IDENTIFICATION OF A POSSIBLE NEW WAP ALLELE IN A ROMANIAN LOCAL PORCINE BREED

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ABSTRACT

In the milk of almost all livestock species there are six major milk proteins. Four of them belong to the casein fraction (αs1-, αs2-, β- and κ-casein) and two are whey proteins (β-lactoglobulin and α-lactalbumin). In porcine milk a third major whey protein was discovered named whey acidic protein (WAP).

WAP is the major whey protein in the case of mouse, rat, rabbit and camel milk. This protein has only recently been identified in sow's milk. The mature protein contains 113 amino acids (aa), has a molecular mass of 22985.86 Da and an isoelectric point pI=5.04 (signal peptide not included – 19 aa). The accession number of this protein in Swiss-Prot data base is O46655. The gene encoding porcine WAP has a length of 2087 bp (Genbank accession number AF3200306) and has been localized using FISH in the telomeric region of the two chromosomes 18.

The purpose of our study was to use the isoelectric focusing technique (IEF) in order to investigate milk protein polymorphisms in porcine breeds reared in Romania, in order to detect new genetic variants. Using this technique we have previously identified a polymorphism in the β-casein region of the gel, two variants (A and B) being reported.

In this paper, using IEF, as described in our previous studies, we report a polymorphic variant in the case of porcine WAP. The milk sample which contains this new variant belongs to a Mangalita breed individual (a local Romanian porcine breed). Studies at DNA level are in progress in order to characterize this new porcine WAP variant, which has been named: B^{MS}.

Key words: WAP, porcine milk, milk proteins, isoelectric focusing, polymorphism

INTRODUCTION

In the milk of almost all livestock species there are six major milk proteins. Four of them belong to the casein fraction (αs1-, αs2-, β- and κ-casein) and two are whey proteins (β-lactoglobulin and α-lactalbumin). In porcine milk a third major whey protein was discovered named whey acidic protein (WAP). The purpose of our study was to use the isoelectric focusing technique (IEF) in order to investigate milk protein polymorphisms in porcine breeds reared in Romania, in order to detect new genetic variants. To our knowledge this aspect has never been investigated in our country so far, and little work was done abroad. During our studies we found different migrating profiles at the β-casein locus, which indicates that there could be two genetic variants at this locus. We have named the fastest migrating allele A and the slower migrating one B. Further studies at DNA level are required in order to confirm and fully characterize this polymorphism.

As is true for most species (except certain marine mammals), the most abundant constituent (77 %) of porcine (*Sus scrofa*) colostrum is water. Other major constituents include proteins (13.8 %), lipids (4.7 %), lactose (3.6 %), and minerals (0.7 %). As the transition from colostrum to mature milk occurs, only the relative proportion of proteins decreases (5.3 %), whereas water (81.7 %), lipid (6.6 %), lactose (5.5 %), and mineral (0,8 %) contents all increase. Caseins are the most abundant proteins (>60 % in swine) in most mammalian milks and consist primarily of a group of heterogeneous phosphoproteins (Aimutis et al., 1981).

β-lactoglobulin was the first milk protein in which a polymorphism was identified (A and B alleles), on paper electrophoresis by Aschaffenburg and Drewry (1955).

In porcine milk the first genetic polymorphisms were reported by Glasnak (1966) and Gerrits et al. (1967, 1969).

Glasnak (1966) reported a polymorphism in the β-casein fraction of sow's milk. Three variants were labeled as alleles A, B, and C. Gerrits et al. (1967, 1969) also noted the polymorphism in the β-casein fraction of sow's milk; however, only two codominant autosomal alleles designated as Cn3A and Cn3B were reported. These two alleles correspond to the B and C alleles reported by Glasnak (1966). In a subsequent study, Glasnak (1968) reported that his fastest moving component designated allele A was probably an artefact caused by precipitation of proteins from solution.

Kraeling and Gerrits (1969) reported a polymorphism in a protein of sow's whey. Two variants were identified, giving rise to the three possible phenotypes. The faster moving component was identified as Wh1A and the slower as Wh1B. Kalan et al. cited by Althen (1971) isolated each of these whey variants from sows homozygous for the individual variant. These variant proteins have been characterized for amino acid composition, C-terminal and N-terminal amino acids, molecular weight, and isoionic point. Kessler and Brew have also reported a whey protein from pig's milk which appears to be similar, if not identical, to the polymorph designated AA in the reports of Kalan (Althen, 1971).

According to Gallagher (1996) it is difficult to interpret the work of early authors on porcine milk proteins as no official protein nomenclature system was used, and therefore, some of the proteins were incorrectly named. For example, Glasnak (1966, 1968) reported the presence of β-I and β-II caseins, while Gerrits et al. (1969) reported a casein3 protein. However, β-I and casein3 protein were later

identified as β-casein. Also, the whey protein identified by Kraeling and Gerrits (1969) and Althen and Gerrits (1971) is the same protein, identified later as β-lactoglobulin.

We started our study having in mind the inconsistencies between work previously done in the case of porcine milk, due to the lack of a standard protein nomenclature and to the different methods used for identifying milk protein polymorphisms. Our aim is to identify polymorphisms that occur in porcine milk proteins using the isoelectric focusing technique (IEF). This electrophoresis method, as described by Balteanu et al. (2007), allows the separation of proteins according to their electric charge.

The technique is based on protein separation according to their electric charge, into a pH gradient. By applying a high power electric field (up to 20 W), the proteins migrate into a polyacrylamide gel until they reach the point where their net charge is zero, named isoelectric point (Vlaic et al., 2008). This way 6 major milk proteins and their alleles can be easily identified after staining with Commassie blue. The high resolution of IEF makes it very useful in milk proteins polymorphisms studies, in any breed or specie. In the future, in order to fully characterize the observed polymorphisms we aim to study them at DNA level.

MATHERIALS AND MOTHODS

Four sows belonging to Bazna and Mangalita breeds (local Romanian unimproved breeds) were milked in order co compare these breeds with highly improved breeds. Milk sample collection was done individually, in 15 ml Falcon tubes. No preservatives were added. Samples were stored during transport at 4 ˚C and then frozen at -20 ˚C.

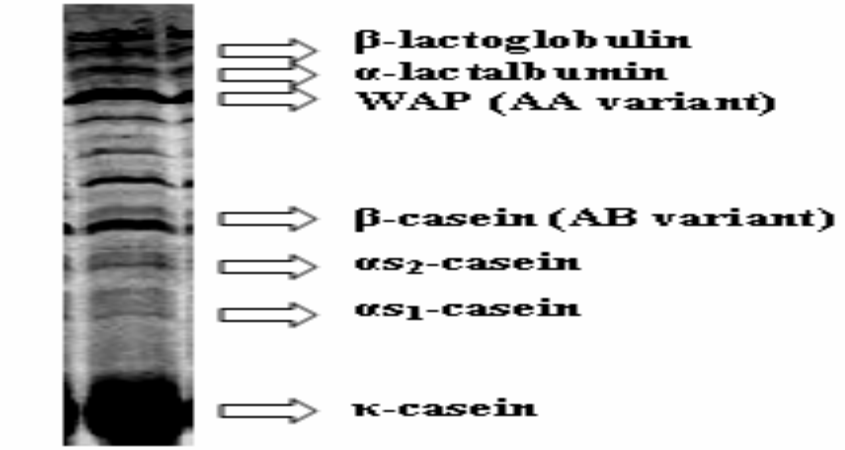
IEF was carried out, as described in our previous studies (Balteanu et al.,2007, Şuteu et al.,2009), in 4 % ultrathin (0.5 mm) polyacrylamide gels containing 8 M urea and a mixture of three ampholytes (Pharmacia LKB, Sweden): pH = 2.5 – 5; pH = 4.2 – 4.9 pH = 5.0 – 7.0. Ten microliters of each skim milk sample (obtained by centrifugation 15 minutes at 5000 rcf.), were diluted 1/10 with a solution containing 8 M urea and three microliters of β-mercaptoethanol. Whole casein was obtained by acid precipitation. The samples were vortexed and incubated at 4 ˚C for 24 hours. After the incubation period they were applied close to the anodic end of the gel, on small filter papers (5 x 6 mm).

IEF was carried out with a Multiphor II Electrophoresis System (Pharmacia LKB, Sweden), in 0.5 mm thick gels and 124 x 258 mm in size. After prefocusing at 14 ˚C and constant power (9 W) for 10 minutes, focusing was carried out at 16 W for 1 h and 20 minutes. During the run, the voltage rouses from 350 V to 2500 V. After the run, the proteins were fixed in gel by immersing it in 10 % triclor acetic acid for 30 minutes and then stained for 10 minutes in a solution containing 0.2 % (v/v) Commassie blue G-250, 50 % methanol and 10 % acetic acid in water. Destaining was carried out in an aqueous solution of 30 % methanol, 8 % acetic acid and 10 % glycerol, until the background was clear.

RESULTS AND DISCUSSION

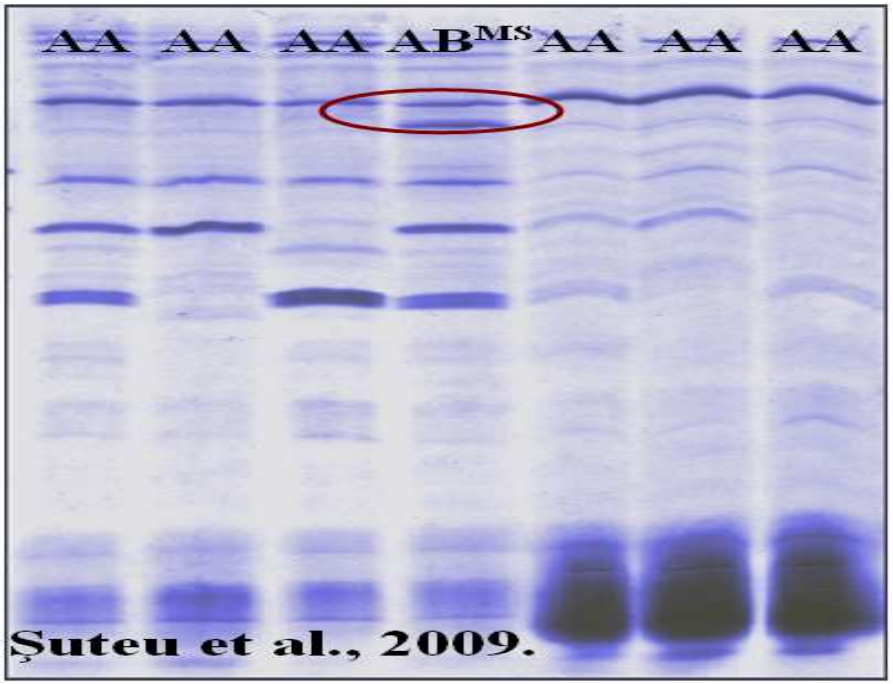
The migrating order in IEF gels, derived from the proteins' isoelectric points, is as follows: β-lactoglobulin; α-lactalbumin; WAP; β-casein; αs2-casein; αs1-casein; κ-casein (Fig. 1.)

Fig. 1. IEF migrating profiles of porcine milk proteins (Şuteu et al.,2009)



Because of the higher isoelectric point values (8.68) the κ-casein variants can't be visualized using these migrating conditions, the ampholytes covering only the 2.5 – 7 pH range.

Fig. 2. IEF migrating profile of porcine WAP B^{MS}.



In this study we report a new porcine WAP variant (Fig. 2.). We have named this new variant B^{MS}. Further studies are in progress in order to characterize this new variant at DNA level. The sample in which we have identified this new variant belongs to one of the Mangalita sows.

Because of the relatively small number of individuals taken into account (about 30 individuals belonging to different breeds, so far) we can not state that this is a breed specific variant, but in our studies this variant hasn't been identified in any other breed.

CONCLUSIONS

IEF is a very accurate and fast way of detecting milk protein polymorphisms. This study reveals the techniques applicability in the case of porcine milk. A slight modification in the pH range (4 - 10) we'll allow us to visualize the κ-casein variants too.

Main result of this study was to determine a porcine WAP polymorphism in the milk of Mangalita sows. From our knowledge no other polymorphisms have (up to date) been documented in the case of porcine WAP.

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