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Survey of β-Lactoglobulin and α_{S1}-Casein polimorphisms in Hungarian dairy sheep breeds and crosses on DNA level
(short communication)

Summary

Beta-lactoglobulin (LGB) and the Welsh α_{S1}-casein (α_{S1}-casein D) types have been determined using PCR-RFLP tests in DNA isolated from blood samples collected from four dairy sheep breeds (Awassi, British Milk Sheep, Tsigaia, Lacaune), from Hungarian Merinos and from various crossbreeds {(Awassi×Merino) F_1, (Merino×Langhe) F_1, (Merino×Pleven Blackhead) F_1, (Merino×Pleven Blackhead) F_1× Black East Friesian}. The following LGB^A frequency values have been obtained: Awassi 0.3478; British Milk Sheep 0.6857; Tsigaia 0.5650; Lacaune 0.4730; Hungarian Merino 0.6767; (Awassi× Merino) F_1 0.4694; (Merino×Langhe) F_1 0.7777; (Merino×Pleven Blackhead) F_1 0.5945; (Merino×Pleven Blackhead) F_1×Black East Friesian 0.6026.
The Welsh α_{S1}-casein variant was found in the Hungarian Merino breed only (4 homozygous and 16 heterozygous ewes). Plans for studies on the possible effects of these milk protein types, on milk yield and composition as well as on cheese making properties of milk and cheese yields are outlined.

Key words: sheep, milk proteins, PCR-RFLP

Zusammenfassung

Titel der Arbeit: **β-Lactoglobulin und α_{S1}-Casein Polymorphismus bei ungarischen Milchschafrassen und Kreuzungen, untersucht anhand von DNA-Analysen** (Kurzmitteilung)
Varianten des Beta-Lactoglobulins (LGB) und das Welsh α_{S1}-Casein (α_{S1}-Casein D) wurden mit Hilfe von PCR-RFLP Analysen anhand von DNA Präparaten aus Blutproben von vier Milchschafrassen (Awassi, Britisches Milchschaf, Tsigaia, Lacaune), dem Ungarischen Merinoschaf und verschiedenen Kreuzungen {(Awassi×Merino) F_1, (Merino×Langhe) F_1, (Merino×Pleven Schwarzkopf) F_1, (Merino×Pleven Schwarzkopf) F_1×Ostfriesisches Milchschaf}, bestimmt. Für LGB^A wurden dabei folgende Häufigkeiten ermittelt: Awassi 0,3478; Britisches Milchschaf 0,6857; Tsigaia 0,5650; Lacaune 0,4730; Ungarisches Merinoschaf 0,6767; (Awassi×Merino) F_1 0,4694;(Merino×Langhe) F_1 0,7777; (Merino×Pleven Schwarzkopf) F_1 0,5945; (Merino×Pleven Schwarzkopf) F_1×Ostfriesisches Milchschaf 0,6026.
Die Variante Welsh α_{S1}-Casein wurde nur in der Rasse Ungarisches Merinoschaf gefunden (4 homozygote und 16 heterozygote Mutterschafe).
Die Untersuchungen zu den Auswirkungen dieser Milchprotein-Varianten auf den Milchertrag, auf die Milchzusammensetzung sowie auf die Käseproduktion werden diskutiert.

Schlüsselwörter: Schaf, Milchproteine, PCR-RFLP Analyse

Introduction

LGB is the major whey protein in sheep milk. It is able to bind and transport small hydrophobic molecules (e.g. retinol and fatty acids) but its biological role is still unknown (GODOVAC-ZIMMERMANN et al., 1988).
Two main types of LGB (A and B) were described by BELL and Mc KENZIE (1967). A study concerning the distribution of LGB in various breeds was made by KING

(1969). KOLDE and BRAUNITZER (1983) described the difference between variants A and B at amino-acid position 20, where variant A has His while variant B has Thr.
ERHARDT (1989) and RECIO et al. (1995) evidenced a third variant (C) at the LGB locus in German Merinoland and Spanish Merino sheep by isoelectric focusing. The variant C is a subtype of A with a single exchange Arg→Glu at position 148 (ERHARDT, 1989).
The LGB was assigned to chromosome 3 in sheep (HAYES et al.,1993).
SCHLEE et al. (1993) detected in DNA the same polymorphism described already in milk by amplifying a DNA fragment of intron I and exon I of LGB by PCR. An RsaI endonuclease restriction site has been evidenced (GT/AC) in the amplified region for allele A while allele B has no such a restriction site.
PRINZENBERG and ERHARDT (1999) and ANTON et al. (1999) developed a PCR-RFLP typing procedure using MspI and Alu I for the detection of both mutations (alleles A, B and C).
Caseins are the only coagulable milk proteins, the yield and the quality of cheese depends primarily on the amount of them. Casein genes are located on the chromosome two (MERCIER et al., 1985).
Two α_{s1}-casein variants „Normal" and „Welsh" had been identified by KING (1967).
FERRANTI et al. (1995) described three genetic variants (A, C, and D) which correspond to simple mutations, involving the degree of phosphorilation of each of them. CHIANESE et al. (1996) detected five variants of α_{s1}-casein (A, B, C, D and E).

Relationship between genetic variants and milk production traits

On one hand there is little information available concerning the relationship between the genetic variants of LGB and milk yield or milk properties, on the other hand, data published in this field are very contradictory.
Some authors e.g. DI STASIO et al. (1992) in Valle del Belice, BARILLET et al. (1993) in Lacaune and RECIO et al. (1995) in Lacha breeds found no relationship between LGB genotypes and milk yield, fat or protein content. GARZON and MARTINEZ (1992) showed that in Manchega breed variants AA and AB are superior in protein and casein content and curd yield. The same results were obtained by LOPEZ-GALVEZ et al. (1994).
On the contrary, BOLLA et al.(1989) in Sardinian breed evidenced that variant BB is associated with higher milk yield than variants AA and AB. FRAGHI et al. (1996) also came to the conclusion that in Sardinian breed variant BB affected favourably milk yield, as well as fat and protein content. According to the results obtained by HERGET et al. (1995) in East Friesian breed, it may be desirable to select for dairy sheep carrying the BB genotype. The milk yield, protein content and the lactation number of these animals was significant higher compared to sheep carrying the AA and AB genotypes. Furthermore, homozyguos BB animals had definitely higher litter size.
With reference to α_{s1}-casein only the D (Welsh) variant is related to modification of the casein content and rennet coagulation (PIREDDA et al., 1993). PIRISI et al. (1995)

showed in Sarda breed that milk from homozygous DD animals had significantly lower total protein content, casein and protein/fat ratio than milk from animals carrying other variants of α_{S1}-casein. This variant has been found in various sheep breeds with very low frequency.

Materials and Methods

1607 blood samples have been collected from different sheep breeds existing in Hungary such as Awassi, British Milksheep, Tsigaia, Lacaune, Hungarian Merino and their crosses. Blood samples have been stored at -20°C until DNA extraction, LGB types and the Welsh variant were determined by PCR-RFLP assay.

All chemicals were purchased from Promega (Madison, Wi, USA) unless indicated otherwise.

The alleles A and B of LGB have been determined using the primers mentioned below:

Forward primer : 5′ CTTCCCACCCCCAGAGTGCAAC 3′

Reverse primer : 5′ TGGGGAGTGGGGGTTCCATGTT 3′

Amplification conditions: 94 °C for 1 min., 94 °C 45 sec., 65 °C 1 min., 72 °C 1 min., cycle number: 31 ; and 72 °C for 10 min. The concentrations of reaction components in 10 µl PCR volume were 200 µM dNTP, 0,2 µM primer, 1,5 mM $MgCl_2$, 0,5 U DyNAzyme polymerase (Finnzymes Oy, Espoo, Finland).

Restriction endonuclease digestion was carried out at 37 °C for 2,5 hours with 5 U RsaI restriction enzyme which differentiates between genotypes A and B at position 1617 (HARRIS et al., 1988).

Since allele C is a subtype of allele A the previously determined AA and AB genotypes have been further analysed by a separate PCR-RFLP assay which can yield evidence for the presence or absence of allele C.

Forward primer: 5′ TCAGGACCCCGGAGGTGGACAAC 3′

Reverse primer: 5′ CCTCCAGCTGGGTCGGGTTGAAG 3′

Amplification conditions : 94 °C for 1 min., 94 °C 15 sec., 60 °C 1 min., 72 °C 10 sec., cycle number: 30 ; and 72 °C for 10 min. The concentrations of reaction components in 10 µl PCR volume were 200 µM dNTP, 0.2 µM primer, 1.5 mM $MgCl_2$, 0.25 U Taq.

Restriction endonuclease digestion was carried out at 37 °C for 2.5 hours with 5 U MspI restriction enzyme, which differentiates between genotypes A and C at position 4626 (HARRIS et al., 1988).

All the primers were designed according to the ovine LGB sequence characterised by HARRIS et al. (1988) and ERHARDT (1989).

The identification of the animals carrying the Welsh variant of α_{S1}-casein was performed according to the method described by RAMMUNO et al. (1997).

Results

The distribution of the genotype and allele frequency values is presented in Table 1 and 2, respectively. The expected number of the individual genotypes has been calcula-

Table 1
LGB genotype frequencies in Hungarian dairy breeds and crosses (Merinos are as controls) (LGB Genotyp-frequenzen bei ungarischen Rassen und Kreuzungen)

breeds/crosses	n	Genotype frequencies AA	AB	BB	χ^2
Awassi	184	27 (22.26)	74 (83.47)	83 (78.27)	2.35
		14.7 %	40.2 %	45.1 %	
British Milksheep	175	94 (82.28)	52 (75.44)	29 (17.28)	16.88
		53.7 %	29.7 %	16.6 %	
Tsigaia	100	36 (31.92)	41 (49.15)	23 (18.93)	2.74
		36 %	41 %	23 %	
Lacaune	148	42 (33.11)	56 (73.79)	50 (41.10)	8.58
		28.4 %	37.8 %	33.8 %	
Hungarian Merino	546	268 (250.03)	203 (238.90)	75 (57.07)	12.31
		49.1 %	37.2 %	13.7 %	
(Awassi x Merino) F_1	98	28 (21.59)	36 (48.82)	34 (27.59)	6.74
		28.6%	36.7%	34.7 %	
(Merino x Langhe) F_1	36	22 (21.77)	12 (12.45)	2 (1.78)	0.03
		61.1 %	33.3 %	5.6 %	
(Merino x Pleven Blackhead) F_1	164	65 (57.96)	65 (79.07)	34 (26.97)	5.18
		39.6 %	39.6 %	20.8 %	
(Merino x Pleven B.) F_1 x Black East Friesian	156	70 (56.65)	48 (74.71)	38 (24.64)	19.92
		44.9 %	30.7 %	24.4 %	

df=2, P= 0.5; The expected values are presented in brackets

Table 2
LGB allele frequencies in Hungarian dairy sheep breeds and crosses (Merinos are as controls) (LGB Allel-frequenzen in ungarischen Rassen und Kreuzungen)

Awassi	(184)	A: 0.3478	B: 0.6522
British Milksheep	(175)	A: 0.6857	B: 0.3143
Tsigaia	(100)	A: 0.5650	B: 0.4350
Lacaune	(148)	A: 0.4730	B: 0.5270
Hungarian Merino	(546)	A: 0.6767	B: 0.3233
(Awassi x Merino)F	(98)	A: 0.4694	B: 0.5306
(Merino x Langhe) F_1	(36)	A: 0.7777	B: 0.2223
(Merino x Pleven Blackhead) F_1	(164)	A: 0.5945	B: 0.4055
(Merino x Pleven B.)F_1 x Black East Friesian	(156)	A: 0.6026	B: 0.3974

ted on the basis of the Hardy-Weinberg equations.
In some cases {Awassi, Tsigaia, (MerinoxLanghe) F1, (MerinoxPleven Blackhead) F1} there was a good agreement between the observed and expected frequencies on the basis of the above mentioned law. The other breeds did not confirm the expectations to fit the Hardy-Weinberg equilibrium.
After typing the 1607 sheep belonging to different breeds and crosses it was found that the Welsh variant of the α_{s1}-casein was present only in the Hungarian Merino breed. The examination in this breed evidenced 4 homozygous and 16 heterozygous ewes of 546 which represent 0.7and 2.9, respectively.

Discussion

Hungary has a sheep population of about one million. Most of the sheep are mutton-type Hungarian Merinos and the lambs produced are exported to various EU countries, predominantly to Italy.

To improve profitability, some farmers initiated dairy programmes in recent years to produce Kaskhaval type hard cheese, which is exported to many countries in Europe and to the United States of America.

Since the results for the possible use of milk protein types in selection to improve milk yield as well as milk composition and cheese yield are few in number and rather contradictory, it has been decided to carry out studies in the existing Hungarian dairy sheep population with the aim to provide additional data to this particular subject.

The selected PCR-RFLP based methods proved to be suitable for milk protein typing.

There is little information available concerning the distribution of LGB genotypes in Hungarian sheep breeds. The allelic frequencies obtained in Tsigaia breed are fairly similar to the results published by ERHARDT (1989).

The frequency of allele A in Lacaune and Merino breed are considerably lower than the values obtained by BARILLET et al. (1993) and KING (1969), respectively.

Contrary to expectations the examination did not confirm the presence of allele C of LGB in the examined Hungarian Merino and (MerinoxPleven) F1 populations.

Only LGB A and B have been demonstrated in our sheep tested so far, genotype and allele frequencies are tabulated. British Milk Sheep had the highest frequency of allele A, but the distribution of the two alleles gives good possibility for correlation studies in all examined breeds and crosses.

The presence of Welsh variant of α_{S1}-casein in Hungarian breeds was not studied earlier.

The used method can yield evidence for the presence or absence of allele D and offers the possibility of an early examination as a considerable selection tool for breeders.

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References

ANTON, I.; ZSOLNAI, A.; FÉSÜS, L.:
Identification of the variant C of β-lactoglobulin in sheep using a PCR-RFLP method. J. Anim. Breed. Genet. (1999) (accepted)

BARILLET, F.; MAHE, M.F.; PELLEGRINI, O.; GROSCLAUDE, F.; BERNARD, S. :
Genetic polimorphism of milk proteins in the French Lacaune breed. 5 th Int. Symp. of Machine Milking of Small Ruminants, Budapest, Hungary, 1993, 199-207

BELL, K.; Mc KENZIE, H.A.:
The whey proteins of ovine milk: β-lactoglobulins A and B. Biochim. Biophis. Acta, **147** (1967), 123-134

BOLLA, P.; CAROLI, A.; MEZZELANI, A.; RIZZI, R.; PAGNACCO, G.; FRAGHI, A.; CASU, S.:
Milk protein markers and production in sheep. Anim. Genet., **20** (1989), Suppl. 1. 78

CHIANESE, L..; GARRO, G.; MAURIELLO, R.; LAEZZA, P.; FERRANTI, P.; ADDEO, F.:
Occurence of five α_{S1}-casein variants in ovine milk. J. Dairy Research, **63** (1996), 49-59

DI STASIO, L.; GIACCONE, P.; PORTOLANO, B.; RASERO, R.:
β-lactoglobulin types and milk production in sheep. Anim. Genet., **23** (1992), Suppl. 1. 106-107

ERHARDT, G. :
Evidence for a third allele at the β-Lactoglobulin locus of sheep and its occurence in different breeds. Anim. Genet., **20** (1989), 197-204

FERRANTI, P.; MALORNI, A.; NITTI, G.; LAEZZA, P.; PIZZANO, R.; CHIANESE, L.; ADDEO, F.:
Primary structure of ovine α_{S1}-caseins: localization of phosphorylation sites and characterization of genetic variants A, C and D. J. Dairy Res., **62** (1995), 281-296

FRAGHI, A.; CARTA, A.; PILLA, F.; SANNA, S.R.; PIREDDA, G.:
β-Lactoglobulin polymorphism in Sarda dairy sheep: influence on milk yield. Proc. 47th Meeting of the European Assoc. for Anim. Prod., Lillehammer, Norway, 25-29. Aug. 1996, 42

GARZON, A. I.; MARTINEZ, J.:
β-Lactoglobulin in Manchega sheep breed. Relationship with milk technological indexes in handcraft manufacture of Manchego cheese. Anim. Genet., **23** (1992), Supplement 1. 106

GODOVAC-ZIMMERMANN, J.:
The structural motif of β-Lactoglobulin and retinol-binding protein: a basic framework for binding and transport of small hydrophobic molecules. Trends in Biochem. Sci., **13** (1988), 64-66

HARRIS, S.; ALI, S.; ANDERSON, S.; ARCHIBALD, A.L.; CLARK, A.J.:
Complete nucleotide sequence of the genomic ovine β-Lactoglobulin gene. Nucl. Acids Res., **16** (1988), 10379-10380

HAYES, H.C.; PETIT, N.; LEMIEUX, N.; DUTRILLAUX, B.:
Chromosomal localisation of ovine β-Lactoglobulin gene by non-isotopic in situ hybridization and R-banding. Cytogenet.Cell Genet., **61** (1993), 286

HERGET, I.; SCHMOLL, F.; BREHM, G.; SCHELLANDER, K.:
β-Lactoglobulin Polymorphism in East Friesian Milk Sheep. Proc. 46th Meeting of the European Assoc. for Anim. Prod., Prague, Czech Republic, 4-7. Sept. 1995, 21

KING, J.W.B. :
The caseins of sheep's milk. Polymorphismes Biochimiques des Animaux, Paris, France, 1967, INRA 427-431

KING, J.W.B.:
The distribution of sheep β-Lactoglobulins. Anim. Prod., Edinburgh **11** (1969), 53-57

KOLDE, H.J.; BRAUNITZER, G. :
The primary structure of ovine β-Lactoglobulin. Milchwirtschaft, **38** (1983), 70-72

LOPEZ-GALVEZ, G.; AMIGO, G.; RAMOS, M.:
Genetic polymorphism of whey proteins in two ovine breeds. Milchwirtschaft, **49** (1994), 123-125

MERCIER, J.P.; GAYE, P.; SOULIER, S.; HUE-DELAHAIE, D.;VILOTTE, J.L.:
Construction and identification of recombinant plasmids carrying cDNAs coding for ovine α_{S1}-,α_{S2}-, β-, κ-casein and β-lactoglobulin. Nucleotide sequence of α_{S1}-casein cDNA. Biochimie, **67** (1985), 959-971

PIREDDA, G.; PAPOFF, C. M.; SANNA, S.R.; CAMPUS, R.L.:
Influence of ovine α_{S1}-casein genotype on the phisicochemical and lactodynamographic characteristics of the milk. Scienza e Technica Lattiero-Casearia, 44 (1993), 135-143

PIRISI, A.; PIREDDA, G.; DI SALVO, R.; PAPOFF, C.M.; PINTUS, S.:
Influence of ovine α_{S1}-casein genotype on milk composition and cheese yielding capacity. Proc. Int. Conf. Prod. Utilization of Ewes and Goats Milk, Crete, Greece, 1995, 179-183

PRINZENBERG, E.M.; ERHARDT, G.:
Molecular genetic characterization of ovine β-lactoglobulin C allele and detection by PCR-RFLP. J. Anim. Breed. Genet. **116** (1999), 9-14

RAMMUNO, L.; COSENZA, G.; RANDO,A.; MACCIOTTA, N.P.P.; PAPPALARDO, M.; MASINA, P.:
Identification of carriers of the Welsh Casa I variant using an allele-specific PCR method. Anim.Genet. **28** (1997), 154-155

RECIO, M.I.; FERNANDEZ FOURNIER, A.; RAMOS, M.:
Genetic polymorphism of the whey proteins for two Spanish ovine breeds. Influence of genetic polymorphism on renneting properties. IDF Seminar on the Implications of genetic polymorphism of milk proteins on production and processing of milk, Zürich, Switzerland, 1995, 15

SCHLEE, P.; KRAUSE, I.; ROTTMANN,O.:
Genotyping of ovine β-lactoglobulin alleles A and B using the PCR. Arch. Tierz., Dummerstorf **36** (1993), 519-523

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