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
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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.

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by

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

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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.

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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_{s_1}-casein	A)		
	)		
	B)	1962	Thompson et al. (1962)
	)		
	C)		
	D	1966	Groschaude et al. (1966)
β-casein	A	1961	Aschaffenburg (1961) Comprising A^1, A^2 and A^3
	A^1)		
	)		
	A^2)	1966	Kiddy et al. (1966)
	)		
	A^3)		
	B	1961	Aschaffenburg (1961)
	B_z	1968	Aschaffenburg et al. (1968)
	C	1961	Aschaffenburg (1961)
	D	1968	Aschaffenburg et al. (1968)
γ-casein	A)		
	)	1967	El-Negoumy (1967)
	B)		Groves (1967)
κ-casein	A)		Neelin (1964)
	)	1964	Schmidt (1964)
	B)		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 Kopfler, 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 imformation 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*

Breed	No. Tested	α_{s_1}- A	α_{s_1}- B	α_{s_1}- C	β- A	β- B	β- C	κ- A	κ- 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 cross-breeding 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			Total Cows Sampled
Sire	X	Dam	
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 ($-10^{o}C$) 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.