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# Nutraceutical properties of goat milk: *In silico* analysis of the casein sequences

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**Abstract.** Milk proteins are of particular interest from the point of view of human nutrition, because they carry peptides with particular biological activities, and also causing symptoms of allergies. The identification of protein allergens is an important tool for human food safety. An *in silico* approach was followed in order to find biopeptides and epitopes already identified in the bovine species within the goat casein sequences and their genetic variants. Out of biopeptides shared with bovine milk, 9 show opioide activity, 5 are mineral carriers, 23 show hypotensive activity, 3 are antithrombotic and 6 are immunomodulators. All the goat genetic variants were considered and differences in the aminoacid sequence of the biopeptides were found in 5 of them. The epitope analysis was carried out for $\alpha_{s1}$-casein. With the exception of the 5$^{th}$ major and the 1$^{st}$ minor epitope, several differences were found both between species and among goat genetic variants.

**Keywords.** Goat – Casein – Biopeptides – Human nutrition – Genetic polymorphism.

***Propriétés nutraceutiques du lait de chèvre : analyse* in silico *des séquences de caséine***

***Résumé**. Les lactoprotéines contiennent des peptides présentant des actions biologiques spécifiques et causant aussi des symptômes d'allergie. L'identification des protéines qui causent des allergies est très importante pour la nutrition et la santé humaines. Une analyse* in silico *a été conduite pour rechercher la présence de peptides et d'épitopes déjà identifiés chez l'espèce bovine, en utilisant les séquences caséiniques caprines et leurs variants génétiques. Parmi les biopeptides en commun avec le lait de vache, 9 montrent une activité opioïde, 5 sont des transporteurs minéraux, 23 ont un rôle hypotensif, 3 ont un rôle anti-thrombose et 6 sont immunomodulateurs. On a trouvé des différences dans les séquences des aminoacides de 5 variants génétiques. L'analyse des épitopes a été conduite sur la $\alpha_{s1}$-caséine. Sauf pour le 5$^{ème}$ épitope majeur et le 1$^{er}$ épitope mineur, plusieurs différences ont été remarquées entre l'espèce bovine et caprine et parmi les variants génétiques de la chèvre.*

***Mots-clés.** Chèvre – Caséine – Biopeptides – Nutrition humaine – Polymorphisme génétique.*

## I – Introduction

Milk proteins are of particular interest in human nutrition because they exert a wide range of nutritional, functional and biological activities. Different studies identified a great number of peptide sequences in the major milk proteins with specific bioactivities affecting the digestive, cardiovascular, immune and nervous systems (Korhonen and Pihlanto, 2004). Most milk proteins are also potential allergens. Epitopes on milk proteins are short fragments widely spread throughout hydrophobic parts of the molecules. They are highly conserved sequences responsible for IgE cross-reactivity with corresponding milk proteins of other mammals, including humans (Wal, 2004).

Almost 50 genetic variants were described in bovine milk protein genes (Farrell *et al.*, 2004) and also in goat the number of genetic variants is continually raising (Caroli *et al.*, 2007). This variation is usually not considered in studies on nutraceutical properties of milk, even if aminoacid exchanges or deletions genetically determined could alter the biological function of both bioactive peptides and antigens. In fact, Hernández-Ledesma *et al.* (2004) demonstrated

the formation of an ACE-inhibitory peptide specific for the bovine β-casein $A^1$ variant, not present in other β-casein alleles. Similarly the immune response could be differently modulated as a function of the genetic polymorphism.

This work presents an *in silico* analysis of goat casein sequences in order to find out bioactive peptides and epitopes already described in cattle. This study is a first step to evaluate the possibility to include particular genetic variants in goat selection strategies.

## II – Material and methods

Goat and cattle casein sequences were researched on Swissprot ($\alpha_{S1}$-casein P18626 and P02662; β-casein P33048 and P02666; $\alpha_{S2}$-casein P33049 and P02663; κ-casein P02670 and P02668) databases and aligned with Bioedit (Hall, 1999). Goat genetic polymorphism was also taken into account to notice if some variants could contain modified peptides. As for the allergens, the six major and three minor epitopes described in bovine $\alpha_{S1}$-casein by Chatchatee *et al*. (2001) were investigated. The B variant of bovine $\alpha_{S1}$-casein (P02662) was compared with goat A, B, C, D, E and F $\alpha_{S1}$-casein (P18626) to check if their aminoacidic differences could change the epitopes and influence the IgE binding process.

## III – Results and discussion

Among more than a hundred peptides described in cattle (Lorenzini *et al*., 2007) 40 bioactive peptides, six showing two different biological activities, were found also in most of the goat casein sequences. In particular 9 showed opioid activity, 5 were mineral carriers, 23 showed hypotensive activity, 3 were antithrombotic and 6 were immunomodulators. In some goat genetic variants the peptides sequences were characterized by aminoacid exchanges (Table 1). Therefore it has to be ascertain if those peptides maintain a biological activity.

**Table 1. Peptides characterized by aminoacid exchanges (*italics*) due to genetic polymorphisms**

| Biological Function | Casein | Bioactive peptides | Differences in goat variants |
|---|---|---|---|
| Opioid | κ | LPYPY*Y*, YPY*Y* | LPYPY*C*, YPY*C* in the J variant |
| | $\alpha_{S1}$ | *TT*MPLW | T*A*MPLW in the C variant<br>*AA*MPLW in the E variant |
| Anti-hypertensive | β | *A*VP | VVP in the C variant |
| | $\alpha_{S2}$ | AMK*P*W | AMKRW in the E variant |
| Immuno-modulator | | *TT*MPLW | TAMPLW in the C variant<br>AAMPLW in the E variant |

For $\alpha_{S1}$-casein epitopes only the first minor epitope was identical in cattle and goat, whereas the other seven epitopes showed many differences. Comparing goat sequences with the first, third, fourth and fifth major and the third minor epitopes found in cattle (Chatchatee *et al*., 2001), 3, 2, 1, 1 and 3 aminoacidic differences were found respectively in all the goat alleles analyzed (Table 2). The goat sequences showed also different levels of homology with the second and sixth major epitopes and the second minor epitope of cattle depending on the alleles (Chessa *et al*., 2008). Theoretically, the allergy response to $\alpha_{S1}$-casein could be not only different between cattle and goat species, but also among goats carrying different genotypes at $\alpha_{S1}$-casein.

## IV – Conclusions

Similarly to bovine milk, goat milk is also a source of bioactive peptides and its nutraceutical properties could be exploited to valorize goat milk production. In particular, the differences found in the genetic variants have to be further investigated with adequate experimental

conditions, *in vitro* or *in vivo*, to understand the biological effect of the mutated peptides. The differences found in $\alpha_{S1}$-casein epitopes should be further investigated to understand the real allergic potential of goat milk. The differential distribution of genetic variants among breeds could be also taken into account, to valorize local breeds showing casein variation of interest for human nutrition and health.

**Table 2. IgE-binding epitopes of bovine $\alpha_{S1}$-casein and comparison with the corresponding goat $\alpha_{S1}$-casein sequences of A, B, C, D, E and F variants**

| Bovine epitopes $\alpha_{S1}$-casein | Sequence and aminoacid exchanges |
|---|---|
| 1st major | NENLLRFFVAPFPEVFGKEK<br>*******V********R**N |
| 3rd major | LEIVPNSAEERL<br>*****K****Q* |
| 4th major | NQELAYFYPELFRQF<br>*********Q***** |
| 5th major | YPSGAWYYVPLGTQY<br>********L****** |
| 3rd minor | MKEGIHAQQK<br>****NP*H** |

**Aminoacid not mutated.*

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