STUDIES ON BIOCHEMICAL POLYMORPHISM OF MILK PROTEIN AS GENETIC MARKERS IN PIGS[1]

E. R. Chung[2], S. K. Han[3], Y. C. Shin, H. Y. Chung and J. E. Kim

Department of Dairy Science, College of Agriculture
Sang Ji University, Wonju 220-702, Korea

Summary

Biochemical polymorphisms of sow's milk proteins, β-casein (β-CN), β-lactoglobulin (β-LG), post-lactoglobulin (post-LG), α-lactalbumin (α-LA) and X-protein, as genetic markers for major pig breeds (Landrace, Yorkshire, Duroc, Hampshire and crossbred) in Korea were determined by starch gel electrophoresis. Phenotype and gene frequencies at all marker loci were estimated and genetic differences among breed populations were analyzed. Three β-CN phenotypes (AA, AB and BB) controlled by two codominant alleles (β-CN^A and β-CN^B), four β-LG phenotypes (AA, AC, AC$^{\pm}$ and CC) controlled by two codominant alleles (β-LG^A and β-LG^C) and ten X-protein phenotypes (AA, BB, CC, DD, AB, AC, AD, BC, BD and CD) controlled by four codominant alleles (X^A, X^B, X^C and X^D) were identified. In addition, a genetically controlled polymorphism of post-LG was found for the first time in sow's milk protein. Three different phenotypes (AA, AB and BB) were found in this system. These types were assumed to be under genetic control by two codominant alleles designated post LG^A and post-LG^B. Of the five marker loci examined, α-LA locus was observed to lack any individual variation in all breeds studied. All populations were in Hardy-Weinberg equilibrium for all loci. There were marked breed differences for phenotype and gene frequencies in the post-LG and X-protein marker loci. However, there were little differences between breeds in the gene frequencies at the β-CN and β-LG marker loci.

(Key Words: Milk Proteins, Biochemical Polymorphisms, Genetic Markers, Pigs)

Introduction

The six lactation specific proteins in bovine milk are the four caseins (αS_1-, αS_2-, β- and κ-casein) which make up approximately 80% of the protein, and the whey proteins, β-lactoglobulin (β-LG) and α-lactalbumin (α-LA). Since the discovery of genetic polymorphism in β-LG (Aschaffenburg and Drewry, 1955), genetically controlled variation of all six major milk proteins has been demonstrated (Eigel et al., 1984). Milk protein genetic polymorphisms have received considerable attention in recent years because of possible associations between milk protein genotypes and economically important traits in dairy cattle. A number of workers have reported that milk protein variants are associated with milk yield (Bech and Kristiansen, 1990; Brum et al., 1968; Gonyon et al., 1987; Haenlein et al., 1987; Lin et al., 1989; Ng-Kwai-Hang et al., 1984), milk composition (Aleandri et al., 1990; Lin et al., 1986; McLean, 1987; McLean et al., 1984; Ng-Kwai-Hang et al., 1986) and cheese production (Aleandri et al., 1990; Marziali and Ng-Kwai-Hang, 1986; McLean, 1987; Pagnacco and Caroli, 1987; Schaar et al., 1985). Therefore, biochemical markers such as milk protein genetic variants highly related to production characteristics could be used to predict future animal performance and could thus serve as additional selection criteria for genetic improvement (Gonyon et al., 1987).

However, in contrast to the large number of cow's milk protein polymorphisms, there are only few and conflicting data on genetic polymorphisms of sow's milk protein. Genetic polymorphism in the β-casein (β-CN) of sow's milk was first described by Glasnak (1966). Three variants were labelled as alleles A, B and C. Gerrits and Kraeling (1967) also noted the polymorphism

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[2]Address reprint requests to Dr. E. R. Chung Department of Dairy Science, College of Agriculture Sang Ji University, Wonju 220-702, Korea.

[3]Department of Dairy Science, College of Animal Husbandry, Kon Kuk University, Seoul 133-701, Korea.

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in the β-CN fraction of sow's milk; however, only two codominant autosomal alleles designated as A and B were reported. These two alleles correspond to the B and C alleles reported by Glasnak (1968). Using polyacrylamide gel electrophoresis and isoelectric focusing, Erhardt and Senft (1987) determined that β-CN polymorphism was controlled by two autosomal codominant alleles $\beta\text{-CN}^A$ and $\beta\text{-CN}^B$ in German Landrace, Edelschwein and Pietrain breeds. Recently, Erhardt (1989[a]) using SDS-gradient and polyacrylamide gel electrophoresis observed two genetic variants, β-CN A and β-CN B, in β-CN locus. On the other hand, genetically controlled polymorphism of whey protein in pigs was reported by Kraeling and Gerrits (1967, 1969). They proposed that the sow whey protein polymorphism was controlled by two codominant alleles designated Wh_1^A and Wh_1^B. In the course of an electrophoretic examination of a large number of sow milk samples, Bell et al. (1981[a]) observed two homozygous genetic variants in β-LG locus, designated porcine β-LG A and β-LG C. Erhardt and Senft (1987) observed genetic polymorphism of β-LG which seemed to be controlled by three codominant autosomal alleles, which they designated $\beta\text{-LG}^A$, $\beta\text{-LG}^B$ and $\beta\text{-LG}^C$. Data of Erhardt (1989[b]) by means of various separation techniques confirmed the existence of this genetic polymorphism. In the α-LA system, many workers (Schmidt and Ebner, 1972; Quarforth and Jenness, 1975; Kessler and Brew, 1970) did not find polymorphism for this protein in the samples of sow's milk they examined. Erhardt and Senft (1987) was also unable to find genetic polymorphism in α-LA of sow's milk using polyarcylamide gel electrophoresis and isoelectric focusing. However, Bell et al. (1981[b]) demonstrated the presence of two genetic variants of porcine α-LA and designated them A and B. The occurrence of additional whey protein polymorphism, disignated $whey_2$ protein, in sow's milk was reported by Althen and Gerrits (1971), and this sow $whey_2$ protein polymorphism was controlled by three codominant alleles Wh_2^A, Wh_2^B and Wh_2^C. The existence of post β-lactoglobulin (post-LG) in sow's whey protien has been identified (Bell et al., 1981[b]), but, until now, genetic polymorphism of this whey protein has not been reported. Genetic markers in milk and blood proteins provide a unique opportunity to study the difference in the phenotypes and gene frequencies among breed populations. This study was undertaken to determine biochemical polymorphisms of sow's milk protein as new additional genetic markers, to investigate genetic difference among breed populations using marker gene of milk protein and to get fundamental data on the relationships between genetic markers of milk protein polymorphism and economic traits of pigs in future. In addition, evidence of genetic polymorphism for post-LG locus in sow's milk protein was first documented in this report.

Materials and Methods

Milk Samples

Milk samples from a total of 416 sows of various breeds (Landrace, Yorkshire, Duroc, Hampshire and crossbred) were collected in the second to sixth week of lactation at piggeries in Kyung Gi Do and Kang Won Do districts.

Milk ejection was stimulated by 10 USP units of oxytocin injected into the ear vein. Milk was taken from each lactating gland for a representative sample from each sow. The milk fat was removed by centrifugation at 3,000 rpm for 30 min and the skimmilk stored at −20℃ until electrophoresis.

Electrophoretic Analysis

In order to analysis the genetic polymorphisms of sow's milk protein, horizontal starch gel electrophoretic technique was applied in this study.

The genetic variants of the β-CN locus were determined by urea starch gel electrophoresis, using both alkali and acid gels, according to Aschaffenburg and Thymann (1965) and Aschaffenburg (1966). Electrophoresis for typing of β-LG locus was carried out with phosphate buffer system originally designated by Bell et al. (1981[a]). Phenotyping of post-LG and α-LA was determined by a modification of the method recommended by McKenzie (1971) and Bell et al. (1981[b]). The X-protein typing performed using the buffer system described by Arave (1967) with a slight modification.

Statistical Analysis

Gene frequencies at the four milk protein loci were determined by simple gene counting method (Pirchner, 1983). Chi-square tests for goodness

of fit by the Hardy-Weinberg equilibrium were according to Pasteur et al. (1988).

Results and Discussion

β-Casein Types

Figure 1 illustrate a representative electrophoregram of starch gel electrophoretic separation of β-CN locus. The two different zones were clearly resolved and these were named β-CN A and β-CN B in order of increasing electrophoretic mobility. The homozygotes possess only one band and the hetrozygotes possess a combination of two bands to give a total of three possible phenotypes, AA, BB and AB. Based on genetic polymorphisms already found in sow's milk (Gerrits and Kraeling, 1967), the hypothesis was formulated that β-LG polymorphism is controlled by two codominant alleles, β-CN^A and β-CN^B, at a single locus. The electrophoretic pattern obtained in the present study was similar to the data reported by Gerrits and Kraeling (1967) and Erhardt (1989[6]).

The distribution of the observed phenotypes of β-CN and gene frequencies in population of five pig breeds is shown in table 1. Among three different phenotypes, the homozygous β-CN

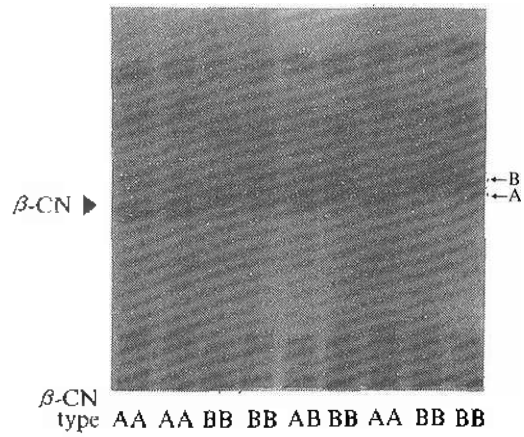

Figure 1. Electrophoregram of β-CN phenotypes in sow's milk (acid gel).

phenotype BB was the most common type in all breeds. Average frequency of appearance of β-CN BB type for the breeds was about 77.9%. In pig breeds examined, the hightest frequency of the β-CN BB type was observed in Landrace breed. The frequency of the heterozygous β-CN phenotype AB in Landrace and Duroc breeds was somewhat lower than that of the other breeds. It is noteworthy that the homozygous β-CN phenotype AA was found in Duroc and crossbred only. However, the frequency of this

TABLE 1. PHENOTYPE DISTRIBUTION AND GENE FREQUENCIES OF β-CN LOCUS

Breed	No. of sows	Phenotypes			Gene frequencies		X^2	p[1]
		AA	AB	BB	β-CN^A	β-CN^B		
Landrace	81	0 (.24)[2]	9 (8.49)	72 (72.27)	.056	.944	.282	.75>p>.50
Yorkshire	69	0 (.81)	15 (13.38)	54 (54.81)	.109	.891	.225	.75>p>.50
Duroc	72	3 (1.14)	12 (15.75)	57 (55.11)	.125	.875	2.328	.10>p>.05
Hampshire	12	0 (.18)	3 (2.64)	9 (9.18)	.125	.875	.587	.50>p>.25
Crossbred	182	2 (3.72)	48 (44.56)	132 (133.72)	.143	.857	.475	.50>p>.25
Total	416	5 (5.66)	87 (85.70)	324 (324.64)	.117	.883	.098	.90>p>.75

[1] One degree of freedom.
[2] Figures within parenthese are the expected numbers according to the Hardy-Weinberg equilibrium.

phenotype was very low (4.2 and 1.1%, respectively). The observed numbers of the phenotypes were in good agreement with expected numbers on the basis of Hardy Weinberg equilibrium in all breeds studied (table 1). Gerrits and Kraeling (1967) reported that in Duroc, Yorkshire and crossbred, the frequency of β-CN variant was 7.4% for AA types, 35.9% for AB type and 56.7% for BB type. By Bogdanov et al. (1973), the distribution of β-CN phenotype in Large White and Belorussian Black Pied breeds was estimated to be 26% for AA type, 46% for AB type and 28% for BB type. The results of the present study were quite different from the data given by these workers. This phenomenon may be due to the different genetic constitution within and between breeds.

As shown in table 1, in all breeds the frequency of the β-CN^B allele ranging from .857 to .891 was considerably higher than that of the β-CN^A allele ranging from .109 to .143. Especially, the β-CN^B allele has a high frequency in Landrace breed with a frequency of .944 compared to those in other breeds. The average gene frequency of β-CN^A for the five breeds was .117 and that of β-CN^B .883. Gerrits and Kraeling (1967) reported the gene frequencies of β-CN on 217 sow's milk smaples. According to this report, allele frequencies for β CN^A and β-CN^B were .28 and .72 for Duroc, .22 and .78 for Yorkshire and .27 and .73 for crossbred, respectively. As a whole, these β-CN^A gene frequencies were higher than those of present study, whereas β-CN^B gene frequencies tended to be lower. By the study of Erhardt and Senft (1987) in German Landrace breed, the frequency of the β-CN allele was known to be .015 for β-CN^A and .985 for β-CN^B. This value is similar to the value obtained in this study. They also reported that the frequencies of β-CN^A and β-CN^B alleles were .235 and .765 in German Edelchwein breed and .031 and .969 in Pietrain breed, respectively. Therefore, the predominant gene of β-CN in most pig breeds was β-CN^B in over 70%.

β-Lactoglobulin Types

Two bands with different mobility were detected in the β-LG locus as shown in figure 2. Four very distinct phenotypes were observed. These are designated β-LG A, B-LG C, β-LG AC and β-LG AC$^{\pm}$ in accordance with nomenclature suggested by Bell et al. (1981[a]). The A type was characterized by one major band. The C type had a similar pattern of one major band but with corresponding slower mobility. The AC type had both the major bands of A and C types. However, the AC$^{\pm}$ type was characterized by a reduced amount of band A compared with type A and a very much reduced amount of C compared with type AC. This electrophoretic patterns are in good agreement with the those reported by Bell et al. (1981[a]). Therefore, the synthesis of these β LG phenotypes was assumed to be controlled by two codominant alleles designated as β-LG^A and β-LG^C at a single autosomal locus.

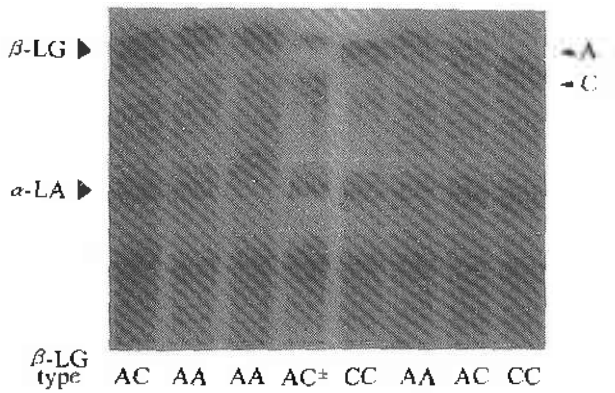

Figure 2. Electrophoregram of β-LG phenotypes in sow's milk.

The phenotype distribution and gene frequencies of β-LG in samples from various breeds are shown in table 2. The β-LG AA type in all breeds studied was found to have the highest frequency. The distribution of β-LG AA types was 100 and 95.8% for Hampshire and Duroc breeds, respectively, while the frequency in other breeds ranged from 80.2 to 85.2%. The frequencies of β-LG AC type were 5.5% for crossbred, 2.9% for Yorkshire, 9.9% for Landrace, 4.2% for Duroc and 0% for Hampshire. The frequencies for β-LG AC$^{\pm}$ type in Yorkshire, crossbred and Landrace breeds were 13.0, 12.1 and 4.9%, respectively, but this phenotype was not found in Duroc and Hampshire breeds. The β-LG CC type was found in only a few crossbred sows. Thus, there were significant differences between the five breeds of pig with respect to the frequencies of β-LG phenotypes. As shown in table 2, there was good agreement between the observed and

TABLE 2. PHENOTYPE DISTRIBUTION AND GENE FREQUENCIES OF β-LG LOCUS

Breed	No. of sows	Phenotypes			Gene frequencies		X^2	p[1]
		AA	AC($AC^{\pm}$)	CC	β-LG^A	β-LG^C		
Landrace	81	69 (69.44)[2]	12(4) (11.11)	0 (.44)	.926	.074	.015	.95>p>.90
Yorkshire	69	58 (58.44)	11(9) (10.12)	0 (.44)	.920	.080	.023	.90>p>.75
Duroc	72	69 (69.04)	3(0) (2.93)	0 (.03)	.979	.021	.009	.95>p>.90
Hampshire	12	12 (12)	0(0) (0)	0 (0)	1.000	.000		—
Crossbred	182	146 (144.20)	32(22) (35.60)	4 (2.20)	.890	.110	1.050	.50>p>.25
Total	416	354 (352.64)	58(35) (60.74)	4 (2.62)	.921	.079	.856	.50>p>.25

[1] One degree of freedom.
[2] Figures within parenthese are the expected numbers according to the Hardy-Weinberg equilibrium.

expected distribution of β-LG phenotypes in each of the five breeds and in the total breeds. Bell et al. (1981[a]) reported that the frequency of each phenotype in Yorkshire and Duroc breeds was 83.0 and 87.0% for A type, 1.4 and 6.3% for AC type, 15.1 and 5.7% for $AC^{\pm}$ type and .5 and 1.0% for C type, respectively. These results are similar to those of the present study. However, the frequencies of Yorkshire breed in this study differed markedly from those obtained by Kraeling and Gerrits (1969), who reported that phenotypic frequency in Yorkshire was AA type 12, AB type 26 and BB type 62%.

As can be seen in table 2, the highest frequencies for all breeds are of allele β LG^A ranging from .891 to 1.000. In all breeds except Hampshire gene frequencies of β-LG^A allele ranged from .890 to .979, whereas those of β-LG^C allele were very low. Only the β-LG^A allele was recognized in the Hampshire breed, but this result may be due to the small number of samples. Therfore, further tests are needed to obtain a firm conclusion. The frequencies of β-LG^A and β-LG^C alleles calculated in the present study were similar to those reported by Bell et al. (1981[a]) in Yorkshire and crossbred and by Erhardt and Senft (1987) in German Landrace. However, Kemmer (1969) reported that the frequencies of β-LG^A and β-LG^B in German Landrace were .67 and .33, respectively. Kraeling and Gerrits (1969) also reported that the gene frequencies of β-LG^A and β-LG^B were: Duroc, .95 and .05; Yorkshire, .25 and .75; and crossbred, .71 and .29.

Post Lactoglobulin Types

The presence of genetic polymorphism of post-LG protein has not yet been reported. However, in the present study, a genetically controlled heterogenity of post-LG was found for the first time in sow's milk protein. From the results shown in figure 3, it is clear that the post-LG locus shows the existence of two distinct bands on electrophoresis. This protein zone shows a slightly slower electrophoretic mobility than β-LG in alkaline gel electrophoresis and it is clearly visible by using a general protein stain. Two post-LG bands were detected in the post-LG region. These bands were tentatively designated as A and B in order of decreasing mobility. Types A and B showed a single band and type AB consisted of the bands of both types. Thus, three different post-LG phenotypes were established in this system. The three possible phenotypes and their corresponding genotypes are A (A/A), B(B/B) and AB(A/B). Although family

data were not available, it was assumed that the post-LG phenotypes are genetically controlled from a single locus by two codominant alleles, post-LG^A and post-LG^B.

The frequencies of the different phenotypes as well as the gene frequencies found in the five breeds studied are summarized in table 3. Among the three different phenotypes, the homozygous AA type was most common in all breeds except Hampsire. In Hampshire breed the AB phenotype was much more frequent that the AA phenotype. However, because of the small numbers of individuals tested changes in the distribution of the post-LG phenotypes may be expected. Therefore, to achieve a phenotypic frequency comparable to that described in the other pig

TABLE 3. PHENOTYPE DISTRIBUTION AND GENE FREQUENCIES OF POST-LG LOCUS

Breed	No. of sows	Phenotypes			Gene frequencies		X^2	p[1]
		AA	AB	BB	P-LG^A	P-LG^B		
Landrace	81	64 (64.88)[2]	17 (15.22)	0 (.89)	.895	.105	.292	.75>p>.50
Yorkshire	69	31 (29.97)	29 (31.01)	9 (8.02)	.659	.341	.048	.90>p>.75
Duroc	72	33 (33.39)	32 (31.28)	7 (7.33)	.681	.319	.033	.90>p>.75
Hampshire	12	3 (3.53)	7 (5.96)	2 (2.52)	.542	.458	.339	.75>p>.50
Crossbred	182	102 (103.20)	70 (67.70)	10 (11.10)	.753	.247	.079	.90>p>.75
Total	416	223 (231.51)	155 (157.65)	28 (26.84)	.746	.254	.108	.75>p>.50

[1] One degree of freedom.
[2] Figures within parenthese are the expected numbers according to the Hardy-Weinberg equilibrium.

breeds further tests by larger sample would be necessary. The BB phenotype was not found in the Landrace breed. Observed values for the phenotype distribution show good agreement with those calculated under Hardy-Weinberg expectations on all breeds.

Among the two alleles for post-LG, the frequency of the post-LG^A allele was considerable higher than that of the post-LG^B in all breeds examined. The frequencies of the post-LG^A allele ranged from .542 to .895. The Landrace breed has relatively high frequencies of post-LG^A and a lower frequency of post-LG^B compared with the other breeds. The average gene frequency of post-LG^A for the five breeds was 75% and that of post-LG^B 25%. This new post-LG protein, by virture of its high degree of polymorphism, should be a useful genetic marker for studying breed relationships and population studies in pigs.

On the other hand, Bell et al. (1981[b]) have been able to demonstrate the presence of two genetic variants of porcine α-LA and designated α-LA A and B. However, as shown in figure 3, all individuals had the same α-LA type in this study. This type has been assumed to be all homozygous for the α-LA^B allele. Variation similar to that described previously (Bell et al., 1981[b]) was seen in only a few milk samples but the types were not differentiated clearly enough to allow genetic interpretation. Therefore, no genetic polymorphism for α-LA was recognized in the present study. However, to obtain a firm conclusion further tests on more samples using other kind of electrophoretic method would be necessary. For the phenotyping of α-LA protein, the modified electrophoretic methods using different conditions and buffer systems are also needed.

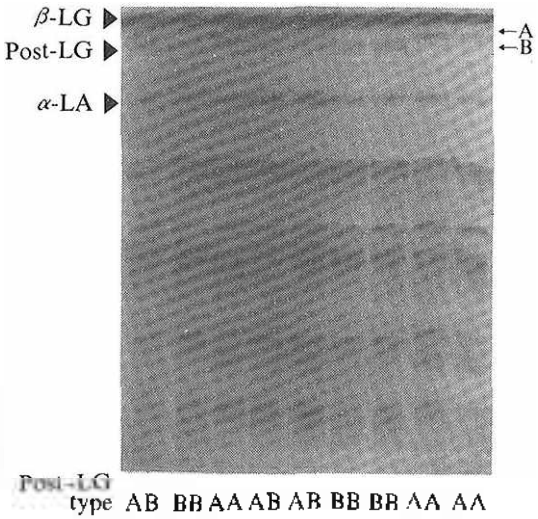

Figure 3. Electrophoregram of Post-LG phenotypes in sow's milk.

X-protein Types

During the investigation of the occurrence of genetic variants in milk proteins by various electrophoretic conditions, we have observed a variety of genetic variants in a new protein zone. As shown in figure 4, this polymorphic protein zone was slower electrophoretic mobility than the β-CN zone in starch gel using a modification of the acid buffer system. Because of an unidentified milk protein component in pigs, this protein zone was tentatively called as X-protein. A similar polymorphism in whey$_2$ protein has also been demonstrated by Althen and Gerrits (1969). On the basis of its electrophoretic patterns, the whey$_2$ proteins are likely to correspond with the X-protein observed in the present study. However, further studies are needed to identify the homogeneity between the X-protein and whey$_2$ protein. Each of the homozygous types showed one band, while the heterozygous types showed two bands as shown in figure 4. They were designated as A, B, C and D in order of decreasing mobility. Four homozygous types AA, BB, CC and DD and six heterozygous types AB, AC, AD, BC, BD and CD were observed in milk samples from the different pig breeds. Although the breeding data were not completed, it was assumed that the X-protein system is controlled by one autosomal locus with four codominant alleles X^A, X^B, X^C and X^D.

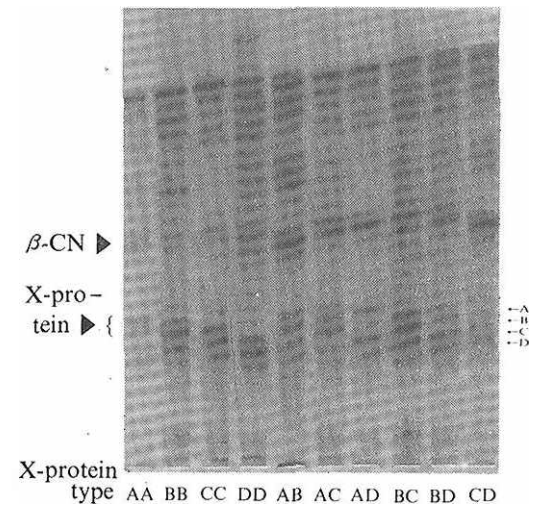

Figure 4. Electrophoregram of X-protein phenotypes in sow's milk.

The distribution of phenotypes and gene frequencies of the four alleles in X-protein based on the analysis of random samples from the different breeds studies are shown in table 4. The highest frequencies for Yorkshire, Duroc and crossbred were of heterozygote AB. The highest frequencies of BC and BB types were found in Landrace and Hampshire, respectively. The occurrence of the homozygous DD was observed in Landrace breed. This type was absent from the other breeds studied. However, the DD type had a very low frequency (1.2%) compared with the other types. The observed phenotype distributions were in good agreement with those expected according to the Hardy Weinberg equilibrium in all breeds.

Among the four alleles, the X^B gene was most common in all breeds ranging from 34.0 to 79.2%. However, the frequencies of X^D allele were the lowest. There was a somewhat difference in the frequency of the X-protein allele between the breeds. The Landrace breed has lower frequencies of X^A and X^B and higher frequencies of X^C and X^D compared with the other breeds. The Yorkshire breed has relatively high frequencies of X^A. From the above results, the genetic differences were recognized to exist between breeds. The X-protein locus thus appeared to be a useful marker for the study of relationship betweeen the different breeds of pig. Extensive studies are in progress to obtain more information on the genetic polymorphism of milk proteins in the pig breeds.

TABLE 4. PHENOTYPE DISTRIBUTION AND GENE FREQUENCIES OF X-PROTEIN LOCUS

Breed	No. of sows	Phenotypes										Gene frequencies				X^2	p
		AA	BB	CC	DD	AB	AC	AD	BC	BD	CD	X^A	X^B	X^C	X^D		
Landrace	81	4	10	9	1	12	12	4	16	7	6	.222	.340	.321	.117	1.170	.99>p>.97
		(4.0)[3]	(9.3)	(8.3)	(1.1)	(12.2)	(11.6)	(4.2)	(17.7)	(6.5)	(6.1)						
Yorkshire	69	9	15	4	0	22	7	2	8	1	1	.355	.442	.174	.029	3.398	.90>p>.75
		(8.7)	(13.5)	(2.1)	(.1)	(21.6)	(8.5)	(1.4)	(10.6)	(1.8)	(.7)						
Duroc	72	5	15	3	0	18	9	3	12	5	2	.278	.451	.202	.069	.636	p>.99
		(5.6)	(14.7)	(2.9)	(.3)	(18.0)	(8.1)	(2.8)	(13.1)	(4.5)	(2.0)						
Hampshire	12	0	7	0	0	2	0	0	3	0	0	.083	.792	.125	.000	1.583	.25>p[2]>.10
		(0)	(7.5)	(.5)	(0)	(1.6)	(0)	(0)	(2.4)	(0)	(0)						
Crossbreed	182	15	29	14	0	40	27	4	38	9	6	.278	.398	.272	.052	1.386	.97>p>.95
		(14.0)	(28.9)	(13.5)	(.5)	(40.2)	(27.5)	(5.3)	(39.4)	(7.6)	(5.1)						
Total	416	33	74	32	1	94	55	13	77	22	15	.274	.410	.254	.062	3.506	.75>p>.95
		(31.2)	(69.9)	(26.8)	(1.6)	(93.5)	(57.9)	(14.1)	(86.6)	(21.2)	(13.1)						

[1] Six degress of freedom

[2] One degree of freedom.

[3] Figures within parenthese are the expected numbers according to the Hardy-Weinberg equilibrium.

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