ISOCARD 2015

ISOCARD ҚОҒАМЫНЫҢ «ЖІБЕК ЖОЛЫ ТҮЙЕЛЕРІ: ТҰРАҚТЫ ДАМУДА КАМЕЛИДТЕРДІ ЗЕРТТЕУ»

ALMATY

4ШІ КОНФЕРЕНЦИЯСЫ

4TH CONFERENCE OF ISOCARD "SILK ROAD CAMEL: THE CAMELIDS, MAIN STAKES FOR SUSTAINABLE DEVELOPMENT"

4АЯ КОНФЕНЦИЯ ISOCARD «ВЕРБЛЮДЫ ШЕЛКОВОГО ПУТИ: ИССЛЕДОВАНИЯ КАМЕЛИДОВ ДЛЯ УСТОЙЧИВОГО РАЗВИТИЯ»

8-12 МАУСЫМ JUNE ИЮНЯ

ДЕМЕУШІЛЕР / SPONSORS

ҰЙЫМДАСТЫРУШЫЛАР / ORGANISATIONS

РГП «Институт микробиологии и вирусологии» КН МОН РК

АРНАУЛЫ ШЫҒАРЫЛЫМ • СПЕЦИАЛЬНЫЙ ВЫПУСК • SPECIAL ISSUE

ISSN 1999-3951

The differences in the PCR results of the isolates X1, X3, X23 suggest these isolates were distinguished genetically, but they have the same physiological and biochemical properties by conventional method. The same environmental factors could explain similarity of the morphology, the physiology and the biochemical features of X1, X3 and X23 isolates..

Conclusion

The gene difference among these yeast isolates is showing that, the yeast isolates might be mutated and started to belong to different species of taxonomy during evolution. And therefore the combined research using conventional or traditional and advanced methods should be more effective for identification of yeasts.

References

1. Fleet G.H, "Yeasts in dairy products" *.Journal of .Appl. Bacteriology,* 1990, 68, pp. 199-211
2. Irena Savova, Maria Nikolova, "Isolation and taxonomic study of yeast strains from Bulgarian dairy products" *.Journal of culture collections,* Vol.3, 2000-2002, pp. 59-65
3. Watanabe Koichi et al, "Diversity of LAB and yeasts in Airag and Tarag, traditional fermented milk production" *World Journal of microbiology and biotechnology,* Vol. 24, 2008, pp. 1313-1325
4. Delgermaa S, Enkhtuul Ts, *Identification methods of yeasts and LAB.* Ulaanbaatar, 2012
5. Enkhtuul Ts, Delgermaa S "Isolation and taxanomic study of lactose-fermenting yeasts from Mongolian dairy products", The 8th international forum on Stratgy technology 2013, proceedings Volume p495
6. C. Andrighetto1 , E. Psomas2 , N. Tzanetakis2 , G. Suzzi3 and A. Lombard "Randomly amplified polymorphic DNA (RAPD) PCR for the identification of yeasts isolated from dairy products" Letters in Applied Microbiology 2000, 30, 5–9

Acknowledgments

Part of this work was supported by the Project Reg. 03/2012 (MUST, SIT) 'Pure culture of useful microorganisms in foods'

BIOCHEMICAL AND MOLECULAR ANALYSES OF ALPHA S1-CASEIN POLYMORPHISMS IN CAMEL (*CAMELUS DROMEDARIUS)* AND DESCRIPTIONS OF BIOLOGICAL ACTIVE PEPTIDES AND ALLERGENIC EPITOPES

Erhardt G.[1], Shuiep E.S.[1,2], Lisson M.[1], Giambra I.J.[1], El Zubeir I.E.M.[3], Pauciullo A.[1]

1.Department of Animal Breeding and Genetics, Justus-Liebig-University Giessen, Ludwigstr. 21B, 35390 Giessen, Germany, Email: georg.erhardt@agrar.uni-giessen.de. 2. Department of Animal Production, University of Nyala, 155 Nyala, Sudan. 3. Department of Dairy Production, University of Khartoum, P. O. Box 321 Khartoum, Sudan

Abstract

Milk samples of 93 camels (*Camelus dromedarius*) from different regions of Sudan were screened for casein variability by isoelectric focusing. Kappa casein and beta-casein were monomorphic, whereas two protein patterns, named α_{s1}-casein A and C were identified. The major allele A revealed frequencies of 0.82 (Lahaoi) and 0.86 (Shanbali) in the two ecotypes.*CSN1S1**A and *CSN1S1**C are both characterized by missing of exon 16 on mRNA-level compared to the already described *CSN1S1**B. Furthermore, *CSN1S1**C shows a single G>T nucleotide substitution in the exon 5, leading to a non-synonymous amino acid exchange (p.Glu30>Asp30). A polymerase chain-restriction fragment length polymorphism method (PCR-RFLP) was established as a DNA-based test for this mutation.The occurrence and differences of IgE-binding epitopes and bioactive peptides between α_{s1}-casein A, B, and C after digestion were analysed *in silico*. The amino acid substitutions and deletion affected the arising peptide pattern and thus modifications between IgE-binding epitopes and bioactive peptides of the variants were found. The allergenic potential of these different peptides will be investigated by microarray immunoassay using sera from milk-sensitized individuals, as it was already demonstrated for bovine α_{s1}-casein variants.

Keywords: milk proteins, genetic polymorphisms, CSN1S1, IgE-binding epitopes, bioactive peptides

ТҮЙЕ СҮТІНДЕГІ ALPHA S1- CASEIN ПОЛИМОРФИЗМНІҢ БИОХИМИЯЛЫҚ ЖӘНЕ МОЛЕКУЛЯРЛЫ АНАЛИЗДЕРІ МЕН БИОЛОГИЯЛЫҚ БЕЛСЕНДІ ПЕПТИДТЕР МЕН АЛЛЕРГИЯЛЫҚ ЭПИТОПТАРДЫҢ СИПАТТАМАЛАРЫ

Суданның әр түрлі аудандарындағы 93 түйелердің сүтінен алынған сынамалардан казеиннің өзгеруін изоэлектрлік бөліп алу жасалынды. Монохромды каппа казеин мен бэта казеиндер ақуыз өрнектері ретінде, αs1-casein A және C болып табылды. Екі экотипте негізгі A бөлігі 0.82 (Lahaoi) және 0.86 (Shanbali) аллельдері болды.
CSN1S1*A және CSN1S1*C екеуі де 16 mRNA бөлімде салыстырмалы түрде CSN1S1*B сипаттайды. Сонымен қатар, CSN1S1*C G>T жеке нуклеотид ретінде 5 эксонда ауыстырады, амино қышқылдық ауыстыруда синонимді емес орын ауыстыруға жетелейд(p.Glu30>Asp30). Полимеразды шынжырлы реакция ұзақ полимфоризм әдісінің бөлігі ретінде (PCR-RFLP) ДНК негізді тест ретінде осы мутация үшін орналастырылды. Бұл жаңалық осы аллельдер үшін жыныстық қатынас пен лактация кезеңінен бос жануарларды зерттеуге мүмкін болып саналады.
αs1-casein A, B, және C биобелсенді пептидтер мен эпитоптар арасындағы байланыстардың осы жағдайлар мен айырмашылықтары ас қорытудан соң анализден өткізілді.

Түйін сөздер: сүт ақуыздары, генетикалық полимрфизм, CSN1S1, IgE-тұрақты эпитоптары, биобелсенді пептидтер

БИОХИМИЧЕСКИЙ И МОЛЕКУЛЯРНЫЙ АНАЛИЗ ПОЛИМОРФИЗМА ALPHA S1- КАЗЕИНА У ВЕРБЛЮДОВ (CAMELUS DROMEDARIUS) И ОПИСАНИЕ БИОЛОГИЧЕСКИ АКТИВНЫХ ПЕПТИДОВ И АЛЛЕРГЕННЫХ ЭПИТОПОВ

Образцы молока от 93 верблюдиц (Camelus dromedarius) из разных регионов Судана исследовались на изменчивость казеина методом изоэлектрического фокусирования. Каппа-казеин и бета-казеин были мономорфными, в то время как две были идентифицированы как белковые последовательности, типы A и C αs1-casein. Два основных аллеля A проявлялись на частоте 0,82 (Лахаой) и 0,86 (Шанбали) в двух экотипах. CSN1S1*A и CSN1S1*C оба характеризуются недостающим экзоном 16 на уровне мРНК по сравнению с уже описанным CSN1S1*B. Более того, CSN1S1*C показывает единичное G>T замещение нуклеотида в экзоне 5, что влечет несинонимичный аминокислотный обмен (p.Glu30>Asp30). Метод полиморфизма фрагмента части цепи полимеразы (PCR-RFLP) был основан на анализе мутации ДНК. Распространение и расхождения эпитопов и биоактивных пептидов IgE с αs1-казеинами A, B и C были проанализированы in silico после переваривания. Были выявлены аминокислотные замещения и удаления, вызванные возрастающей пептидной последовательностью и, соответственно, модификациями между IgE эпитопами и биоактивными пептидами вариантов. Аллергенический потенциал этих различных пептидов будет исследован методом иммуноферментных микрочипов с использованием сыворотки от молочно-сенситизированных особей, как это было показано на αs1-казеине коровьего молока.

Ключевые слова: белки молока, генетический полиморфизм, CSN1S1, эпитопы и биоактивные пептиды IgE

Introduction

Camel milk plays an important role as protein source for many humans especially for the people living in the arid lands of the world (Konuspayeva et al., 2009). In addition there is a growing interest in usage of camel milk as a healthy food (Nikkah, 2011), and alternative protein source for humans with milk protein allergy (Hinz et al., 2012). However, the knowledge in milk protein of this species and the genetic variation is very limited. Kappeler et al. (1998) described two genetic variants of α_{s1}-CN (CSN1S1*A; CSN1S1*B) by protein- and mRNA-sequencing within Somali camel (Camelus dromedarius).Therefore, the aim of this study was to investigate the occurrence of polymorphisms in camel α_{s1}-CN especially in Sudan. Furthermore, options to describe the allergenic potential as well as bioactive peptides of those protein variants should be demonstrated.

Material and methods

Milk and blood samples from two camel ecotypes Lahaoi (n=65) and Shanbali (n=28) were collected (Shuiep et al. 2013). Fresh milk samples (n=5) of Camelus dromedarius were obtained from Kamelhof Rotfelden (Rotfelden-Ebhausen, Germany) and immediately kept at 4°C. Simultaneous phenotyping of camel milk protein variability on protein level was done by isoelectric focusing (IEF) according to Erhardt (1989). DNA was isolated according to Sambrook et al. (1989) from the blood samples on the filter paper (FTA®Classic Card- Whatman®BioScience, Maidstone, UK). Somatic cells of the fresh milk samples were gained for mRNA extraction according to Boutinaud et al. (2002). Invisorb® Spin RNA Mini Kit (Invitek GmbH, Berlin, Germany) was used for extraction of total RNA which was reverse transcribed to cDNA using Verso™ cDNA kit (Thermo Fisher Scientific, Waltman, MA, USA).For sequencing of CSN1S1 cDNA a set of primers for amplification and sequencing were designed by means of DNAsis-Max ver. 3.0 software (Hitachi Software, San Bruno, CA, USA), using first the complete sequences of camel cDNAs reported by Kappeler et al. (1998) and then the new sequences determined. PCR was used to amplify complete cDNA of CSN1S1. The amplified fragments were afterwards sequenced using a Big Dye Terminator sequencing kit v.1.1 (Applied Biosystems, Forster City, CA, USA). A PCR-RFLP test was developed for the screening of single nucleotide polymorphisms (SNP) identified in CSN1S1 (c.150G>T GenBank ID: JF429138). To determine peptides containing IgE-binding epitopes and differences between the genetic variants after digestion, potential cleavage sites were predicted using the in silico ExPASy tool, Peptide Cutter (http://web.expasy.org/peptide_cutter/). Resulting peptides were compared with epitopes described in bovine α_{s1}-casein (Chatchatee et al., 2001). For identification of bioactive peptides the amino acid sequences of α_{s1}-casein A, B, and C were digested in silico (http://www.uwm.edu.pl/biochemia/) using different enzymes.

Results and discussion

Simultaneous typing of camel milk protein using IEF revealed polymorphic protein pattern named α_{s1}-CN in the most acidic fraction of the gel. Alpha- α_{s1}-casein A revealed the most alkaline isoelectric point (pI), whereas allele C was just focused in its acidic side. Naming was done considering nomenclature of Kappeler et al. (1998). Due to this α_{s1}-CN C seems to be a new variant. Screening of all 93 camel milk samples by IEF revealed dominance of α_{s1}-CN A in comparison to α_{s1}-CN C with frequencies of 0.86 and 0.82 in Lahaoi and Shanbali ecotypes, respectively. Sequencing of the complete coding sequence of camel *CSN1S1* of IEF-pre-typed α_{s1}-CN A cDNA samples showed full sequence similarity to α_{s1}-CN A of Kappeler et al. (1998). Samples with α_{s1}-CN C are characterized by a non-synonymous G>T-SNP (c.150G>T; GenBank ID: JF429138) resulting in the amino acid substitution p.Glu30>Asp30 in the mature protein. *CSN1S1*C* and α_{s1}-CN B (Kappeler et al., 1998) are both characterized by p.Glu30>Asp30 in the deduced mature protein sequence, and are differing by missing or non-missing of exon 16, respectively. The PCR-RFLP test developed confirmed the IEF and can therefore be used for typing camel *CSN1S1* variability independent of age, sex and lactation stage. A comparison of the identified peptides after *in silico* digestion of α_{s1}-casein A, B and C with IgE-binding epitopes described in bovine α_{s1}-casein showed that IgE-binding epitopes f24-43 and f153-168 did not resist digestion in intact form and that the amino acid substitutions and deletion influence the resulting peptide pattern. Thus, peptide f28-40 occurred in all 3 variants. Peptide f151-159 was only identified in variant A and C, whereas peptides f151-158 and f160-167 were found in variant B. These peptides comprise major parts of the IgE-binding epitope f153-168. To determine the allergenic potential of these peptides microarray immunoassay using synthesized peptides and sera from humans with cow milk allergy are currently in progress. A total of 83 bioactive peptides with ACE inhibitory (53), stimulating (8), antioxidative (12), inhibitory (9), and hypotensive (1) activities were found in α_{s1}-casein A, B and C. Due to the deletion of 8 amino acids, two further ACE-inhibitory and

antioxidative peptides (fAY and fHL) were exclusive identified in α_{s1}-casein B. This confirms that genetic variants of camel casein are a source of different bioactive peptides as already described in cattle (Weimann et al., 2009) and reveals the additional potential of milk protein variants for human health.

References

1. Boutinaud, M., Rulquin, H., Keisler, D.H., Djiane, J., Jammes, H., 2002. Use of somatic cells from goat milk for dynamic studies of gene expression in the mammary gland. J. Anim. Sci. 80, 1258-1269.
2. Chatchatee, P., Järvinen, K.M., Bardina, L., Beyer, K., Sampson, H.A., 2001. Identification of IgE- and IgG-binding epitopes on alpha (s1)-casein: Differences in patients with persistent and transient cow's milk allergy. J. Allergy Clin. Immunol. 107, 379-383.
3. Erhardt, G., 1989. κ-Kaseine in der Rindermilch - Nachweis eines weiteren Allels (κ-CN^E) in verschiedenen Rassen. J. Anim. Breed. Genet. 106, 225-231.
4. Kappeler, S., Farah, Z., Puhan, Z., 1998. Sequence analysis of Camelus dromedarius milk caseins. J. Dairy Res. 65, 209-222.
5. Konuspayeva, G., Faye, B., Loiseau, G., 2009. The composition of camel milk: A meta-analysis of the literature data. J. Food Compos. Anal. 22, 95-101.
6. Hinz, K., O'Connor, P.M., Huppertz, T., Ross, P.M., Kelly, A.L., 2012. Comparison of the principal proteins in bovine, caprine, buffalo, equine and camel milk. J. Dairy Res. 79, 185-191.
7. Nikkah, A., 2011. Equidae, camel, and yak milks as functional foods: a review. J. Nut. Food Sci. 1, 116. doi: 10.4172/2155-9600.1000116.
8. Sambrook, J., Fritz, E., Maniatis, T., 1989. Molecular cloning: A laboratory manual. (2nd ed.). Cold Spring Harbor Laboratory Press, New York, NY, USA.
9. Shuiep E.S., Giambra I.J., El Zubeir I.E.M., Erhardt G., 2013. Biochemical and molecular characterization of polymorphisms of α_{s1}-casein in Sudanese camel (Camelus dromedarius) milk. Int. Dairy J. 28, 88-93.
10. Weimann, C., Meisel, H., Erhardt, G., 2009. Bovine κ-casein variants result in different ACE inhibitory peptides. J. Dairy Sci. 92, 1885-1888.

Acknowledgments

This work was partly funded by DAAD by a scholarship to E.S. Shuiep and by German Research Foundation (DFG ER 122/13-2).

LOW MILK CHOLESTEROL IN CAMEL MILK: TRUE OR NOT?

Faye B.[1,2], Bengoumi M.[3], Al-Masaud A.[1], Konuspayeva G.[1,4]

1. Camel project, P.O.Box n°721, 11942 Al-Kharj, Saudi Arabia, faye@cirad.fr; 2. CIRAD-ES, Campus international de Baillarguet, TA C/112 A, UMR SELMET, 34398, Montpellier, France; 3. FAO regional office, Tunis, Tunisia; 4. Al-Farabi Kazakh National University, 71 Al-Farabi av., 050040 Almaty, Kazakhstan

Abstract

Many authors argue that camel milk contains less cholesterol than cow milk while other reported the reverse. To compare the cholesterol content in camel and cow milk in similar farming conditions and to assess the impact of short underfeeding on cholesterol concentration in milk and serum, seven cows and seven camels were sampled (milk and blood) at the middle of lactation at morning and evening milking, then two weeks after distribution of low energy-protein diet, another sampling was achieved. Cholesterol content in camel milk (5.64 ± 3.18 mg $100g^{-1}$) was lower than in cow (8.51 ± 9.07 mg $100g^{-1}$), but the difference was not significant. Moreover, the ratio cholesterol/fat was similar in the two species (225 ± 125 mg $100g^{-1}$ fat in camel and 211 ± 142.4 mg $100g^{-1}$ fat in cow). Serum cholesterol concentration was significantly higher in cow (227.8 ± 60.5 *vs* 106.4 ±28.9 mg $100mL^{-1}$). There was significant difference between morning and evening milking in milk fat composition and concentrations in cholesterol. The present study showed that cholesterol concentration in camel serum is lower than in cow in similar feeding and environmental conditions, but further researches are needed to demonstrate the relationship between feeding and cholesterol content in camel milk.

Key words: camel milk, cholesterol, serum, low diet

ТҮЙЕ СҮТІНДЕГІ ХОЛЕСТЕРОЛ ДЕҢГЕЙІ ТӨМЕН: РАС ПА?

Көптеген авторлар түйе сүтіндегі холестерол пайызы сиыр сүтіне қарағанда төмен деген дәлел келтіруде, осы орайда кейбірі бұл ділелмен келіспеуде. Түйе мен сиыр сүтіндегі холестерол санын салыстыру мақсатында бірдей шаруақожалық жағдайында және қысқа толық қорек бермеудің итеруін сүт пен оның ерітіндісінің холестерол санын бағалауда, 7 сиыр мен 7 түйенің орта лактация кезеңінде, яғни таңғы және түскі сауу ортасында қаны мен сүтінен сынамалар алынды, кейін төменгі ақуыздық диетаның төмендеуі 2 апта таралғаннан кейін,басқа сынамаларда соңына жеттіу. Түйе сүтіндегі холестерол саны (5.64 ± 3.18 мг 100 г-1) сиыр сүтіне қарағанда біршама төмен болды (8.51 ± 9.07 мг 100г-1). Қалай дегенде, екі сынамадағы холестерол мен майдың ара қатынасы шамалас болды (225 ± 125 мг 100г-1 май түйеде және 211 ± 142.4 мг 100 г-1 май сиырда). Холестеролдың саны сиыр сүтінде біршама жоғары болды (227.8 ± 60.5 / 106.4 ±28.9 мг 100 мл-1). Таңғы және кешк сауу арасында алынған сүт майының саны мен холестерол мөлшерінде біршама маңызды айырмашылық болды. Аталмыш мәліметтер бірдей қоршаған орта жағдайы мен қоректендіруде түйе сүтіндегі холестерол саны сиыр сүтіне қарағанда төменірек екендігін көрсетті. Ендігі кезекте зерттеушілерге түйе сүтіне тамақтандыру мен холестерол санының ара қатынасының қатысын көрсетуі қажет.

Түйін сөздер: түйе сүті, холестерол, ертінді, құнарлығы аз диета

НИЗКОЕ СОДЕРЖАНИЕ ХОЛЕСТЕРОЛА В ВЕРБЛЮЖЬЕМ МОЛОКЕ: МИФ ИЛИ НЕТ?

Многие авторы оспаривают утверждение о более низком содержании холестерола в верблюжьем молоке по сравнению с коровьим, тогда как другие утверждают обратное. Для исследования и сравнения содержания холестерола при одинаковых условиях содержания и небольшого недокорма были отобраны две группы по семь верблюдиц и семь коров, в середине периода лактации, от которых отбирались пробы молока и крови на утренней