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β-LACTOGLOBULIN PHENOTYPES IN FINNISH AYRSHIRE AND FRIESIAN CATTLE WITH SPECIAL REFERENCE TO MASTITIS INDICATORS

By

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ATROSHI, F., R. KANGASNIEMI, T. HONKANEN-BUZALSKI and M. SANDHOLM: *β-lactoglobulin phenotypes in Finnish Ayrshire and Friesian cattle with special reference to mastitis indicators.* Acta vet. scand. 1982, *23*, 135—143. — An unselected material consisting of composite milk samples from 1029 Ayrshire and 113 Friesian cows were analysed for the β-lactoglobulin types. The frequencies of β-Lg types were AA 8.3 %, AB 45.5 % and BB 46.3 % for Ayrshire cows and 22.1 %, 45.1 % and 32.8 % for Friesian cows, respectively. The relationship between β-Lg type with milk BSA, somatic cell count, protein percentage, fat percentage and milk production were analysed. AA cows were significantly higher in daily milk yield but lower in protein percentage and fat percentage than BB cows; AB types were intermediate. The annual production was highest in AB-animals. There was a tendency for AA cows to have high somatic cell counts but low milk BSA concentrations.

β-lactoglobulins; milk BSA; somatic cell count; bovine mastitis; bovine milk; milk proteins.

The application of electrophoretic procedures for analysis of milk proteins has produced evidence for the existance of genetic variants of the principal protein constituents. *Aschaffenburg & Drewry* (1957) who used paper electrophoresis, were able to classify β-lactoglobulin (β-Lg) from individual cows into distinct genetic types. So far 3 β-lactoglobulin fractions (A, B and C) have been described (*Aschaffenburg & Drewry, Bell* 1962). The variation is controlled by 3 co-dominant alleles, Lg^A, Lg^B and Lg^C each giving a single protein fraction.

A considerable number of studies have now been carried out in an effort to find out the relationships between genes for milk β-Lg types and mastitis in cattle. The most comprehensive study made so far of this relationship is that of *Osterhoff et al.* (1973) who came to the conclusion that heterozygous cows (β-Lg^{AB}) have a lower prevalence of mastitis than homozygous cows AA or BB, as indicated by negative bacteriology. β-Lg types also appear to play an important role in production traits in cattle (milk yield, fat, protein and lactose) (*Meyer et al.* 1978). It would appear possible that β-Lg phenotypes could be utilized as markers of production performance of the cow. *Meyer et al.* made an interesting observation that the serum albumin (BSA) concentration of milk and β-Lg phenotypes correlated with each other. The Lg^{A} homozygous cows had the highest albumin content in milk, whereas β-Lg^{B} homozygous cows had the lowest. As milk BSA concentration has been suggested as an indicator of the severity of mastitis, it seemed important to establish normal BSA values for different β-Lg phenotypes.

MATERIALS AND METHODS

The total material consisted of 1200 unselected milk samples. Each sample represented a daily composite bucket sample for each cow. The final material consisted of 1029 Ayrshire cows and 113 Friesian cows. From the Ayrshire cows, data about the production characteristics, lactation stage and number of calvings were obtained from the ADP-bank of the National Cattle Recording Scheme. From this material 707 cows had the relative annual milk yield available for the previous calendar year. These were used to study the effects of β-Lg type on the yearly production.

Cell counts

The somatic cell counts were calculated by using the Fossomatic method (*Heeschen* 1975) and are expressed in thousands/ml milk.

Serum albumin (BSA) in milk

Milk BSA was determined by radial immunodiffusion in agar-gels containing porcine anti-BSA (*Mancini et al.* 1965, *Honkanen-Buzalski et al.* 1982). The BSA concentrations are given in mg/ml.

Electrophoretic determination of β-lactoglobulin phenotypes

The gels were prepared by dissolving 0.3 g agarose in 30 ml 0.02 mol/l barbital buffer, pH 8.6, in a bath of boiling water. When the agarose was dissolved, the solution was poured on a 12×20 cm glass plate lying on a horizontal table. A template for preparing 1×15 mm slots for sample application was inserted and the agarose was allowed to solidify in a humid chamber at room temperature for 2 h. Milk samples were delipidated by centrifugation at 2000 g/10 min at 4°C. To 1 ml of delipidated milk, 0.1 ml rennin dilution was added (1 ml commercial rennin + 15 ml 0.1 mol/l Na-acetate buffer, pH 5.0) and the mixture was incubated for 4 h at 37°C and recentrifuged. Twenty µl of the supernate was transferred into the slot of the agarose gel and separated under a voltage gradient of 150 V/cm for 3 h at 4°C. After this the β-Lg bands had migrated 6—7 cm from the original as shown in Fig. 1. After electrophoresis the agar-plate was fixed with picric-acetic acid mixture, dried under pressed filter paper and stained with Amido Black (*Weeke* 1973).

Statistical analyses

The data was analysed by the least squares method according to the principles of *Searle* (1971), using a Hewlett-Packard 1000 computer.

The following fixed model was used in studying the effect of β-Lg types on different traits, milk yield and milk constituents.

$$y_{ijkl} = \mu + a_i + b_j + c_k + e_{ijkl}$$

where, μ = constant

a_i = effect of i^{th} β-Lg type, $i = 1, 2, 3$

b_j = effect of j^{th} stage of lactation (classified), j = 4—14, 15—30, 31—60, 61—90, 91—150, 151—210, 211—270, 271 days and over

c_k = effect of k^{th} number of calving, k = 1, 2, 3, 4, 5, 6, 7 and over

e_{ijkl} = error assumed to be N $(0,\sigma_e^2)$.

The somatic cell count in thousands and BSA concentration (mg/ml) were transformed to natural logarithms due to a skewed distribution of the original values. Significance in frequency distribution of β-Lg type vs. cell count and BSA-concentration was tested using G-statistic (*Sokal & Rohlf* 1969).

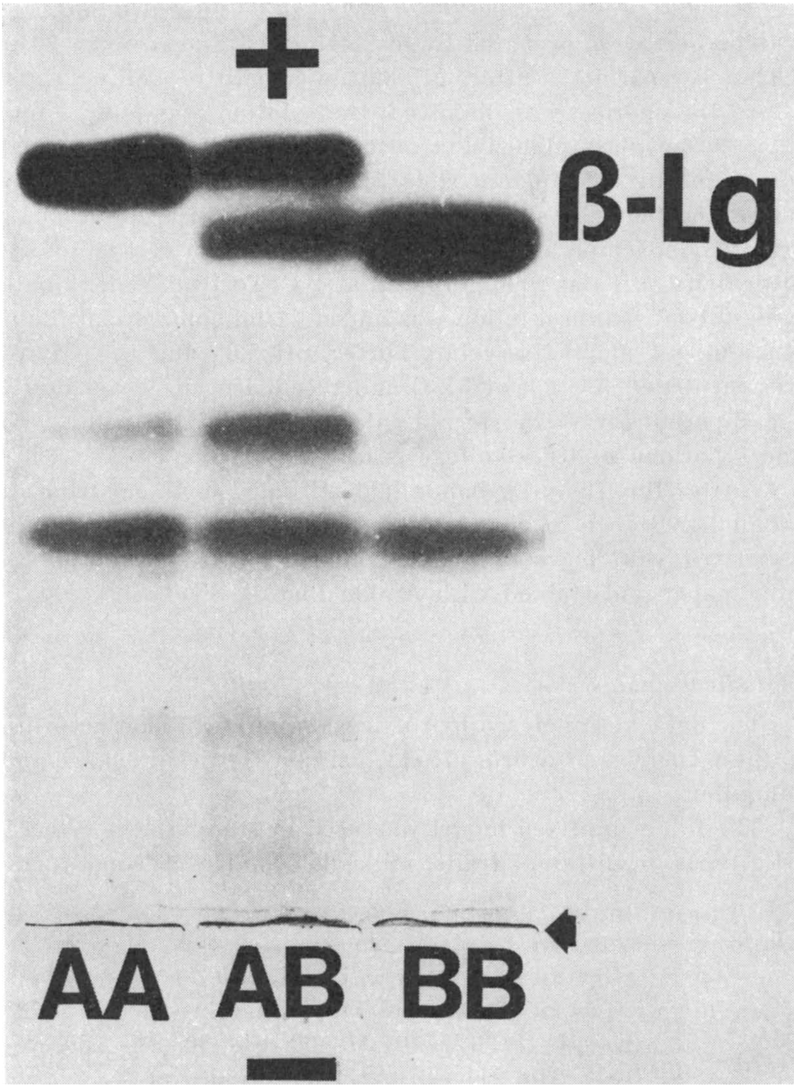

Figure 1. Separation of different β-Lg types by agarose electrophoresis. The point of application is marked by an arrow.

RESULTS

The means and standard deviations for each trait are shown in Table 1. The distribution of β-Lg types and their genefrequencies are listed in Table 2. β-Lg type BB cows were predominant (46.3 %) in Ayrshires. The frequency of β-Lg^B-allele in the Fin-

Table 1. The means and standard deviations of different traits of 1029 Finnish Ayrshire cows.

Trait	$\bar{x}$	s
Somatic cell counts × 1000	347	746
ln (somatic cell count × 1000)	4.88	1.30
BSA, mg/ml	0.234	0.123
ln (BSA, mg/ml)	—1.56	0.43
Milk yield, kg/day	18.8	6.5
Milk fat, %	4.41	0.72
Milk protein, %	3.45	0.43

nish Ayrshire was 0.69 while that of allele β-Lg^{A} was 0.31. The frequencies of β-Lg types in the 113 Friesian cows were β-Lg^{A} was 0.45 and β-Lg^{B} 0.55.

For the illustration of the possible association of lactoglobulin types with mastitis indicators, a crude grouping was done on the basis that about 30 % of the cows would become included in the "high" groups. This figure would roughly represent the prevalence of chronic mastitis in Finnish herds. The threshold levels became 250,000 cells/ml for somatic cells and 0.25 mg/ml for BSA. Table 3 gives a distribution of cows of different β-Lg types belonging to "high" or "low" groups of somatic cell count and BSA-concentration. A tendency of AA homozygous having higher cell counts and lower albumin values can be seen. However, the differencies are not statistically significant.

As regards the β-Lg type and milk constituents, the following conclusions can be drawn from Table 4: In comparison with other groups, AA cows had high somatic cell counts, low milk

Table 2. The frequency of β-lactoglobulin phenotypes and β-Lg gene frequencies in Finnish Ayrshire and Friesian cattle.

Lactoglobulin phenotype	Ayrshire		Friesian	
	number	%	number	%
AA	85	8.26	25	22.1
AB	468	45.48	51	45.1
BB	476	46.26	37	32.8
Total	1029	100.00	113	100.0
Gene β-Lg^{A}	0.31		0.45	
frequency β-Lg^{B}	0.69		0.55	

Table 3. Distribution of somatic cell counts and albumin groups according to β-Lg types in Ayrshire cows. Figures in brackets give the number of animals.

Lactoglobulin phenotype	Somatic cell count		BSA	
	< 250,000	> 250,000	< 0.25	> 0.25
AA	63.5 % (54)	36.5 % (31)	77.6 % (66)	22.4 % (19)
AB	71.8 % (336)	28.2 % (132)	70.7 % (331)	29.3 % (137)
BB	70.6 % (336)	29.4 % (140)	67.4 % (321)	32.6 % (155)
Significance	$\delta^2 = 2.29$, n.s		$\delta^2 = 4.06$, n.s	

BSA, high milk yield, but were low in fat and protein percentages. AB cows proved to be intermediate between AA and BB in all traints. The differences between β-Lg types were significant only in daily milk yield, fat and protein percentages as shown in Table 4. A study of the relative milk yield for the last calender year (707 cows) showed that AB cows produced more (AB 101.7 %, AA 99.6 % and BB 99.0 %) as calculated from the relative milk yield within farms.

Table 4. Least squares deviations of β-Lg types in different traits of Ayrshire cows. The deviations are from the mean of the BB type. The figures are adjusted for the effect of stage of lactation and calving number.

β-lactoglobulinln phenotype	somatic cells	ln BSA	milk yield kg/day	fat %	protein %
AA	+0.12	—0.10	+1.19	—0.25	—0.11
AB	+0.01	—0.04	+0.67	—0.11	—0.01
BB	5.09	—1.62	19.72	4.56	3.46
F-value	0.3	2.5	3.6	6.0	4.1
significance	n.s	n.s	*	**	*

* $P < 0.05$
** $P < 0.01$
n.s = non-significant

DISCUSSION

The present results show the existence of $\beta\text{-Lg}^A$ and $\beta\text{-Lg}^B$ alleles in the Finnish Ayrshire and Friesian cattle breeds. The frequency of β-Lg types in Finnish Ayrshire cattle are similar to those obtained by *Aschaffenburg* (1965) in Ayrshire cattle in

England. If the expected gene frequencies for AB-type cows are calculated in the present Ayrshire material starting either from BB or AA cows, AB cows represent about 3—4 % higher frequency than expected. Because AB types showed a higher annual production, the heavy selection for higher milk yield could result in an increased number of AB type as seen in the present material.

When relationships between the β-lactoglobulin types and production characteristics are considered, cows of AA type were found to be significantly higher in daily milk yield. However, the fat and protein percentages in milk were the lowest in AA animals. The negative correlations between milk yield and fat and protein percentages result in the fact that when calculated on daily fat and protein yield basis, the effects of β-Lg types on fat and protein disappeared.

When considering milk somatic cell contents and BSA concentrations, the present results are in agreement with the data of *Meyer* (1978). Relatively fewer AA cows were included in the high BSA-group (Table 3), whereas relatively more AA animals were included in the high somatic cell group.

Milk somatic cell counts and BSA have been used as indicators of mastitis. Generally milk BSA and somatic cell count are positively correlated and can be used as mastitis indicators (*Schalm et al.* 1971, *Giesecke & Viljoen* 1974, *Smith et al.* 1979, *Honkanen-Buzalski & Sandholm* 1981). There was a tendency towards lower BSA in AA animals in samples with a high somatic cell contents although the differencies were not significant. However, observations that AA type may differ from the general correlation, raises the question of reliability of the cell counts and albumin determination as mastitis indicators as such.

The BSA is thought to diffuse from blood to the alveolar space through the separated tight junctions between the endothelial and epithelial cells mainly due to mediators from activated inflammatory cells.

When the present results of low albumin contents in AA type milk samples with high somatic cell content are combined with earlier reports of lower mastitis frequency (negative bacteriology) (*Osterhoff et al.* 1973, *Kriventzov et al.* 1975) in AB or BB cows, one could expect that a greater degree of somatic cells in AA type animals are not inflammatory cells (neutrophils, macrophages). If they are these do not become activated to the same extent than as in AB or BB groups.

It should be pointed out that the present investigation was carried out on "normal" composite milk samples; the effects of mastitis would become more clear if quarter milk samples were analysed separately when the effect of mastitis on individual quarter were not shadowed by dilution effects.

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SAMMANFATTNING

β-lactoglobulin fenotyper i finska Ayrshire och Friesiska kor med speciell hänsyn til mastitindikatorer.

Ett material av komjölkprover från 1029 Finska Ayrshire kor och 113 Friesiska kor analyserades gör att bestämma β-lactoglobulin typ. Frekvensen av β-lactoglobulin typer var AA 8.3 %, AB 45.5 % och BB 46.3 % för Ayrshire kor och 22.1 %, 45.1 % och 32.8 % för Friesiska kor. Korrelationen mellan β-lactoglobulin typ och mjölkalbumin (BSA), cellhalt, proteinhalt, fetthalt och mjölkproduktion analyserades. Den årliga mjölkproduktionen var högst hos AB kor. När mjölkens cellhalt och BSA halt analyserades hos de olika lactoglobulin typerna, kunde man se en tendens att AA kor hade högre cellhalt men lägre albuminvärden än väntat.

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