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Distribution of α_{S1}-casein "welsh" variant in some Slovak and Czech sheep breeds*

Abstract

Occurrence of the α_{S1}-casein D allele (formerly "welsh" variant) in sheep breeds Tsigai, Improved Valachian and Slovak Merino was investigated by means of isoelectric focusing in ultra thin polyacrylamide gel. This variant in herds of the above mentioned breeds was observed as follows: Improved Valachian 3.64%; Tsigai 5.57%; Slovak Merino 12.45%. In the last mentioned breed in a single animal α_{S1}-casein was not found at all (0.34% of the total). There is no mention of existence of null allele of sheep α_{S1}-casein in literature so far. In case of the Tsigai breed in one individual (0.24%) the "welsh" variant in homozygous form was observed.

Key Words: sheep, milk, "welsh" variant, α_{S1}-casein

Zusammenfassung

Titel der Arbeit: **Vorkommen der α_{S1}-Kasein „welsh“ Variante in drei Slowakischen und Tschechischen Schafrassen**

Es wurde das Vorkommen des α_{S1}-Kasein D Allels (vormals Welsch-Variante) in den Schafrassen Tsigai, Veredelte Walachische und Slowakische Merino mittels isoelektrischer Fokussierung in ultradünnem Polyakrylamidgel untersucht. In diesen Populationen fanden sich bei den drei Rassen veredelte Walachische 3,64 %, Tsigai 5,57 % und Slowakische Merino 12,45 % dieser Allele. In letztgenannter Rasse traten bei 0,34 % aller Tiere keine mit α_{S1}-Kasein auf. In der Literatur gibt es bis jetzt keinen Hinweis auf die Existenz des Nullallels des Schaf-α_{S1}-Kaseins. Bei der Tsigai Rasse fand sich die Welsch-Variante in homozygoter Form bei einem Tier (0,24 %).

Schlüsselwörter: Schaf, Milch, Welsch-Variante, α_{S1}-Kasein

Introduction

Though electrophoretic mobilities of various casein fractions in sheep milk, by reason of their different glycosylations and phosphorylations, were different, nevertheless their genetic variability was low (KING, 1969; ARAVE et al., 1973; RICHARDSON and CREAMER, 1976; RUSSO et al., 1981; CHIOFALO et al., 1982). However, on the basis of experiments with several endonucleases and bovine cDNA probes was found, that heterogeneity in related areas of ovine DNA was of high degree (Di GREGORIO et al., 1989; 1991; LEVÉZIEL et al., 1991; PHUA et al., 1992).

After gel electrophoresis of ovine caseins two additional bands of "α-casein" were marked as "welsh" variant (KING, 1966). As was proven later, "welsh" variant is a true genetic variant of α_{S1}-casein (MAURIELLO et al., 1990; DAVOLI et al., 1990), and the allele was indicated as α_{S1}-CN^{D} (FERRANTI et al., 1995).

On the present six variants (A, B, C, D, E and F) of the ovine α_{S1}-casein are known, which, similarly than in the case of caprine α_{S1}-casein, quantitative variations exist (ADDEO et al., 1992; CHIANESE et al., 1990, 1996, PIRISI et al., 1999). BOLLA et al. (1989) published data on negative correlations among "welsh" variant and fat, or

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protein content in sheep milk. In milk samples with "welsh" variant casein content was reduced. A worsening of milk clotting properties was observed (e.g. softer curd consistency) in homozygous animals and to a lesser extent, in heterozygous animals (PIREDDA et al., 1993). In such milk higher levels of seroproteins and worse lactodynamographic parameters were observed as well.

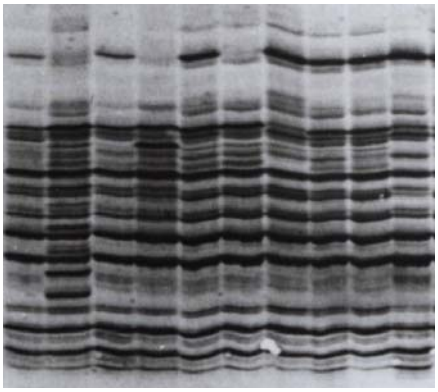

Fig. 1: Separation of proteins in sheep milk by PAGIF. In the lane marked W under β-casein (β-CN) is a "welsh" variant of α_{S1}-casein (Trennung des Milchproteins mittels PAGIF, der Strich markiert W des β-Kaseins (β CN) ist eine Welschvariante des α_{S1}-Kaseins)

Particular fractions of sheep caseins can be separated by polyacrylamide gel electrophoresis the best at pH value 5.6, where two bands of the "welsh" variant are well distinguishable. The order of anodic mobility decreased from A to E as follows: A>B>C>D>E.

The isoelectric focusing (IEF) pattern of the ovine α_{S1}-casein, concerning number of bands in gel, can be very heterogeneous (Di LUCCIA et al., 1989; CHIANESE et al., 1996). This electrophoretic complexity is caused by coexistence of its isoforms in milk with different length of the chain, by different grades of phosphorylation, which might change electric charge of the single peptide. By IEF there can be seen an increase in the isoelectric points of α_{S1}-CN variants from variants A to E.

Allele specific RFLP methods on identification of "welsh" variant were published as well (RAMUNNO et al., 1997; PILLA et al., 1998).

The aim of this study was to investigate distribution of α_{S1}-casein “welsh variant” in three Slovak and Czech sheep breeds.

Materials and methods

Collecting and preparation of individual milk samples, as well as IEF, was carried out by description in KRAUSE et al. (1988). Milk samples from Tsigai breed (413 animals) came from sheep farm at Proč, Slovak Merino (289) from Šaca (Košice), and Improved Valachian (302) from the farm at Nálepkovo.

Samples were collected in the spring period of the years 1991-1995, stored at -30°C, and gradually processed and analysed in the rest time of the same years.

On separation of milk proteins as well as identification of "welsh" variant of the α_{S1}-casein ultra thin polyacrylamide gel (265x125x0.25 mm), containing a mixture of ampholytes and 8 M urea, was applied. Bidistilled water was used for preparing gel monomer (%T=5, %C=3) and for ampholyte mixture: 1.2% pH 4.2-4.9; 0.9% pH 2.5-

5; 0.3% pH 5-6.5, Pharmacia. In the course of ampholyte solutions preparing we followed descriptions by MAHÈ and GROSCLAUDE (1993).
Determination of the "welsh" variant has been realised on the basis of literary description (MAURIELLO et al., 1990). In this traditional interpretation "welsh" variant is considered two additive bands between β-casein and the slower bands of the α_{S1}-casein at the bottom of the gel (Fig. 1).

Results

In the investigated population of the Slovak Merino "welsh" variants of the α_{S1}-casein was found in a larger amount: 12.45% (Tab.).

Table
Presence of "welsh" variant (W) in the three examined ovine breeds (Vorkommen der Welsch-Variante (W) in den untersuchten Rassen)

Breed	Number of animals	W	W%
Slovak Merino	289	36	12.45
Tsigai	413	23	5.57
Improved Valachian	302	11	3.64

In this breed in a single case (0.34%) no intensive band was found on the gel in the appropriate area of the α_{S1}-casein appearance (Fig. 2). Repetitive isoelectric focusing in polyacrylamide gel (PAGIF) analysis of the milk sample from the same identical animal gave result. Till now, no publication on the existence of ovine null allele of sheep α_{S1}-casein was issued.
Occurrence of the "welsh" variant in breeds Improved Valachian and Tsigai was far smaller: 3.64 and 5.57%, respectively (Tab.). In a single case homozygous form of the "welsh" variant was found in the latter breed, with a ratio of 0.24% (Fig. 3).

Discussion

The α_{S1}-CN D allele has been found in various breeds with a very low or low, 0.006-0.133, frequency (MAURIELLO et al., 1990; ARAVE et al., 1973; THOMAS et al., 1989; BARILLET et al., 1993; CHIANESE et al., 1996; DI STASIO, 1983; RUSSO et al., 1981; SERRANO MOYANO et al., 1999; CHIOFALO and MICARI, 1987). In the Sarda breed it was 0.03 (PIREDDA et al., 1993) or 0.027 (PAPOFF et al., 1997).
On the other hand, in some breeds the variant showed a relatively high frequency. In different flocks of Sarda 0.078, 0.22, 0.253 and 0.32, with number of animals in the flock 336, 356 and 207, respectively (CAROLI et al., 1989; PIRISI et al., 1999; BOLLA et al., 1989). Frequencies in Mutton and Stavropol Merino were 0.27 and 0.44, respectively (MÁCHA and HORÁK, 1972); in Altamurana breed 0.23 (MAURIELLO et al., 1990).
This variant was not found at all in breeds like the Chios (MICARI et al., 1986), Comisana (CHIOFALO and MICARI, 1987), Polish Merino (MROCZKOWSKI et al., 2002), Dorset (ARAVE et al., 1973), Manchega and Segureña (LÓPEZ-GÁLVEZ et al., 1999). No “welsh” variant was found in Lacaune, Tsigai, Awassi and British milksheep breeds, analysed by PCR-ASA technique (ANTON et al., 1999).

X

Fig. 2: PAGIF separation of milk proteins in sheep. No α_{S1}-casein is found in a sample (lane marked X) (PAGIF Trennung des Schafmilchproteins, Beispiel für fehlendes α_{S1}-Kasein (markiert durch X))

After electrophoresis of milk proteins frequency of the "welsh" variant in Improved Valachian was found 0.167, in Tsigai of 0.0315 (MÁCHA and HORÁK, 1972).
Our observed frequency value of the „welsh“ variant in Slovak Merino (12.45%), as compared to the frequencies in breeds listed above, doesn’t obtain an average. Despite it, in light of milk quality (in the first place: lower synthesis of the α_{S1}-casein) loss of proteins at 12.45% of herds could be significant. In a similar comparison, value found in the Tsigai does not seem to be high: the frequency falls into the area of low prevalence (MÁCHA and HORÁK, 1972). On the other hand, in the Improved Valachian authors, in contradiction to our results, found an occurrence near of 17%, however at relatively low number of animals.

H

Fig. 3: Likely homozygous form of "welsh" variant, lane H, in an individual of Tsigai breed on the PAGIF gel (Wahrscheinlich homozygote Form der Welschvariante (Linie H) bei einem Tier der Rasse Tsigai)

The fat, protein or mineral content of milk can essentially influence its manufacturing properties (GRAML and PIRCHNER, 2003). Different alleles of milk proteins can alternatively impress processing of the milk and can also affect the quality or quantity of final products (MROCZKOWSKI et al., 2004; PABST, 1998; PANICKE et al., 1996; HANUŠ et al., 1995). In case of the "welsh" variant of ovine α_{S1}-casein probably the partial dephosphorylation (FERRANTI et al., 1995; FERRANTI et al., 1998; CHIANESE at al., 1996) influences negatively concentration of caseins and

curd consistency. As was reported, loss of phosphate groups reduces partly the ability of proteins to couple mutually in solution (BINGHAM et al., 1972), and partly increases possibility for caseins to remain in soluble state, despite the presence of calcium ions in solution (VAN HEKKEN and STRANGE, 1993). Unfavourable influence of "welsh" variant on coagulation ability of sheep milk or on firmness of curd can also be explained by reduced levels of caseins in it.

Our observation of the D allele's homozygous form in the Improved Valachian herd (0.24%) might be an interesting finding. This rare phenomenon was mentioned in Sarda sheep (BOLLA et al., 1989). MÁCHA and HORÁK (1972) also gave notice on unique occurrence of homozygous form of the discussed allele, but they did not indicate in which breed (or breeds) it has occurred. Unambiguous evidence on α_{S1}-casein null allele in Slovak Merino should require a more detailed research, as there is no mention on existence of such a mutation of α_{S1}-casein in sheep so far.

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