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Short communication

A 44 amino acid porcine β-casein isoform

[1,2]Mihai Şuteu, [1]Augustin Vlaic, [2]Robert Renaville

[1] Animal Genetics Department, University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Faculty of Animal Sciences and Biotechnologies, Cluj-Napoca, Romania; [2] Animal and Microbial Biology Unit, University of Liege, Gembloux Agro-Bio Tech, Gembloux, Belgium. Corresponding author: M. Şuteu, suteu_usamv@yahoo.com

Abstract. One of the major proteins encountered in porcine milk is β-casein. An alternative splicing phenomenon, caused by the substitution of the first nucleotide of exon 6, leads to the formation of a 44 amino acid porcine β-casein isoform (encoded by *CSN2*). The present paper further characterizes the predicted 44 amino acid isoform. Based on the amino acid sequence we computed for this variant, in non-phosphorylated form, a molecular weight of 5016.46 Da. The Tricine-SDS-PAGE migrating profile of this protein confirms our previous findings and indicates a molecular weight slightly higher than the theoretical one.
Key Words: porcine, β-casein, *CSN2*, alternative splicing, isoform.

One of the most abundant milk proteins, in most mammals, is β-casein, having an average content of 37% in bovine milk and more than two fold compared with αs1-casein in porcine milk (Lee et al 2008; Gallagher et al 1997). Porcine β-casein, as a mature protein, consists of 217 amino acids (Alexander & Beattie 1992; Şuteu 2011), the coding gene (*CSN2*) being located on SSC8 (Archibald et al 1994; Ballester et al 2005; Şuteu 2011; Şuteu et al 2011). Several authors reported different molecular weights for this protein, from 24900 Da (Mulvihill & Fox 1979, cited by Gallagher et al 1997) to 24363.30 (Şuteu et al 2011) or 24397 Da (Alexander & Beattie 1992). The last two values were computed based on the amino acid sequences, in non-phosphorylated form.

Investigating porcine β-casein polymorphisms at cDNA level, Şuteu et al (2011a) documented an alternatively splicing phenomenon leading, in certain sows, to the formation of a 44 amino acid porcine β-casein isoform. The putative mutation for this phenomenon (c.175G>A) occurs at the intron–exon junction. In individuals where the mRNA is spliced normally, the first nucleotide of exon 6 is G, similar to EU242520. When the first nucleotide is A (similar to EU025876), the mRNA is spliced alternatively (Şuteu et al 2011a). In order to further characterize this isoform and highlight its presence in AA sows we opted for the Tricine-SDS-PAGE technique, as it is the preferred electrophoretic system for the resolution of proteins smaller than 30 kDa (Schägger 2006).

A sow genotyped (as described in detail by Şuteu et al 2011a) and proven to be an AA homozygous, concerning *CSN2* c.175G>A polymorphism, was milked. The milk sample was centrifuged at 5000 *x* g for 15 minutes and the fat layer was discarded. The skim milk was diluted using distilled water (1:5 – as proposed by Aimutis et al 1982) and 500 µL reducing sample buffer (Buffer A – Schägger 2006) were added per 100 µL diluted skim milk. The samples were incubated at 37 °C for 30 min.

The electrophoresis followed the protocol proposed by Schägger (2006), with the following mentions/differences:
- 4% stacking gel and 16% separating gel.
- 10 µL sample and 10 µL molecular weight marker loaded onto the gel.
- Staining solution: 50% methanol, 10% acetic acid, 0.2% Coomassie dye.
- Distaining solution: 7.5% methanol, 10% acetic acid.

A wide molecular weight marker was used (Sigma-Aldrich), since it contains a 6500 Da fragment.

Figure 1 presents the Tricine-SDS-PAGE electrophoretic profile of the 44 amino acid porcine β-casein variant. We computed a theoretical molecular weight for this β-casein isoform of 5016.46 Da, based on its amino acid sequence, in non-phosphorylated form (GenBank Acc. No. HM114445).

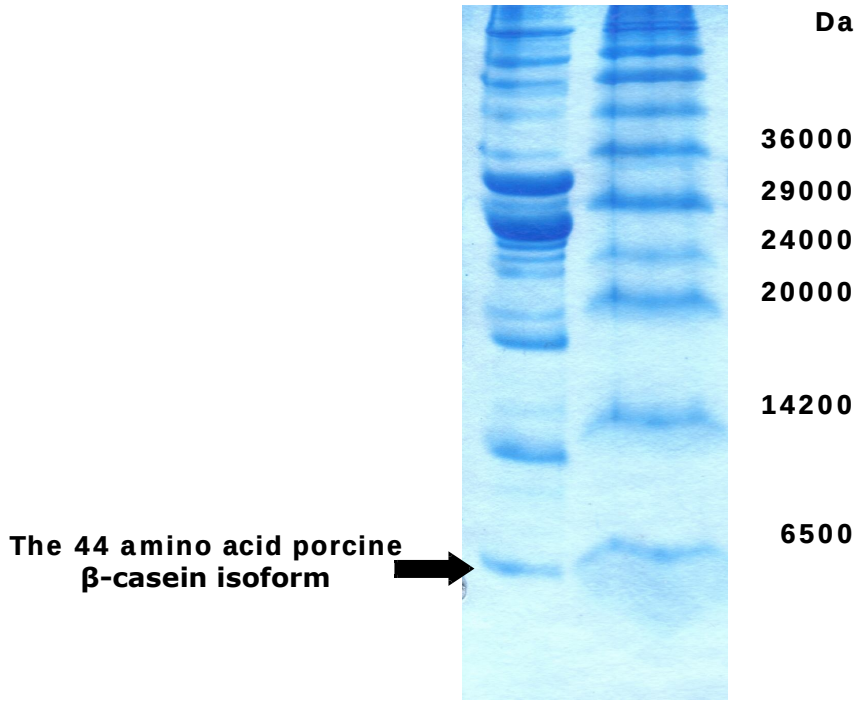

Figure 1. Left lane: Tricine-SDS-PAGE migrating profile of a milk sample from a sow with AA genotype, concerning *CSN2* c.175G>A. The 44 amino acid porcine β-casein isoform is indicated by an arrow. Right lane: molecular weight marker (Wide Range, Molecular Weight 6,500-200,000 Da – Sigma-Aldrich).

Comparing the migrating profile of this new β-casein variant to the 6500 Da fragment of the marker we can state that the actual molecular weight of the 44 amino acid β-casein isoform is slightly higher than the computed value, of 5015.46 Da, due to phosphorylation.

This is the first evidence, at protein level, of the existence of this β-casein isoform in sow's milk. Further studies aim to determine the biological role of this new isoform.

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References

Aimutis W. R., Kornegay E. T., Eigel W. N., 1982 Electrophoretic and biochemical comparison of casein and whey protein from porcine colostrum and milk. J Dairy Sci 65: 1874-1881.

Alexander L. J., Beattie C. W., 1992 The sequence of porcine β–casein cDNA. Anim Genet 23: 369-371.

Archibald A. L., Couperwhite S., Haley C. S., Beattie C. W., Alexander L. J., RFLP and linkage analysis of the porcine casein loci – *CASAS1*, *CASAS2*, *CASB* and *CASK*. Anim Genet 25: 349-351.

Ballester M., Sancez A., Folch J. M., 2005 Assignment of the β-lactoglobulin (*BLG*) gene to porcine chromosome 1. Anim Genet 36: 356-358.

Gallagher D. P., Cotter P. F., Mulvihill D. M., 1997 Porcine milk protein: a review. Int Dairy J 7: 99–118.

Lee P., Chung H. G., Lee H. G., Lee H. C., Woo J. S., Lee S., Jo S. J., Chang W. K., Lee H. T., Kwon M., Park J. K., 2008 Cloning and characterization of 5'-untranslated region of porcine beta casein gene (*CNS2*). Dom Anim Endocrinol 35: 245-253.
Schägger H., 2006 Tricine-SDS-PAGE. Nature Protocols 1(1): 16-22.
Şuteu M., 2011 Porcine milk protein polymorphisms. Porc Res 1(1): 1-112.
Şuteu M., Vlaic A., Renaville R., 2011 Characterization of porcine β-casein G allele (*CSN2*G). ABAH Bioflux 3(2): 105-109.
Şuteu M., Vlaic A., Bâlteanu V. A., Wavreille J., Renaville R., 2011a Evidence of alternative splicing of porcine β-casein (*CSN2*). Anim Genet doi: 10.1111/j.1365-2052.2011.0281.x.

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Authors:
Mihai Şuteu, Animal Genetics Department, University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Faculty of Animal Sciences and Biotechnologies, 3-5 Calea Manastur Street, Cluj-Napoca 400488, Romania, European Union, e-mail: suteu_usamv@yahoo.com;
Augustin Vlaic, Animal Genetics Department, University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Faculty of Animal Sciences and Biotechnologies, 3-5 Calea Manastur Street, Cluj-Napoca 400488, Romania, European Union, e-mail: avlaic@usamvcluj.ro;
Robert Renaville, Animal and Microbial Biology Unit, University of Liege, Gembloux Agro-Bio Tech, B-5030 Gembloux, Belgium, European Union.

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