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STUDIES ON MILK PROTEIN POLYMORPHISM IN DANISH CATTLE AND THE INTERACTION OF THE CONTROLLING GENES

By

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The application of gel-electrophoresis in studies of genetically controlled grouped protein variations, protein polymorphism, has revealed the existence of variations in haemoglobin (9), various serum proteins (6, 7, 13, 24), serum enzymes (8, 14, 17) and milk proteins in cattle.

In milk, genetically controlled variations have been observed in whey proteins and in each of three casein fractions. In 1955 *Aschaffenburg & Drewry* (1) demonstrated in the β-lactoglobin two components designated A and B after decreasing electrophoretically mobility. The appearance of the two bands were by family investigations shown to be controlled by two codominant allele genes (2, 21). Later a third and electrophoretically slower component, β-lactoglobulin C, was observed by *Bell* (10).

In α-lactalbumin from Zebu cattle *Blumberg & Tombs* (11) demonstrated two components. Only the slowest one is, however, observed in Western breeds (4, 21). Consequently the α-lactalbumin has not been included in the present study. The remaining polymorphic systems known today in milk protein occur in the casein, which is a phosphoprotein consisting of four main components, α_{s1}-, β-, γ-, and κ-casein.

α_s-casein, the calcium sensitive part of the classical α-casein-complex, accounts for about 45 per cent of whole casein (32).

By means of electrophoresis in starch-urea-gels, *Thompson et al.* (25) demonstrated grouped heterogeneity of α_s-casein. The three components α_{s1}-A, -B, and -C (nomenclature after *Thompson et al.* (27)), always occur singly or in pairs, and family studies have shown that the three α_{s1}-components are under control of three codominant allele genes (18, 28).

β-casein, which comprises 30 per cent of whole casein (26), was the first casein fraction in which evidence for polymorphism was obtained. Using paperelectrophoresis *Aschaffenburg* (3) proved that there were three variants β-A, -B, and -C under genetic control and determined by three alleles.

The calcium-insensitive fraction of the α-casein-complex, which plays a major role in stabilization of the casein micelles and in the rennin clotting, is termed κ-casein. It accounts for approximately 15 per cent of whole casein (30), and it is the only casein component which contains S-S-linkage (31). *MacKinlay & Wake* (20) consider that κ-casein is originally synthesized as functional units containing free -SH-groups, and that these groups then become randomly cross-linked by S-S-bonds to give a product of higher molecular weight. κ-casein which normally forms a diffuse zone by gel-electrophoresis, after reduction with mercaptoethanol will therefore resolve into sharp bands, where inherited variants can be demonstrated (5, 22, 23, 33). The two variable κ-casein bands, A and B, (5) which occur either singly or in pairs are presumed to be under genetic control of allelomorphic genes.

Recently there have been indications that the three casein type-systems mentioned above are not transmitted independently. *King et al.* (19) found that the distribution of α_{s1}- and β-casein phenotypes is not in accordance with the distribution expected from random combination of genes at two loci; from family data, *Grosclaude et al.* (15) consider the α_{s1}- and β-casein types to be controlled by closely linked geneloci. The data presented by the authors in a preliminary report (29) tended to show that the three casein type-systems, α_{s1}, β and κ are products of a single locus rather than closely linked loci.

In the present study data on the occurrence of α_{s1}-, β- and κ-casein types and β-lactoglobulin types in Danish cattle breeds are given, and the mutual relationship of the milk protein systems is elucidated and discussed.

MATERIAL

Milk from 1520 heifers at the Danish bull progeny testing stations and from 542 dams was phenotyped. The heifers were offspring of 82 bulls; the parentage of these heifers was previously controlled by blood grouping tests. The genotypes of the bulls were deduced from their progeny; heterozygosity of a bull was presumed when two variants of the same protein of paternal origin were found among his offspring. If only one variant transmitted from bull to offspring could be demonstrated within a progeny group and the probability of heterozygosity was less than five per cent, then the bull was considered homozygous. Twelve bulls heterozygotic in two or three casein type-systems could be used for studies on the mutual relation of the casein type-systems. It was possible to determine the type transmitted from the sire for 122 offspring from these bulls. Thirty heifers, the offspring from 8 bulls which were heterozygous for β-lactoglobulins and for at least one of the casein systems, were used in analyses of linkage of the β-lactoglobulins and the casein type-systems.

For investigations on the occurrence of rare β-lactoglobulin variants (C and D) an additional material comprising 159 Jersey, 96 Red Danish Dairy Cattle (RDM) and 54 Black and White Danish Dairy Cattle (SDM) was analysed.

TECHNIQUE

All polymorphic milk proteins known in Western cattle breeds, as described by *Aschaffenburg & Thymann* (5), can be analyzed simultaneously by means of electrophoresis in thin starch-urea-gels, when mercaptoethanol is added to samples and gels. The same technique was modified for use of a high-voltage electrophoresis apparatus (Technik A. Hölzel, München) permitting phenotyping of 100 samples in a single run. The modifications were as follows: The hot gel was poured directly on the cooling plate of the high-voltage apparatus, where three pairs of 1.8 mm thick glasslists were stuck to the plate with silicone grease to form three 10 cm wide trays. The gel was prepared from 90 g starch (partially hydrolysed Danish potato starch), 236 g urea, 540 ml buffer (TRIS-hydroxymethylaminomethane 12,1 g/l, Na_2EDTA 1.56 g/l, H_3BO_3 0.92 g/l) and 1 ml mercaptoethanol. Five cross-cuts were made in each geltray 9 cm apart. The samples were inserted in the cross-cuts using Whatmann no. 1 filterpaper. The electrophoresis was consequently performed in a continuous buffer system, where the vesselbuffer was the same as the above gel buffer. The temperature of the cooling plate was 5°C. Electrophoresis was

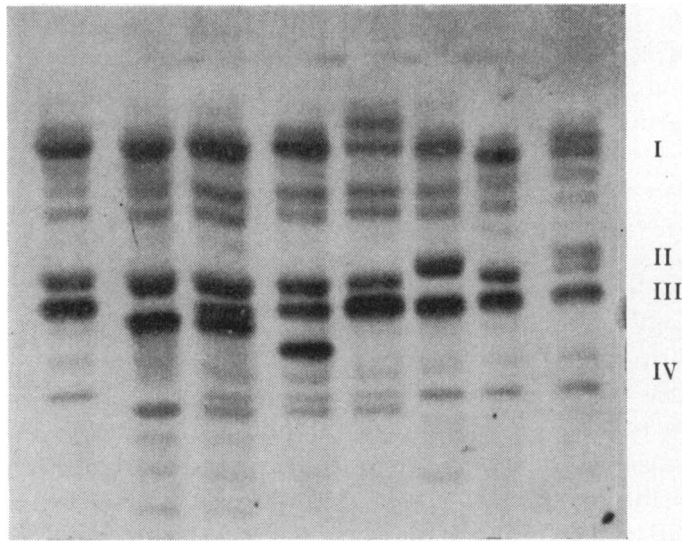

Figure 1. Types of caseins and β-lactoglobulins found in Danish cattle as demonstrated by simultaneous phenotyping of the four proteins. I α_{s1}-casein, 11 β-lactoglobulin, III β-casein, IV $\varkappa$-casein. Phenotypes of samples 1—8 are given below:

Sample no.	Phenotypes			
	I α_{s1}-casein	II β-lactoglobulin	III β-casein	IV $\varkappa$-casein
1	BB	BB	AA	AA
2	BB	BB	BB	BB
3	BB	BB	AB	AB
4	BB	BB	AC	AB
5	AB	BB	AA	AB
6	BB	AA	AA	AA
7	CC	BB	AA	AA
8	BC	AB	AA	AA

continued for six hours using 1000 v (70—100 mamp., 12—15 v per cm).

The separations of the three casein fractions and of the β-lactoglobulin obtained after electrophoresis of whole milk are illustrated in Figs. 1 and 2.

Only two β-lactoglobulin components can be seen from the figures, since when this technique is used the slower β-lactoglobulin variants migrate with the same motility as β-lactoglobulin B. In order to determine whether the rare variant hitherto found only in some Jersey cows (10) occurs in Danish cattle, samples from a smaller

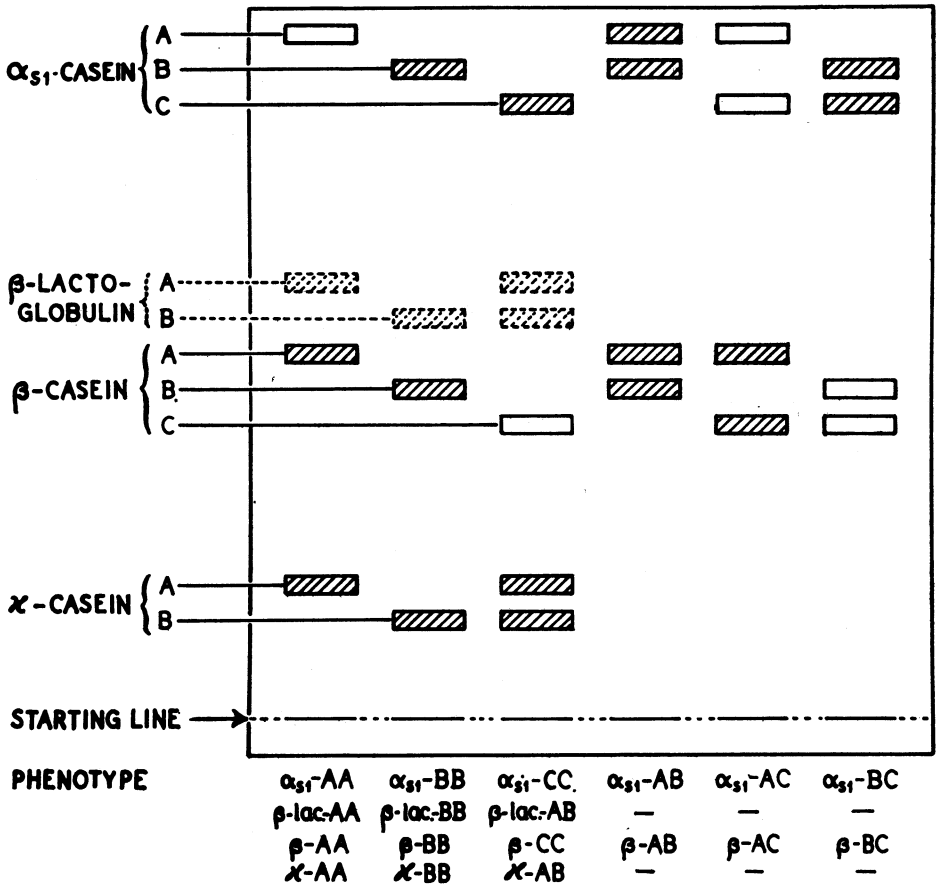

Figure 2. Diagram showing the phenotypes of α_{s1}-, β- and χ-casein and of β-lactoglobulin which can be demonstrated in a single run on thin starch-urea-gel. The hatched squares show the types found in Danish cattle.

material were analysed using traditional horizontal starch-gel electrophoresis omitting urea. The procedure was as follows: The gel contains 11.5 per cent starch (partially hydrolysed Danish potato starch) and a discontinuous lithium buffer was used (12). Gelbuffer contained 10 per cent 0.02 M lithium hydroxide, 0.076 M boric acid and 90 per cent 0.0033 M citric acid, 0.016 M TRIS-hydroxymethylaminomethane. Vesselbuffer: 0.1 M lithium hydroxide, 0.38 M boric acid. Electrophoresis at 5°C takes 3½ hours using 10 v per cm. Fig. 3 illustrates the resolution of β-lactoglobulins after electrophoresis of whole milk.

Table 1. The observed and expected distribution of phenotypes among 542 cows.

Breed	No. of animals	α_{s1}-casein						β-casein						$\varkappa$-casein			β-lactoglob.		
		A	B	C	AB	AC	BC	A	B	C	AB	AC	BC	A	B	AB	A	B	AB
RDM	obs. 282	0	273	0	3	0	6	256	0	0	25	1	0	154	20	108	2	230	50
	exp.	0	273	0	3	0	6	257	0	0	24	1	0	154	19	109	3	228	51
SDM	obs. 118	0	117	0	0	0	1	99	1	0	18	0	0	53	11	54	30	29	59
	exp.	0	117	0	0	0	1	99	1	0	18	0	0	54	12	52	30	29	59
Jersey	obs. 142	0	129	2	0	0	11	58	14	0	70	0	0	37	31	74	22	45	75
	exp.	0	127	0	0	0	14	61	17	0	64	0	0	39	33	71	25	48	69

$P > .20$

RESULTS

Inheritance and distribution of milk proteins

The distribution of α_{s1}-, β- and ϰ-casein phenotypes and the β-lactoglobulin components A and B in populations of three Danish cattle breeds is given in Table 1. The observed and expected numbers of phenotypes show agreement when the four protein systems are considered separately; it is assumed that there are three alleles within both α_{s1}-casein and β-casein, as well as two alleles in ϰ-casein and the β-lactoglobulin.

Table 2. Gene frequencies in three Danish cattle breeds.

Breed	α_{s1}-casein			β-casein			ϰ-casein		β-lactoglob.	
	α_{s1}-Cn^A	α_{s1}-Cn^B	α_{s1}-Cn^C	β-Cn^A	β-Cn^B	β-Cn^C	ϰ-Cn^A	ϰ-Cn^B	Lg^A	Lg^B
RDM	.005	.98	.01	.95	.04	.002	.74	.26	.10	.90
SDM	0	1.00	.004	.92	.08	0	.68	.32	.50	.50
Jersey	0	.95	.05	.65	.35	0	.52	.48	.42	.58

Gene frequencies calculated from the data in Table 1 are given in Table 2. In α_{s1}-casein it is seen that the B-variant is the all over dominating component in each of the three breeds. The α_{s1}-C component is very rare in RDM, SDM and Jersey, even though the frequency is a little higher in Jersey. The α_{s1}-A component, which was considered to be restricted to Holstein cattle (18), was found in three cows and five heifers of RDM, but not in SDM and Jersey. One of the three components of β-casein, the A, is seen to be dominating. Only in Jersey cattle the β-B component has any influence. The β-C variant was found in one cow and two heifers from the same progeny group of RDM. A more equal distribution of the two components, ϰ-A and ϰ-B, is found for ϰ-casein in all three breeds.

The transmission of the components within each of the four milk protein systems was studied in a family material. Details of the data obtained were given in a preliminary report (29). For the α_{s1}- and β-caseins and the β-lactoglobulins, the theory of inheritance advanced by *Thompson et al.* (25), *Aschaffenburg* (3) and by *Aschaffenburg & Drewry* (1) was confirmed.

The data on ϰ-casein are given in Table 3. In all cases the phenotype of the offspring was found to be in accordance with

Table 3. Segregation of $\varkappa$-casein types among offspring from different types of matings.

$\varkappa$-casein type of parents		No. of offspring	Distribution of offspring						$\varkappa^2$	d. f.
			A		AB		B			
♂	♀		obs.	exp.	obs.	exp.	obs.	exp.		
AA	AA	66	66	66	0	0	0	0		
AA	BB	11	0	0	11	11	0	0		
BB	AA	3	0	0	3	3	0	0		
AA	AB	47	29	23.5	18	23.5	0	0	2.57	1
AB	AA	79	37	39.5	42	39.5	0	0	0.32	1
AB	AB	92	19	23	47	46	26	23	1.11	2
BB	AB	9	0	0	4	4.5	5	4.5	0.11	1
AB	BB	29	0	0	12	14.5	17	15.5	0.86	1
BB	BB	10	0	0	0	0	10	10		

the parental type. The segregation ratios of offspring from matings involving heterozygotes were in agreement with the expected. The results presented here and previous findings (22, 23, 33, 5) demonstrate that the two $\varkappa$-casein components are controlled by two allele codominant genes.

Rare β-lactoglobulin variants

β-lactoglobulin variations were investigated by means of traditional starch-gel-electrophoresis in samples from 159 Danish Jersey cows. The well known β-lactoglobulin components A and B and a further two variants with lower mobility were demonstrated (Figs. 3 and 4). The variant with mobility a little lower than B is termed C, since it is presumed that this variant is identical with the component described by *Bell* (10); however, a comparison has not been made so far. The slowest component is, in accordance with previous nomenclature, termed D.

The distribution of the observed phenotypes is given in Table 4, where it can be seen that the C and D variants were found in heterozygotic forms combined with either β-lactoglobulin A or B. A homozygous CC and a heterozygous AD, not included in Table 4, were found in an additional material where these rare variants were expected.

The occurrence of four β-lactoglobulin components indicates that the variations of this protein are controlled by at least four allele genes. Thus, the two not observed phenotypes β-lactoglobu-

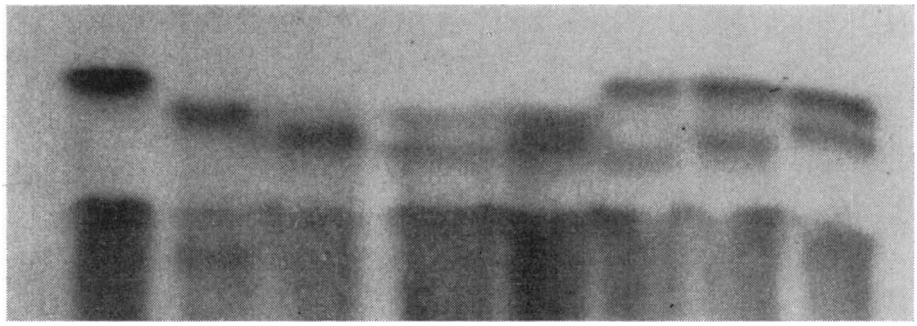

Figure 3. Starch-gel demonstrating β-lactoglobulin phenotypes. From the left the types are: AA, BB, CC, BD, BC, AD, AC and AB.

lin CD and DD are expected, but the low frequencies of the components would explain, why they have not as yet been found. Calculations of gene frequencies based on the data in Table 4 and the above hypothesis gave the following results for Danish Jersey cattle: $Lg^A = 0.39$, $Lg^B = 0.55$, $Lg^C = 0.04$ and $Lg^D = 0.02$.

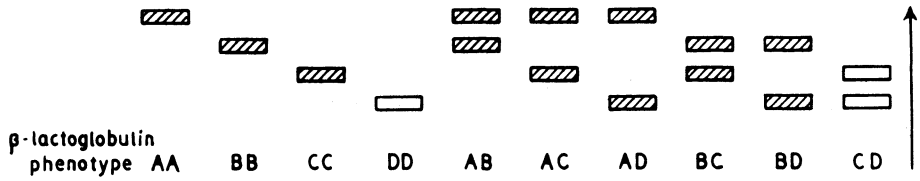

Figure 4. Diagram showing the 10 phenotypes of β-lactoglobulin according to the hypothesis of four β-lactoglobulin alleles. The hatched squares show the types hitherto found, and the arrow indicates way of migration.

From Table 4 the observed and expected numbers of phenotypes are seen to show a good agreement. Using the same technique, milk from 96 RDM and 54 SDM was analysed and neither β-lactoglobulin C nor β-lactoglobulin D were found. Further investigations are, however, necessary before any conclusion can be drawn as to the occurrence of these two variants in RDM and SDM.

The mutual relationship of the milk protein types

In previous investigations, it was demonstrated that the phenotypes of α_{s1}- and β-casein show an association (19, 15). Some combinations were found more frequently than expected and cer-

Table 4. The observed and, on basis of gene frequencies, expected distribution of β-lactoglobulin phenotypes in 159 Jersey cows.

AA		BB		CC		DD		AB		AC*		AD*		BC°		BD°		CD	
obs.	exp.	obs.	exp.	obs.	exp.	obs.	exp.	obs.	exp.	obs.	exp.	obs.	exp.	obs.	exp.	obs.	exp.	obs.	exp.
28	24.2	52	47.6	0	.3	0	.1	59	67.8	8	5.5	1	2.3	6	7.7	5	3.3	0	.3

* and ° indicate classes pooled for χ^2 calculation.
$\chi^2 = 2.2$ (d. f. 2).

Table 5. The segregation of casein types among offspring from bulls heterozygotic in at least two of the casein type-systems.

Code no. and breed of sire	Phenotype of sire	I segreg. of α_{sl}-β		II segreg. of α_{sl}-$\varkappa$		III segreg. of β-$\varkappa$		IV segreg. of α_{sl}-β-$\varkappa$	
	α_{sl}, β, $\varkappa$	α_{sl}, β	α_{sl}, β	α_{sl}, $\varkappa$	α_{sl}, $\varkappa$	β, $\varkappa$	β, $\varkappa$	α_{sl}, β, $\varkappa$	α_{sl}, β, $\varkappa$
5 RDM	BB AB AB					5 AA	1 BB	5 BAA	1 BBB
28 „	BB AB AB					4 AA	3 BB	4 BAA	3 BBB
50 „	BB AB AB					4 AA	5 BB	4 BAA	5 BBB
75 SDM	BB AB AB					1 AA	4 BB	1 BAA	4 BBB
15 Jersey	BB AB AB					6 AA	6 BB	6 BAA	6 BBB
27 „	BB AB AB					3 AA	6 BB	3 BAA	6 BBB
62 „	BB AB AB					4 AA	7 BB	4 BAA	7 BBB
70 „	BB AB AB					2 AA	4 BB	2 BAA	4 BBB
31 „	BC AB BB	3 BB	7 CA					3 BBB	7 CAB
74 „	BC AA AB			3 BA	2 CB			3 BAA	2 CAB
30 „	BC AB AB	4 BB	5 CA	4 BB	4 CA	4 AA	4 BB	4 BBB	4 CAA
66 „	BC AB AB	14 BB	12 CA	16 BB	11 CA	17 AA	13 BB	13 BBB	11 CAA
Total		21	24	23	17	50	53	52	60

tain combinations were not seen. These findings were confirmed in a material of Danish Jersey cows, and in addition the β- and ϰ-casein types were found not to be randomly distributed (29).

In Table 5, a family material relevant for studies on the mutual relation of the three casein type-systems is given. The table lists offspring from 10 bulls heterozygotic in two of the three casein type-systems, and offspring from two bulls being heterozygous for all three systems. Columns I, II and III show the combinations of α_{s1}-, β- and ϰ-casein types transmitted from sire to offspring when two systems are examined, and in column IV all three systems are considered. The number of offspring with the various types observed is also given. From the table it appears that each sire produces two types of offspring only. If the three casein type-systems are controlled by genes at separate loci, four types of gametes may be expected in all four columns, except for the last two bulls, which are expected to produce 8 types of gametes in column IV.

The fact that only two types of offspring are found in each family and the appearance of association between the phenotypes of the three casein type-systems strongly suggests that the α_{s1}-, β- and ϰ-casein types are controlled by genes at the same locus. Further evidence for this hypothesis is given by 10 of the bulls in Table 5, heterozygotic βAB ϰAB (column III), being of the same phase (βAϰA/βBϰB). In the case of linkage, assuming an equilibrium between coupling and repulsion gametes, half of these bulls are expected to be βAϰB/βBϰA. In a preliminary report (29) two offspring from bull no. 27 were found to be of a non-parental β-ϰ type. Determinations on new samples, however, showed that these two animals had been misclassified.

The data so far obtained do not exclude recombination of the α_{s1}-, β- and ϰ-casein types, but the above finding and the family data on the segregation of α_{s1}-, β- and ϰ-casein published by *Grosclaude et al.* (15, 16) suggest that the grouped variations within the three casein fractions are controlled by genes at a locus of similar nature to the one controlling the bovine B-blood group system, the E system of swine and several others of the blood group systems known in man and animals.

Since one locus is considered to be responsible for the casein types known today, attempts have been made to postulate the minimum number of alleles which could explain the casein phenotypes observed among 521 Danish cows having one offspring.

Table 6. The minimum number of casein alleles and their frequencies in Danish cattle breeds.

Allele		Frequency		
Designations in use α_{s1} β $\varkappa$	Proposed designations α_{s1} β $\varkappa$	RDM	SDM	Jersey
A A B	A G S	.006	—	—
B A A	B G P	.71	.65	.51
B A B	B G S	.23	.26	.09
B B B	B I S	.04	.09	.33
B B A	B I P	.002	.005	—
B C B	B L S	.002	—	—
C A A	C G P	.01	.005	.01
C A B	C G S	—	—	.06

The results can be seen from Table 6 where the frequencies of the postulated alleles are also given. It appears that only 8 alleles have to be postulated to account for the phenotypes so far found in Danish cattle breeds. Seven of these seem to be present in RDM and five in SDM. In Jersey five alleles were postulated, but their frequencies differ significantly from those of the two other breeds.

In Table 7, a comparison is made between the observed casein phenotypes and those expected considering the alleles of Table 6 to be present. In all three cow populations the figures are seen to show a good agreement.

Since the α_{s1}-, β- and $\varkappa$-casein types can be regarded as the product of a single locus, it would be more convenient not to use the same letters for classification of the electrophoretically determined bands within the three casein fractions and therefore, the following changes in nomenclature are proposed:

α_{s1}-casein: α_{s1}-A, -B and -C to be maintained,

β-casein: β-A, -B and -C to be changed to G, I and L respectively,

$\varkappa$-casein: $\varkappa$-A and -B to be termed P and S respectively.

To illustrate the convenience of these designations the alleles listed in Table 6 have also been written according to the proposed nomenclature.

Table 7. The observed and expected distribution of phenotypes considering one locus controlling the three casein type-systems.

Breed		Phenotypes																	
	α_{s1}	AB	AB	BB	BB	BB	BB	BB	BC	BC	BB	BB	BC	BC	BB	BC	BC	BC	CC
	β	AA	AA	AA	AA	AB	AB	AC	AA	AA	AA	AB	AA	AA	BB	AB	AB	AB	AA
	$\varkappa$	AB	BB	AA	AB	AB	AA	AB	AA	AB	BB	BB	AB	BB	BB	AB	BB	AA	BB
RDM 252	obs.	3	0	129	80	13	1	1	3	0	12	7	3	0	0	0	0	0	0
	exp.	2	1	128	81	15	1	1	4	0	13	5	1	0	0	0	0	0	0
SDM 109	obs.	0	0	44	40	13	0	0	0	0	6	4	0	0	1	0	0	1	0
	exp.	0	0	46	36	12	1	0	1	0	7	5	0	0	1	0	0	0	0
Jersey 160	obs.	0	0	43	13	55	0	0	2	8	2	10	0	0	17	1	8	0	1
	exp.	0	0	42	14	55	0	0	2	10	1	9	0	2	18	1	6	0	0

Investigation on the relationship between the β-lactoglobulins and the casein types showed that the phenotypes of the β-lactoglobulins were distributed independently of the casein types. It was found that a linkage of less than five per cent recombination between the β-lactoglobulin and the casein is very unlikely.

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SUMMARY

In milk samples from 1520 heifers and 542 dams the α_{s1}-, β- and $\varkappa$-casein types and the β-lactoglobulin components, A and B, were determined simultaneously by means of electrophoresis in thin starch-urea-gels.

The theory of inheritance advanced within each system from previous investigations was confirmed. Family data on the transmission of the two $\varkappa$-casein components A and B are given (Table 3). The frequencies of the controlling genes in Danish cattle were computed for each of the systems (Table 2).

With traditional starch-gel-electrophoresis, four components, termed A, B, C and D, could be demonstrated in the β-lactoglobulin (Figs. 3 and 4). The four components are presumed to be controlled by four allele genes.

The α_{s1}-, β- and $\varkappa$-casein phenotypes were not independently distributed and the segregation of casein types among offspring from bulls heterozygotic in two or three casein type-systems strongly suggests that the genetic variations of the α_{s1}-, β- and $\varkappa$-casein are controlled by genes at a single locus. Then, a minimum of 8 alleles may account for the phenotypes observed among 521 Danish cows (Table 6).

Linkage of less than five per cent recombination could be excluded between the casein types and the β-lactoglobulin types.

ZUSAMMENFASSUNG

Untersuchungen betreffend Milchproteinpolymorphie bei dänischem Vieh und das gegenseitige Verhältnis der kontrollierten Gene.

Mit Hilfe einer Elektrophorese in dünnen Harnstoff-Stärkegelen wurde in Milch von 1520 Stärken auf den Nachkommenschaftprüfungsstationen und 542 Mutterkühen eine Bestimmung von α_{s1}-, β- und $\varkappa$-Casein sowie β-Lactoglobulintypen vorgenommen. Die Erbhypothesen innerhalb jedem dieser vier Systeme, die auf Grundlage von früheren Untersuchungen vorausgesetzt worden waren, konnten bestätigt werden. Die Häufigkeit der kontrollierten Gene bei dänischem Vieh ist in Tabelle 2 angegeben.

Anhand der traditionellen Stärkegelelektrophorese sind vier β-Lactoglobulinkomponente festgestellt worden. Diese bekamen die Bezeichnungen A, B, C und D, und es wird angenommen, dass sie von vier allelen Genen kontrolliert werden.

Die α_{s1}-, β- und χ-Caseintypen zeigen nicht unabhängige Vererbung, und die Ausspaltungsverhältnisse unter den Nachkommen von heterozygoten Stieren lassen vermuten, dass die Variationen in den drei Caseintypsystemen von Genen kontrolliert werden, die demselben Locus angehören. Unter dieser Voraussetzung lassen die beobachteten Phänotypen bei 521 Kühen sich durch ein Minimum von 8 Allelen erklären. Eine Kopplung mit weniger als 5 % Überkreuzung zwischen den Caseintypen und den β-Lactoglobulintypen kann ausgeschlossen werden.

SAMMENFATNING

Undersøgelser over mælkeproteinpolymorfi hos dansk kvæg og de kontrollerende geners indbyrdes relationer.

Ved elektroforese i tynde urinstof-stivelsegeler er der foretaget en bestemmelse af α_{s1}-, β- og χ-kasein samt β-laktoglobulintyper i mælk fra 1520 afkomsprøvekvier og 542 mødrekøer. De ud fra tidligere undersøgelser fremsatte arvehypoteser inden for hver af de fire systemer har kunnet bekræftes, og hyppigheder for de kontrollerende gener hos dansk kvæg er anført (Tabel 2).

Ved hjælp af traditionel stivelsesgelelektroforese er der påvist fire β-laktoglobulinkomponenter. Disse er benævnt A, B, C og D og antages at være kontrolleret af fire allele gener.

α_{s1}-, β- og χ-kaseintyperne viser ikke uafhængig nedarvning, og udspaltningsforholdene blandt afkom efter heterozygote tyre tyder stærkt på, at variationerne i alle tre kaseintypesystemer er kontrolleret af gener tilhørende samme locus. Under denne forudsætning kan de observerede fænotyper hos 521 køer forklares ved et minimum af 8 alleler. Kobling med under 5 % overkrydsning kan udelukkes mellem kaseintyper og β-laktoglobintyper.

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