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RESEARCH ARTICLE

Study of gene expression for milk proteins genes in Saudi camel breeds.

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Abstract

Camel milk is one of the main components of the human diet in Saudi Arabia and most of the Middle East countries. Unfortunately, Camel did not receive enough attention as a breeding animal. The present study is an attempt to explore the existence of genetic variation in milk proteins genes of Saudi camel. Camels from Taif governorate were sampled as representative of camel population in KSA. Gene expression of some related genes of milk protein (casein) were examined, namely, exon3-exon1 mRNA and exon6-exon7. The densitometric analysis of gene expression varied greatly among all individuals. The results showed great linkage of the expression of all studied genes, such that individual with high expression in one gene are also high in the other genes. To our knowledge the present work is the first record in the gene expression of some camel milk casein. As with available resources in our hands no previous work was done with the local habitat Saudi she-camel. We examined gene expression of CD-CSN1S1 mRNA and some selected exons in the gene control alpha casein protein in the Saudi camel milk to explore presence or absence of individual variation in gene expression of selected genes. Based on these results we could conclude that, though high level of inbreeding within Saudi camels, still these populations possess the needed level of genetic variation required for genetic improvement program in this population.

INTRODUCTION

According to estimates of the United Nations Food and Agriculture Organization (FAO), the number of the world's population is expected to exceed nine billion people by 2050 (FAOSTAT, 2013).

The population of the Saudi Arabia is now nearly 29 million people this population is projected to reach more than 44 million people by 2050 (FAO-STAT, 2013). Against these challenges high lights the science of genetics as one of the ways to ensure that any state meet the demand sustainably in many areas such as, food and livestock. One of potential livestock resources that did not received any attention are Camels.

Camel has historically and economically been an important species worldwide and especially in Arab peninsula. Saudi camels comprise 16% of the animal biomass. Geographically, the camel is distributed throughout the tropical and subtropical dry zones of North Africa, western Asia and north west India and Australia as well In a review by Faye and Bonnet (2012) Camel products are regularly increased but this trend is mainly due to an increase in the camel population and nor the dairy yield (331 to 337l/100KgLW/year) have changed significantly between 1961 and 2009.

In Saudi Arabia at Qassim region Abbas et al. (2000) described four distinct herding strategies, namely, semi intensive with herd size averaged 1260 heads of camels, where this type of production marketed milk, meat, and young camels in regular basis. Type two herds were unspecialized merchants who kept medium sized herds (mean =86 camels) for family use without apparent commercial benefit. The third type of herders consisted of pastoralists oragro-pastoralists who kept smaller herds (mean= 14 camels) always with other animals (mainly sheep and goats, and occasionally cattle). Type four herds were classical camel merchants who also kept a relatively small group of camels (mean= 17 camels) in feed lot for sale at a profit at the first opportunity.

Milk is the main food obtained from a herd of camels (Dahl and Hjort, 1979a). That is the reasons behind the importance of camel milk could summarized as follow: (i) the main part of the production is self-consumed and thus, contributes to the food security of arid lands, (ii) there is a growing interest for camel milk from the urbanized population in those are as and then an increasing market opportunity, (iii) there is a trend to the development of dairy camel intensive system which could be profitable for settled producers (Faye et al., 2004).

As milk from the lactating camel must provide nourishment for her young calf as well as for human, not a great deal will be left for milk products. Moreover, the composition of camel milk does not allow for making some of the accepted products that are made from cow, sheep and goat milk. Nevertheless, milk products are made from camel milk and the milk it self is used for purposes other than simply nutrition.

As in most pastoral communities, where milk for human consumption is obtained from more than one domesticated species of animal, milk products are made after milk of various animals in mixed. It is often unclear therefore if some of the products can be made from camel milk alone (Dahl and Hjort, 1979b) or if the milk used is a mixture. This is often the case when camel milk is mixed with fresh or churned goat milk. This mixture is made with certain quantities of camel milk added until the required taste is obtained.

When camel milk is not consumed fresh it must be processed as soon as possible both because its keeping quality seems to be poor and as it is further adversely affected by the climate it soon goes bad if not treated. Many superstitions and beliefs have evolved a round camel milk and milking. Only specific members of the family can graze the animals (Hartley, 1979) and the milk is considered a shaving medicinal as well as mystical properties.

In India camel milk is used therapeutically against dropsy, Jaundice, problems of the spleen, tuberculosis, asthma, anaemia, and piles (Rao et al., 1970). The chal and other lung ailments has proven beneficial in the treatment of tuberculosis (Akhundov et al., 1972). A clinic has been established in which milk is used for treatment (Urazakov and Bainazarov, 1974). Patients with chronic hepatitis had improved liver function after being treated with camel milk (Sharmanov et al., 1978). In fact, camel milk was as effective as ass milk and superior to treatment with only medication or a diet consisting of cow milk proteins.

The camel milk works as a laxative on people unaccustomed to drinking this milk (Rao et al., 1970). Apparently stomach upsets only occur when the milk is drunk while still warm. When it is cool, no ill effects have been noted. The milk also apparently has slimming properties (Yasin & Wahid, 1957). In Ethiopia camel milk is considered as having aphrodisiac powers (Rao et al., 1970). In Somalia, among the pastoral tribes, it Is believed that milk drunk on the night when the camels first drink water, following along period of thirst, has magical powers. He who drinks milk on this night from a thirst-quenched camel will lose the thorns that have penetrated his feet, even from childhood.

Most camel milk is drunk fresh. It is also consumed when slightly sour or strongly soured. Camels' milk is generally opaque white (Yagil & Etzion, 1979). Normally it has a sweet and sharp taste, but some times it is salty. Fresh camels' milk has a high pH (Ohris & Joshi, 1961). The pH of milk is between 6.5- 6.7 which is similar to the pH of sheep' milk (Shalash, 1979).

The chemical composition of camel milk has been studied in different countries (Al-haj & AlKanhal, 2010, Haddadin et al., 2008 and Shuiep et al., 2008). Total protein content ranges from 2.4 to 5.3% (Konuspayeva et al., 2009, Nikkah, 2011a and Nikkah, 2011b), and is divided into caseins (CN) and whey proteins (Kappeler et al., 2003). The casein fraction composes 52-89% (Al-haj & AlKanhal, 2010 and Ereifej et al., 2011) and distributes into four fractions: α_{s1}-,α_{s2}-,β-, and κ-CN (ElAgamy, 2006 and Ochirkhuyag et al., 1977) encoded by four autosomal genes, CSN1S1, CSN1S2, CSN2 and CSN3, (Kappeler et al., 1998). These four genes are clustered in DNA stretch of about 250 k band mapped on chromosome 6 in cattle, sheep and goat (Rijnkels, 2002). The genes have been very well characterized both at DNA and protein level. Goats and cows represent the most polymorphic species, for which many alleles associated with different rates of protein synthesis have been identified (Caroli et al.,2006, Caroli et al.,2009 and Ramunno et al., 2005). In camel milk α_{s1}-CN (22%) is the second main fraction after β-CN (65%) and before α_{s2}-CN (9.5%) and κ-CN (3.5%) (ElAgamy, 2006).

Sequence analyses of camel caseins on protein, mRNA and DNA-level are rare (Begetal., 1985 and Kappeler et al., 1998). Camel casein mRNA-sequences were clarified by Kappeler et al. (1998) (GenBank ID: AJ012628 to AJ012630, Y10082) but no complete DNA-sequence of any camel casein is available; only some gene parts are published (GenBank ID: AF165632, CDU53902, AM259943, AF108120). Multi-species alignment of CSN1S1 mRNA sequences of 11 eutherian species showed higher sequence similarities between camel and swine CSN1S1 than between camel and cattle, goat, and sheep (Martin et al., 2003).

Concerning camel CSN1S1 gene, two genetic variants were described by Kappeler *et al.* (1998) by protein-and mRNA-sequencing within Somali camel (*C. dromedarius*). The mature protein of α_{s1}-CAN is encoded by 207 amino acid residues, while α_{s1}-CNB is characterized by an insertion of 8 amino acid residues (EQAYFHLE) between Gln154 and Pro155 of A and an amino acid exchange in position 30 (p.Glu>Asp) of the mature protein, which is not mentioned in UniProt-database O97943.

Except these two variants, to our knowledge, there is no further variability described within camel caseins. As associations between milk protein variability and milk performance traits are reported in ruminants (Caroli et al., 2009, Giambra et al., 2011 and Martin et al., 2002) further research is needed for camel milk proteins. In addition, milk protein variants are also used for breed characterization (Ceriotti et al., 2004).

The genetic variation of α_{s1}casein (Kappeler et al., 1998) and recently a new protein variant have been identified for such protein fraction (Shuiep et al., 2013). It is located predominantly on micellar surface and it is the specific substrate of the chymosin, which is responsible for the hydrolyzation of the κ-CN into the para-κ-CN and the caseino-macropeptide (CMP). At least 16 alleles corresponding to 13 κ-CN variants have been identified in goat (Caroli et al., 2006), and at least 19 alleles corresponding to 14 κ-CN variants so far in cattle (Caroli et al., 2009)

Study the gene expression of major caseins genes in Saudi camels. Explore genetic variability at casein genes of Saudi camels. Assessment and investigating the occurrence of polymorphism in Saudi camels on molecular level of casein genes. Investigating functional gene expression of casein genes in Saudi camels.

Materials and Methods

Materials

Ethidium bromide and agarose were purchased from Sigma-Aldrich (St. Louis, MO, USA). The deoxyribonucleic acid (DNA) 100 bp ladder was purchased from MBI, Fermentas, Thermo Fisher Scientific, USA. Qiazol for RNA extraction and oligo dT primer were purchased from QIAGEN (Valencia, CA, USA).

Sampling

All animal procedures were approved by the Ethical Committee Office of the dean of scientific affairs of Taif University, Saudi Arabia. Twenty one female camels were used for this study. The camels used in this study were within same age (5 years old), weighting 450-550 kg and within same breeds. Animals were handled for 2 weeks for hand milking 2 times per day (morning at 7.00 am and evening at 7.00 pm). Under sterile conditions and after washing udder, milk samples (500 ml for each animal) were collected and subjected for somatic cell isolation.

Somatic cell isolation from camel milk

The collected milk is placed in a 50 ml centrifuge tube, adding EDTA so that the final concentration to 0.5 mM (to prevent precipitation of casein), 4°C under the conditions, respectively, centrifuged with 1500 X g 15 min for (centrifugal milk samples divided into three layers, the upper layer of fat, skim milk layer in the middle and the lower milk somatic cell layer); discard the upper and middle layer (fat and skim milk), and carefully wipe with alcohol cotton ball attached to the wall of the butterfat, disperse the cell mass with 20 ml of PBS-EDTA solution, washed once, and then re-suspended .Add about 10 ml of PBS and gently shaken so that a body attached to the bottom of the cells were completely suspended; centrifugation at 4℃ 1500 xg for 10 min, again discarded upper suspended solids, carefully wipe with alcohol cotton ball attached to the wall of butterfat; 2 ml PBS-EDTA suspension cells, -80°C and stored; using a hemocytometer counting and trypan blue dye somatic cell counts.

Remove the cell suspension, add 1mL Qiazol to Eppendorf tubes, mix allowed to stand for 5 minutes, the cells full after lysis, make RNA extraction.

RNA Extraction from somatic cells

RNA was extracted as previously described (Soliman et al., 2014). **Complementary deoxyribonucleic acid (cDNA) synthesis:**

A mixture of 3 µg total RNA and 0.5 ng oligo dT primer (Qiagen Valencia, CA, USA) were used for cDNA synthesis in a total volume of 11 µl sterilized DEPC water was incubated in the Bio-Rad T100^{TM} Thermal cycle at 65°C for 10 min for denaturation. Then, 2 µl of 10X RT-buffer, 2 µl of 10 mM dNTPs and 100 U Moloney Murine Leukemia Virus (M-MuLV) Reverse Transcriptase (SibEnzyme. Ak, Novosibirsk, Russia) were added and the total volume was completed up to 20 µl by DEPC water. The mixture was then re-incubated in BIO-RAD thermal cycler at 37°C for one hour, then at 90°C for 10 min to inactivate the enzyme.

Semi-quantitative PCR analysis:

For semi-quantitative RT-PCR analysis, specific primers for examined genes (Table 1) were designed using Oligo-4 computer program and synthesized by Macrogen (Macrogen Company, GAsa-dong, Geumcheon-gu. Korea). PCR was conducted in a final volume of 25 µl consisting of 1 µl cDNA, 1 µl of 10 pM of each primer (forward and reverse), and 12.5 µl PCR master mix (Promega Corporation, Madison, WI, USA), the volume was brought up to 25 µl using sterilized, deionized water. PCR was carried out using Bio-Rad T100^{TM} Thermal Cycle machine with the cycle sequence at 94°C for 5 minutes one cycle, followed by 31 cycles (Table 1) each of which consists of denaturation at 94°C for one minute, annealing at the specific temperature corresponding to each primer (Table 1) and extension at 72°C for one minute with an additional final extension at 72°C for 7 minutes. As a reference, expression of glyceraldehyde-3-phosphate dehydrogenase (G3PDH) mRNA was examined (Table 1). PCR products were visualized under UV light after electrophoresis on 1.5% agarose (Bio Basic, Markham, ON, Canada) gel stained with ethidium bromide in TBE (Tris-Borate-EDTA) buffer. PCR products were photographed using gel documentation system. The intensities of the bands were quantified densitometrically using Image J software version 1.47 (http://imagej.en.softonic.com/).

PCR cycle of respective genes are shown, while temperature and time of denaturation and elongation steps of each PCR cycle were 94°C, 30 sec and 72°C, 60 sec, respectively.

Results and discussion

Table 1. PCR conditions of examined genes

mRNA expression	Forward primer 5'-3' (name, sequence, GenBank ID and location)	Reverse primer5'-3' (name, sequence, GenBank ID and location)	PCR cycles and annealing conditions
hline 1 (784bp)	CD-CSN1S1-mRNA-F CTTCTTCCCAGTCTTGGGTTC (AJ012628; r.10-30)	CD-CSN1S1-mRNA-R ATAGGCGTGGAGGAGAAA TTTAG (AJ012628; r. 757-735)	40 cycles, 59 °C for 1 min
DNA exon3-exon (507bp)	CD-CSN1S1-Ex3-F TCCTCTCAGGTACCCAGAAG TC (AJ012628; r. 102-123)	CD-CSN1S1-Ex4-R CTCTATGCTGTCTGGTTCAT T (AJ012628; r. 150-130)	39cycles, 55 °C for 1 min
DNA exon6-exon7 (511bp)	CD-CSN1S1-Ex6-F TTTCACCCATTCAGTTTAGAC A (AJ012628; r. 191-212)	CD-CSN1S1-Ex7-R TTCCTAGTATCCTTCAGTTC ATCG (AJ012628; r. 245-222)	37 cycles, 55°C for 1 min
G3PDH (309p)	AGATCCACAACGGATACATT'	TCCCTCAAGATTGTCAGCA A	26 cycles, 52 °C 1 min

Regrettably, there are a shortage in official and scientific reports describing farming and husbandry of camels in KSA. Therefore, based upon our survey at Taif governorate, camels are kept in small herds, each herd consists of around 15 to 20 camel females with one or no males. These herds are usually scattered in the desert, where infrastructural facilities for genetic improvement are not exist, let alone veterinary services, transportation or recording systems. These conditions dictate deep and intensive inbreeding, which will result in losing genetic variations, that in turn make any effort for genetic improvement unsuccessful. The present study is an attempt to explore the existence of genetic variation in milk proteins genes of Saudi camel. This exploration of genetic variation was carried out through inspecting expression of milk protein genes.

Camels from Taif governorate were sampled as representative of camel population in KSA. That is, gene expression of some related genes of milk protein (casein) were examined. Figure (1) shows the level of CD-CSN1S1- mRNA expression local she-Camels. The densitometric analysis of gene expression varied greatly among individual of She-Camel with the highest value recorded for She-Camel number 5 followed by number 4 and 7 with the same level of gene expression. The lowest value was obtained for individual number 6.

The expression levels of exon3-exon1 mRNA was also examined as it is shown in figure (2) shows the level of exon3-exon1 mRNA expression baladi She-Camel in Taif governorate with same age and milking period. The densitometric analysis of gene expression was varied between individual She-Camel with the highest value recorded with She-Camel number 7 followed by number 4 and 5 with the same level of gene expression approximately. While the lowest value was obtained with individual number 1.

The obtained results showed the highest gene expression of some milk casein gene was higher in she-camel number 7 and lowest in the She-camel number 1.

To our knowledge the present work is the first record in the gene expression of some camel milk casein. As with available resources in our hands no previous work was done with the local habitat Saudi she-camel. We examined gene expression of CD-CSN1S1 mRNA and some selected exons in the gene control alpha casein protein in the Saudi camel milk to explore presence or absence of individual variation in gene expression of selected genes. Based on these results we could conclude that, though high level of inbreeding within Saudi camels, still this population possess the needed level of genetic variation required for genetic improvement program in this population.

Based upon the findings of the present work, and in the absence of Artificial Insemination and recording programmes, institutional herds offer the only opportunity to carry out recording and selection. Cunningham (1979) has proposed a kind of open nucleus breeding scheme that may be suitable for this situation. The plan operates around a central herd which should preferably be under government control and carry out proper recording and breeding practices. The supporting base population is the scattered herds which provide females to replace about 10 percent of the females in the central herd annually. Selection from scattered herds will be by simple procedures involving judgement by eye, milking ability, size, conformation and condition. Where feasible, test milkings may also be done. The selection of these animals will be done by officers who would tour many villages to select the best animals from as wide a population as possible. Males are bred from the best females and selected bulls in the central herd. They are then evaluated on their own growth rates and dams' and milk yields and the best are chosen as sires. The lowest yielding 10 percent of the females are replaced with new animals drafted from village herds. The latter animals are recorded during the following year with the remainder of the central herd and re-evaluated.

The benefits to the cooperating farmers from this scheme will accrue from the sale of selected bulls from the central herd. Assuming that the central herd has 200 camel females and that the best third of the bulls born annually are chosen, about 2025 bulls will be available for sale to farmers after meeting the central herd’s requirements. This may satisfy the requirements of the cooperating farmers but will have hardly any impact among farmers outside the scheme. This is a serious shortcoming of programs involving central herds where supporting AI services are inadequate.

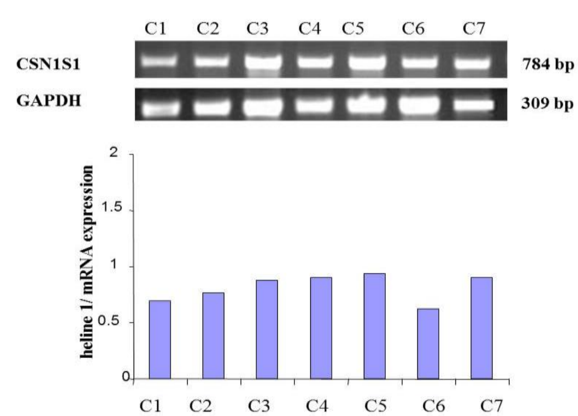
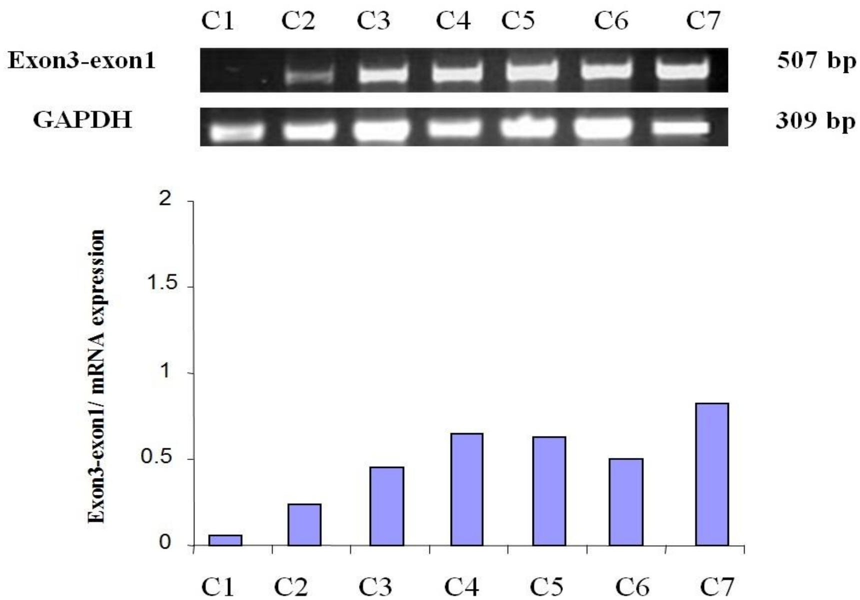


Fig. 1. RT-PCR analysis of CD-CSN1S1 geneexpression in camel milk. RNA was extracted and reverse transcribed (1 µg) and RT-PCR analysis was carried out to examine the expression of CD-CSN1S1-mRNA

Fig. 2. RT-PCR analysis of exon3-exon1 mRNA gene expression in camel milk. RNA was extracted and reverse transcribed (1 µg) and RT-PCR analysis was carried out to examine the expression of exon3-exon1 mRNA.



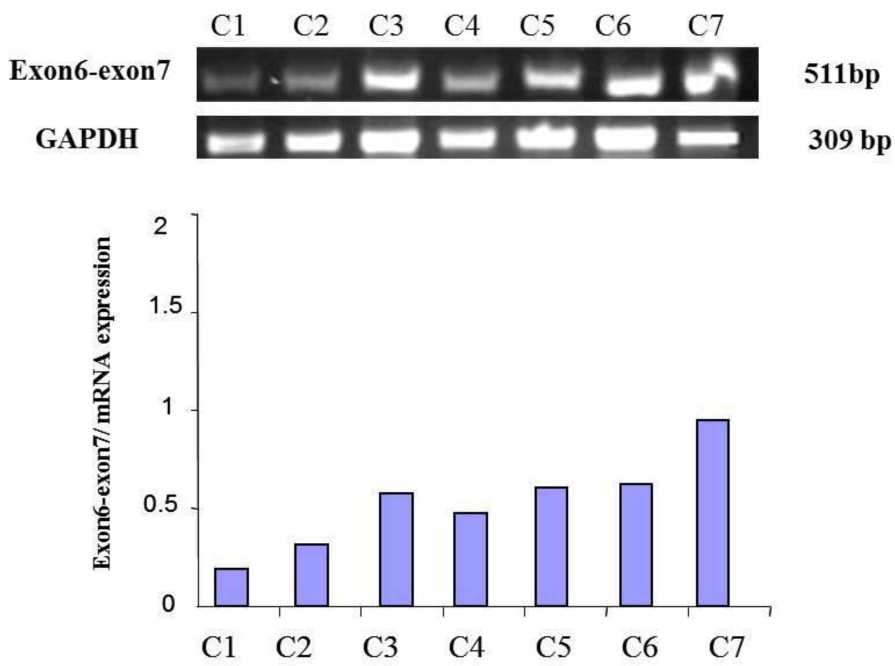


Fig. 3. RT-PCR analysis of exon6-exon7 mRNA gene expression in camel milk. RNA was extracted and reverse transcribed (1 µg) and RT-PCR analysis was carried out to examine the expression of exon6-exon7 mRNA.

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