high frequency. It would require considerably greater numbers and more generations than studied in the present work to estimate the affect of the heterozygote in α_{s_1}-casein, and to recognize any advantage.

In the Hardy-Weinberg equilibrium analysis, the α_{s_1}-casein showed no significant deviation from the expected. This too could be caused by the fact that there is no selective advantage to the heterozygote, or it could be the result of small numbers in the populations studied and gene frequencies near unity.

In the analysis of effect of protein type on calf production, the B/B genotype showed some advantage; however, in the α_{s_1}-casein there was little else with which to compare the effect of the B/B genotype within the α_{s_1}-casein. At this point it is difficult to determine the actual effects of α_{s_1}-casein genotypes in relation to calf production. As more measurements of calf production become available for analysis and as more known genotypic combinations with adequate numbers become available, some of these relationships may be better defined.

III. β-CASEIN

The situation with the A allele of the β-casein was much the same as that of α_{s_1}-casein B. It had a high frequency and all β-casein genotypes seemed to be near equilibrium from the χ^2 analysis. Few of the β-casein genotypes showed significant effects on the production traits measured, and no consistent pattern was shown for heterozygote superiority. In the comparison of gene frequencies in breed crosses with that of parental breeds, the β-casein A allele yielded an atypical picture. Some of the breed crosses' frequencies were lower than either parental frequency (Charolais-Hereford reciprocal crosses and the Hereford X Angus cross). Whether or not this happened completely by chance is not clear. It may be an indication of some selective advantage to the heterozygote which would tend to lower gene frequency in the β-casein A allele. This situation too will need to await further study to verify any cause and effect relationship.

IV. κ-CASEIN

The κ-casein components yielded somewhat unusual results when subjected to the various analyses reported here. The frequencies of the κ-casein A allele allowed much greater opportunity for selection to occur than either the α_{s_1}- or β-casein allelic frequencies. The κ-casein A and B allele frequencies were between 0.56-.75 and 0.44-.25, respectively. Except for one comparison, the breed cross frequencies for the κ-casein A allele were all lower than the parental frequencies. A greater fitness to the heterozygote in relation to both homozygotes is suggested by these findings.

The investigation of the κ-casein genotypic frequencies by Hardy-Weinberg equilibrium produced a consistent pattern of deviation of observed genotypes from expected. Three comparisons were found to be highly significant ($P < .01$) and one was significant ($P < .05$) out of a total of seven comparisons. In all analyses, the homozygotes (A/A and B/B) contained fewer than the expected and the heterozygote exceeded the expected. These data strongly suggest a heterozygote superiority in the κ-casein system.

The analysis of the effect of κ-casein genotype on calf production presented a mixed picture with respect to the heterozygote. There was no consistent effect of genotype on the production traits in breeds or breed crosses. Where the κ-casein genotypes showed a significant effect on production, the A/A or B/B genotypes had superior effects as measured by least squares means . The κ-casein A/A genotype showed the high effects in four of the analyses.

V. APPLICATION OF INFORMATION

The most significant finding of this study was in the Hardy-Weinberg equilibrium analysis of the κ-casein genotypes. The data have suggested that some selection pressure was operating with respect to the heterozygote. Since the cows were all mated within their specified breeding groups with no knowledge of casein genotype (i.e. randomly), artificial selection would not be responsible for the gene frequency deviations from expected. In the differences detected with the χ^2 analysis, the pattern as well as the magnituide of the deviations indicate that chance alone is not responsible. The interpretation that these data suggest is one of heterosis or heterozygote superiority. This greater fitness of the heterozygote could be caused by an epistatic relationship of the alleles controlling κ-casein variation with other alleles. It could also be due to a pleiotropic effect of the κ-casein genes or to a favorable linkage relationship between the κ-casein genes and other genes.

If future research supports these conclusions, then selection for specific genetic loci of milk proteins could become important (Smith, 1967). Its importance will depend on the magnitude of the heterosis effect, its courses, and whether it is present at other milk protein loci. Additional investigation is needed to establish causes for the κ-casein A/B situation observed in this research.

APPENDIX

APPENDIX TABLE I. DISTRIBUTION OF CASEIN GENOTYPES AMONG BREEDS AND BREED CROSSES

Breed or Cross	Total Cows	αs_1-casein genotype[1], cows typed			
		B/B	C/C	A/B	B/C
Hereford (inbreds & crosses)	161	156	0	1	4
Hereford	26	26	0	0	0
Angus	30	26	1	0	3
Charolais	34	29	0	0	5
H X A[2]	19	18	0	0	1
A X H	22	21	0	0	1
A X C	17	15	0	0	2
C X A	27	25	0	0	2
H X C	17	15	0	0	2
C X H	18	17	0	0	1
H X B S	10	6	0	0	4
A X B S	9	7	0	0	2
C X B S	8	7	0	0	1
Total	398	368	1	1	28
Percent		92.4	0.3	0.3	7.0

Breed or Cross	Total Cows	β-casein genotype[1], cows typed			
		A/A	B/B	A/B	A/C
Hereford (inbreds & crosses)	161	101	5	55	0
Hereford	26	18	0	8	0
Angus	30	30	0	0	0
Charolais	34	29	0	5	0
H X A[2]	19	13	1	5	0
A X H	22	19	0	3	0
A X C	17	16	0	1	0
C X A	27	23	0	4	0
H X C	17	8	0	9	0
C X H	18	12	0	6	0
H X B S	10	5	0	5	0
A X B S	9	4	0	4	1
C X B S	8	5	0	3	0
Total	398	283	6	108	1
Percent		71	1.5	27	0.5

APPENDIX TABLE I. (CONTINUED)

Breed or Cross	Total Cows	κ-casein genotype[1], cows typed		
		A/A	B/B	A/B
Hereford (inbreds & crosses)	161	82	4	75
Hereford	26	13	1	12
Angus	30	15	1	14
Charolais	34	16	1	17
H X A[2]	19	6	0	13
A X H	22	12	2	8
A X C	17	9	0	9
C X A	27	11	0	16
H X C	17	5	0	12
C X H	18	5	1	12
H X B S	10	5	0	5
A X B S	9	1	0	8
C X B S	8	4	1	3
Total	398	183	11	204
Percent		46	3	51

---

[1] Actual cows observed with each genotype are listed, only those genotypes typed are reported.

[2] H, A, C, and B S refer to Hereford, Angus, Charolais and Brown Swiss, respectively.

APPENDIX TABLE II. LEAST-SQUARES ANALYSIS OF VARIANCE FOR WEANING WEIGHT, 180-DAY GAIN, AND 180-DAY ADJUSTED WEANING WEIGHT

Source	D.F.	Sum of Squares	Mean Squares	F
		Weaning Weight		
Total	772	5072257.00		
Total Reduction	30	2464200.00	82140.00	23.369
MU-Y	1	154.77	154.77	.044
Alpha	3	12857.82	4285.94	1.219
Beta	3	22673.45	7557.81	2.150
Kappa	2	20718.68	10359.33	2.947[a]
Sex	2	70118.81	35059.40	9.975**
Age of Dam	6	289277.93	48212.98	13.717**
Breed	11	722578.50	65688.93	18.689**
Year	2	236215.75	118107.87	33.602
Remainder	742	2608057.00	3514.90	
		180-Day Gain		
Total	772	3161263.00		
Total Reduction	30	1258463.00	41948.76	16.358
MU-Y	1	8519.12	8519.12	3.322
Alpha	3	8675.36	2891.78	1.128
Beta	3	19918.31	6639.43	2.589[a]
Kappa	2	9078.86	4539.43	1.770
Sex	2	45421.68	22710.84	8.856**
Age of Dam	6	149925.00	24987.50	9.744**
Breed	11	479015.31	43546.84	16.981**
Year	2	123653.62	61826.81	24.109**
Remainder	742	19028.00.00	2564.42	
		180-Day Adjusted Weaning Weight		
Total	772	4229655.00		
Total Reduction	30	1754010.00	58467.00	17.524
MU-Y	1	131.87	131.87	.040
Alpha	3	14434.60	4811.53	1.442
Beta	3	25011.64	8337.21	2.499[a]
Kappa	2	11966.56	5983.28	1.793
Sex	2	81531.87	40765.93	12.218**
Age of Dam	6	223485.87	37247.64	11.164**
Breed	11	600928.81	54629.89	16.374**
Year	2	185038.62	92519.31	27.730**
Remainder	742	2475645.00	3336.44	

[a] (P < .10)
** (P < .01)

APPENDIX TABLE III. OVERALL ANALYSIS OF VARIANCE FOR 180-DAY GAIN WITH INTERACTIONS

Source	D.F.	Sum of Squares	Mean Squares	F
Total	772	3161263.00		
Total Reduction	37	1276443.00	34498.45	13.453
MU-Y	1	5546.21	5546.21	2.163
Alpha	3	8110.70	2703.56	1.054
Beta	3	1237.20	412.40	.161
Kappa	2	405.98	202.99	.079
Sex	2	14953.95	7476.97	2.916[a]
Age of Dam	6	153300.56	25550.09	9.963**
Breed	11	454915.81	41355.98	16.127**
Year	2	117934.50	58967.25	22.995**
Beta X Kappa	3	5305.13	1768.37	.690
Kappa X Sex	4	9659.57	2414.89	.942
Remainder	735	1884820.00	2564.38	

[a] $(P < .10)$

** $(P < .01)$

LITERATURE CITED

Annan, W. D., and W. Manson. 1969. A fractionation of the α_s-casein complex of bovine milk. J. Dairy Res. 36:259.

Arave, C. W., R.C. Lamb, H. C. Hines. 1971. Blood and milk protein polymorphisms in relation to feed efficiency and production traits of dairy cattle. J. Dairy Sci. 54:106.

Aschaffenburg, R. 1961. Inherited caseins variants in cow's milk. Nature 192-432.

Aschaffenburg, R. 1963. Inherited casein variants in cow's milk. II. Breed differences in the occurrence of β-casein variants. J. Dairy Res. 30:251.

Aschaffenburg, R. 1968. Reviews of the progress of dairy science. Section G. Genetics. 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.

Aschaffenburg, R., and J. Drewry. 1957. Genetics of the β-lactoglobulins of cow's milk. Nature 180:376.

Aschaffenburg, R., A. Sen, and M. P. Thompson. 1968. Genetic variants of casein in Indian and African Zebu cattle. Comp. Biochem. Physiol. 25:177.

Blumberg, B. S., and M. P. Tombs. 1958. Possible polymorphism of bovine α-lactalbumin. Nature. 181:683.

Bouchard, R., and G. J. Brisson. 1969. Changes in protein fractions of ewe's milk throughout lactation. Can. J. Animal Sci. 49:143.

Brinks, J. S., J. J. Urick, O. F. Pahnish, B. W. Knapp, and T. J. Riley. 1967. Heterosis in preweaning and weaning traits among lines of Hereford cattle. J. Anim. Sci. 26:278.

Brunner, J. R., C. A. Ernstrom, R. A. Hollis, B. L. Larson, R. McL. Whitney, and C. A. Zittle. 1960. Nomenclature of the proteins of milk. First revision. J. Dairy Sci. 43:901.

Butterworth, M. H., T. R. Goughton, J. C. Macartney, A. J. Prior, C. P. Middlemiss, and D. E. Edmond. 1968. Some observations on the lactation of Blackhead ewes and the growth of lambs: The composition and yield of milk. J. Agr. Sci. 70(2):203.

Caldwell, J., D. F. Weslei, and T. C. Cartwright. 1971. Occurrence of α_{s1}- and β-casein types in five breeds of beef cattle. J. Anim. Sci. 32:601.

Coccodrilli, G. D., Jr., P. T. Chandler, and C. E. Polan. 1970. Effects of dietary protein on blood lipids of the calf with special reference to cholesterol. J. Dairy Sci. 53:1627.

Corradini, C. 1969. Distribution of the genetic variants of α_{s1}-, β-, and κ-casein in milk from Jersey cows in the Netherlands. Ned. Milk. enZuineligdschr., 23:79.

Comberg, G., M. Groning, and H. Meyer. 1967. Inorganic and organic constituents of bovine milk and their genetic basis. Z. Tierzuech Zuchtungsbiol. 84(1):34 (Engl. Summ.)

El-Negoumy, A. M. 1966. Rapid recovery, preservation, and phenotyping of milk proteins by a modified starch-gel technique of superior resolving power. Anal. Biochem. 15:437.

El-Negoumy, A. M. 1967. Polymorphism in γ-casein fractions from the milk of individual cows. Biochem. Biophys. Acta. 140:503.

El-Negoumy A. M., and H. Via. 1966. A convenient migration chamber for electrophoresis in solid gels. J. Chromatog. 23:325.

Frandsen, J. H. 1958. Dairy Handbook and Dictionary. J. H. Frandsen, Publisher, Amherst, Mass.

Flower, A. E., J. S. Brinks, J. J. Urick, and F. S. Willson. 1963. Comparisons of inbred lines and linecrosses for performance traits in Hereford range cattle. J. Anim. Sci. 22:914.

Grosclaude, F., J. Garnier, B. Ribadeau-Dumas, and R. Jeunet. 1964. Relationship between the loci controlling the polymorphism of α_s- and β-caseins. J. Animal Sci. (Abstr.) 27:505 #3223 (1965)

Grosclaude, F., J. Pujolle, J. Garnier, and B. Ribadeau-Dumas. 1965. Genetic determinism of the κ-caseins of cow's milk: The close linkage between the κ-casein locus and the α_s-casein and β-casein loci. J. Anim. Sci. (Abstr.) 28:150, #956 (1966)

Groves, M. L., and Kiddy, C. A. 1968. Polymorphisms of γ-casein in cow's milk. Archs. Biochem. Biophys., 126:188.

Groves, M. L. and C. H. Kiddy. 1970. β-caseins isolated from milk samples typed β-casein A^1 and A^3. J. Dairy Sci. 53:931.

Hines, H. C., C. A. Kiddy, E. W. Brum, and C. W. Arave. 1969. Linkage among cattle blood and milk polymorphisms. Genetics 62:401.

Hoogendoorn, M. P., J. E. Moxley, R. O. Hawes, and H. F. MacRae. 1969. Separation and gene frequencies of blood serum transferrin, casein and beta lactoglobulin loci of dairy cattle and their effects on certain production traits. Can. J. Anim. Sci. 49:331.

Horvath, I. 1970. Milk casein polymorphism in Hungarian Spotted Cattle and ancestor breeds. I. Examination of α_{s1}-, and β-casein components in the milk of Hungarian Spotted and Simmental cows. Acta. Net. Hung. 20:35. J. Dairy Sci. (abstr.) 33(3):237.

Jenness, R., B. L. Larson, T. L. McMeekin, A. M. Swanson, C. H. Whatnah, and R. McL. Whitney. 1956. Nomenclature of the proteins of bovine milk. J. Dairy Sci. 39:536.

Kalan, E. B., R. Greenberg, and M. P. Thompson. 1966. Analysis of proteolytic digests of genetic variants of α_{s1}-casein. Archs. Biochem. Biophys. 115:468.

Kannan, A., and R. Jenness. 1961. Relation of milk serum proteins and milk salts to the effects of heat treatment on rennet clotting. J. Dairy Sci. 44:808.

Kiddy, C. A., and J. O. Johnston. 1964. Genetic polymorphism in caseins of cow's milk. I. Genetic control of α_s-casein variation. J. Dairy Sci. 47:147.

Kiddy, C. A., R. F. Peterson, and F. C. Kopfler. 1966. Genetic control of the variants of β-casein A. J. Dairy Ci. 49:742.

Kiddy, C. A., M. P. Thompson, J. O. Thompson, and L. Pepper. 1963. Genetic control of α_s- and β-casein. J. Dairy Sci. 46:626.

Krosigk, C. M. von, J. O. Young, and G. A. Richardson. 1960. Genetic influences of the composition of cow's milk. J. Dairy Sci. 43:876. (Abstr.)

Lebaron, A. 1970. Protein payments for milk producers - trend for the future? Utah Sci. 31:13.

Legates, J. E. 1960. Genetic and environmental factors affecting the solids-not-fat composition of milk. J. Dairy Sci. 43:1529.

Lush, J. L. 1960. Improving dairy cattle by breeding. J. Dairy Sci. 43:702.

McKenzie, H. A. 1967. Milk proteins. Recent advances in protein chemistry. 22:56.

Neelin, J. M. 1964. Variants of κ-casein revealed by improved starch-gel electrophoresis. J. Dairy Sci. 47:506.

Pahnish, O. F., J. S. Brinks, J. J. Urick, B. W. Knapp, and T. M. Riley. 1969. Results from crossing beef X beef and beef X dairy breeds: Calf performance to weaning. J. Anim. Sci. 28:291.

Ramshaw, E. H., and E. A. Dunstone. 1969. The flavor of milk protein. J. Dairy Res. 36:203.

Schmidt, D. G. 1964. Starch-gel electrophoresis of κ-casein, Biochem. Biophys. Acta. 90:411.

Schmidt, D. G., P. Both, and P. J. deKoning. 1966. Fractionation and some properties of κ-casein variants. J. Dairy Sci. 49:776.

Smith, C. 1967. Improvement of metric traits through specific genetic loci. Anim. Prod. 9:349.

Smithies, O. 1959. *Advances in Protein Chemistry*. Academic Press, Inc. New York City.

Stern, C. 1960. *Principles of Human Genetics*. Second Edition. W. H. Freeman and Company. San Francisco, Calif. pp. 179.

Tagari, H. 1969. Comparison of the efficiency of proteins contained in lucerne hay and soya-bean meal for sheep. Brit. J. Nutr. 23:455.

Tagari, H., and J. H. B. Roy. 1969. The effect of heat treatment on the nutritive value of milk for the young calf. Brit. J. Nutr. 23:763.

Thompson, M. P., and W. G. Gordon. 1968. Amino acid composition and fingerprints of α_{s1}- and β-caseins from *bos taurus* and *bos indicus*. J. Dairy Sci. 51:947.

Thompson, M. P., and Kiddy, C. A. 1964. Genetic polymorphism in caseins of cow's milk. III. Isolation and properties of α_{s1}-caseins A, B, and C. J. Dairy Sci. 47:626.

Thompson, M. P., C. A. Kiddy, J. O. Johnston, and R. M. Weinberg. 1964. Genetic polymorphism of caseins in cow's milk. II. Confirmation of genetic control of β-casein variation. J. Dairy Sci. 47:378.

Thompson, M. P., C. A. Kiddy, L. Pepper, and C. A. Zittle. 1962. Variations in the α_s-casein fraction of individual cow's milk. Nature 195:1001.

Urick, J. J. 1968. Effects of linecrossing on postweaning performance on beef cattle. U. S. Range Livestock Expt. Sta. Beef Cattle Field Day Report Mimeo. p. 7.

Urick, J. J., J. S. Brinks, R. T. Clark, O. F. Pahnish, and F. S. Willson. 1966. History and performance of inbred lines of Hereford cattle. Mont. Agr. Expt. Sta. Bull. 602.

Wake, R. G., and R. L. Baldwin. 1961. Analysis of casein fractions by zone electrophoresis in concentrated urea. Biochem. Biophys. Acta. 47:225.

Watt, B. K., and A. L. Merrill, 1963. U. S. Dept. of Agriculture Handbook Composition of Foods. No. 8:39.

Waugh, D. F., and P. H. Von Hippel. κ-casein and the stabilization of casein micelles. J. Amer. Chem. Soc. 78:4578.

Webb, G. H., and A. H. Johnson, 1965. Fundamentals of Dairy Chemistry. AVI Pub. Co., Westport, Conn. Chapter 1.

Whiting, F., S. B. Slen, and L. M. Bezeau. 1952. The quantity and quality of mature ewes' milk as influenced by level of protein in the ration. Sci. Agr. 32:365.

Woychik, J. H. 1964. Polymorphism in κ-casein of cow's milk. Biochem. Biophys. Research Commun. 16:267.

Woychik, J. H. 1965. Phenotyping of κ-caseins. J. Dairy Sci. 48:496.

Woychik, J. H., E. B. Kalan, and M. E. Noelken. 1966. Chromatographic isolation and partial characterization of reduced κ-casein components. Biochem. 5:2276.

[redacted]

Weston, Arlo B.
The genetics of certain protein components in milk ..

NAME AND ADDRE

[illegible]

[redacted]