

FAST AND SENSITIVE DETERMINATION OF CAMEL'S AND GOAT'S MEAT AND MILK USING SPECIES-SPECIFIC GENETIC MARKERS

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Abstract: For the fast and sensitive determination of camel's and goat's meat and milk, species-specific regions (SSR) of follicle stimulating hormone receptor (FSHR) gene in both camel and goat were amplified using PCR technique. DNA was extracted from small amount of muscles (0.05 gm) and very little of fresh milk (100 µl) to amplify specific DNA sequences of FSHR gene in both camel and goat using designed species-specific primer pairs. PCR amplified fragment size was 300 bp in camel's meat and milk, while the fragment size in goat's meat and milk was 855 bp. The use of species-specific regions of FSHR gene allowed direct and fast detection of adulteration and authentication of camel's and goat's meat and milk.

Key words: Meat, milk, species-specific, genetic markers, PCR

Introduction

In general, consumers are anxious about some of the issues such as food authenticity and adulteration, especially, when the identity of the ingredients in composite mixtures is not readily apparent (*Lockley and Bardsley 2000; Aida et al. 2005*). However, identification of species origin of meat and milk products has received considerable attention over the last ten years, particularly after the spread of commercial fraud. The common fraudulent practice found in the meat and dairy production line are the use of a less costly type of meat or milk in substitution of more expensive ones (*Calvo et al. 2002*). So, control tests of meat and milk products are very important to assure adulterant free for safe consumption. Previously, determination of species-specific meat and milk products could be achieved using numerous methods such as anatomical differences, enzyme-linked immunosorbent assays, histological differentiation of the hair that may possibly exist in the meat or milk, immune diffusion tests, level of glycogen in muscle tissue, properties of tissue fat, and sensory analysis (*Addeo et al. 1990; Chianese et al. 1990; Moio et al. 1990; Molina et al. 1999*). However, immunological,

electrophoretic and chromatographic methods are often not suitable and less sensitive for food products (meat and milk) identification, as well as complex, high cost and require data about the differences in protein compositions. Recently, food products like meat and milk products can be fast and accurate identified using molecular genetics methods such as PCR and PCR-RFLP (*Abdel-Rahman and Ahmed 2007; Ilhak and Arslan 2007; Abdel-Rahman et al. 2009*). Somatic cells from red meat and in milk (leukocytes and epithelial mammary cells) were used as a source of DNA which can be successfully applied for precise species differential using molecular genetics techniques (*Amills et al. 1997; Maudet and Taberlet 2001*). In this study, fast species-specific PCR technique was developed for determination and detection of camel's and goat's meat and milk.

Materials and methods

DNA extraction from meat. According to *Abdel-Rahman et al. (2009)*, genomic DNA was extracted from camel's and goat's muscle samples, where 50 mg of the tissue was homogenized and suspended in 500 μ L STE (0.1 M NaCl, 0.05 M Tris-HCL and 0.01 M EDTA, pH 8). After adding 30 μ L 10% SDS and 30 μ L proteinase K (10 mg/mL), the mixture was vortexed and incubated at 50°C for 30 min. DNA was extracted by equal volumes of phenol–chloroform–isoamylalcohol (25:24:1) and chloroform–isoamylalcohol (24:1), successively. DNA was precipitated by adding two equal volumes of chilled ethanol (95%). The pellet was washed with 70% ethanol, air-dried and subsequently dissolved in an appropriate volume (50 μ L) of autoclaved double distilled water (addH₂O).

DNA extraction from milk. Camel's and goat's genomic DNA was extracted from little fresh milk samples according to *Abdel-Rahman and Ahmed (2007)*, where 1400 μ L of lyses buffer (10 mM Tris-HCl, 100 mM NaCl, 1 mM EDTA, pH 8.0 and 0.5% SDS) and 30 μ L of proteinase K (20 mg/mL) were added to 100 μ L of camel's and goat's milk sample. The mixture was vortexed and incubated at 55°C for 20 min. DNA was extracted by equal volumes of phenol–chloroform–isoamylalcohol (25:24:1) and chloroform–isoamylalcohol (24:1), successively. DNA was precipitated by adding two equal volumes of chilled ethanol (95%). The pellet was washed with 70% ethanol, air-dried and subsequently dissolved in an appropriate volume (35 μ L) of autoclaved double distilled water (addH₂O).

Species-specific primer pairs designing. Two PCR species-specific primer pairs were designed to amplify a specific segment of follicle stimulating hormone receptor (FSHR) gene coding ovulation in both camel and goat (Table 1). Using the online of the National Center for Biotechnology Information (NCBI), PCR primer pairs were designed (Primer-BLAST program) on the camel 531 bp linear DNA of FSHR gene sequence (GenBank accession number: GU990799.1) to

amplify a fragment of 300 bp (Figure 1). Also, PCR primer pairs were designed on the goat 860 bp linear DNA of FSHR gene sequence (GenBank accession number: AY765375.1) to amplify a fragment of 855 bp (Figure 2).

Table 1. Primer sequences of species-specific DNA regions and their annealing temperatures.

Species	Primer sequence 5' - 3' (Forward/Reverse)	Annealing temperature (°C)
Camel	ACTGGAATCTATCTGCTGCTC/GCTGCTGATGCCAAAGAGG	58
Goat	CGACAAGGCAAAACGGACAC/TCCTGGCAGAGGAAGACTCCA	51

Primers sequences alignment. Using the online BLAST program, the designed species-specific primers were confirmed and aligned to investigate whether there is a match or similarity with other species before proceeding to PCR. Table 2 shows the match of the forward primer which flanking the region of interest of FSHR gene in camel compared the other species using nucleotide sequence database in GenBank (NCBI). As expected, the primer matches to camel specie, but does not have matches to other target species (Animals producing meat and milk), that allow desired PCR amplification. Also in goat, the forward primer which flanking the region of interest of FSHR gene matches to goat specie, but does not have matches to other target species (Table 3).

>gi|329132912|gb|GU990799.1| Camelus bactrianus follicle stimulating hormone receptor (FSHR) gene, partial cds (531 bp).

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1  ACTGGAATCTATCTGCTGCTCATAGCATCCGTTGATATCCACACCAAAAGCCAGTACCACAACATATGCCATTGACTGGCAAACCTGGA 87
5' ACTGGAATCTATCTGCTGCTC3' .....
1  TGACCTTAGATAGACGACGAGTATCGTAGGCAACTATAGGTGTGGTTTTTCGGTCATGGTGTGATACGGTAACGACCGTTTGACCT 87

88  GCAGGCTGTCATGCTGCTGGCTTTTTCACTGTCTTTGCCAGTGAGCTCTCAGTCTACACTCTGACAGCCATCACGCTGGAAAGATGG 174
88  CGTCCGACAGTACAGCAGCAGCAAAAGTGACAGAAACGGTCACTCGAGAGTCAGATGTGAGACTGTCTGGTAGTCGACCTTTCTACC 174

175  CATACCATCACCCACGCCATGCAGCTAGAATGCAAAGTGCAGCTCCGCCATGCTGCCAGTGTCTATGCTGGTAGGCTGGGTCTTTGCT 261
175  GTATGGTAGTGGGTGCGGTACGTCGATCTTACGTTTCACGTCGAGGCGGTACGACGGTCACAGTACGACCATCCGACCCAGAAACGA 261

262  TTTGCACTGCGCCCTCTTTCCCTCTTTGGCATCAGCAGCTACATGAAGGTGAGCATCTGCTTGCCCATGGATATTGACAGCCCTTG 348
.....3' GGAGAAACCGTAGTCGTCG5'
262  AAACGTCAGCGGGAGAAAGGGGAGAAACCGTAGTCGTCGATGTACTTCCACTCGTAGACGAACGGGTACCTATAACTGTGCGGGGAAC 348

349  TCACAGCTTTATGTGATGTCCCTCCTTGTGCTCAATGTCTGCGCTTTATGGTCATCTGTGGCTGCTACACTCACATCTACCTCACA 435
349  AGTGTGCGAAATACACTACAGGGAGGAACACGAGTTACAGGACCGGAAATACAGTAGACACCGACGATGTGAGTGTAGATGGAGTGT 435

436  GTGAGGAACCCCAACATCATGTCTCCTCTAGTGACACCAAGATTGCCAAGCGCATGGCCATGCTCATCTTCACGGACTTCCTCTGC 522
436  CACTCCTTGGGGTTGTAGTACAGGAGGAGATCACTGTGGTTCTAACGGTTTCGCGTACCGGTACGAGTAGAAGTGCCTGAAGGAGACG 522

523  ATGGCACCC 531
523  TACCGTGGG 531

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Figure 1. Primer pairs flanking a fragment (300 bp) of FSHR gene in camel. The forward primer begins from the nucleotide number one, while the reverse primer begins from the nucleotide number 300 in the opposite direction.

>gi|54399507|gb|AY765375.1| Capra hircus follicle-stimulating hormone receptor (FSHR) gene, promoter region and partial cds (860 bp).

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1  TTCCGACAAAGGCAAAACGGACACGTGACCGAGGGTGGGATAGTTGGTGTCTACTAAGCCCAAAACCCAGCCTGACCTGTTGGTCAC 87
5  'CGACAAAGGCAAAACGGACAC3'--
1  AAGGCTGTCCGTTTGGCTGTGCCTGACCTGCCATCCACAGATGATTGGGTTTTTGGGTCGGACTGGACAACCATG 87

88  TCAGCTGAGTCAGTTATCTCTGTAGACTACTCTTAAAGTGCCTCCTACCATCTGTCCAGGGGCTCACTAACCCACTGCCTGTCTTC 174
88  AGTCGACTCAGTCAATAGAGACATCTGATGAGAATTTTCACGGAGGATGGTAGACAGGTCCCCGAGTGATTGGGTGACGGACAGAAAG 174

175  TGCTACACCATATTTGGTTGTTAATTCACGCAAGAAAGAGAATTAGTGGCTTTGACCCAGAAGTTCTGGTTTTGTATCAAGCAGCCT 261
175  ACGATGTGGTATAAACCAACAATTAAGGTCGTTCTTCTCTTAATCACCGAAACTGGGTCTTCAAGACCAAAACATAGTTCGTCGGA 261

262  GGAGGAAGACATTGACACCAAGACTGGAACAGGTCCCTGACCTTCACTGAGGAACCTGTGTAATGATGTTTCACACTGCAGATTGC 348
262  CCTCCTTCTGTAACGTGTGTTCTGACCTTTGTCCAGGGACTGGAAGTGACTCCTTGAGACAATTACTACAAAGTGACGCTCAACG 348

349  ATCTGTTTTGGAGAAAGTCAAGCGTGTCTACTCTGTTTTGAGAAAAAAGAGTGAACCCACAGGGACAGTCTTACAGCGAATTT 435
349  TAGACAAAACCTCTTTGAGTTCGCACAGTGAGACAAAACCTCTTTTTTTTTTTTCACTGGGTGTCCCTGTCAGAAATGTCGCTTAAA 435

436  AATATAAGCTATTCTAGACATGCATCAAGTTTCAATTTGCAAAACCAACCAAAAGGGTAAAGGGACAGCGTATCTTGCCACGCCCT 522
436  TTATATTTCGATAAGATCTGTACGTAGTTCAAAGTTAAACGTTTGGGTGGTTTTTCCCATTTCCCTGTCGCATAGAACGGTGCAGGA 522

523  CTACCTCTCCCTCCCCACCCACCAAAGTCACTGCTGTCACTCAGAAATTTCTGCTATTTGCTGGAAGTGACCGATAAAAAAGAAA 609
523  GATGGAGAGGGGAGGGGTGGGGGTGGTTTCAGTGACGACAGTGAGTCTTTAAGACGATAAACAGACCTTCACTGGCTATTTTTCTCTT 609

610  AAAAGGAACACGCGCCTGGGCGGGTCACTGACCTTACCAGCTCCCAACGCAGACCTCTTCTCAAAGGGCTCAGTGTGGAGCCTCT 696
610  TTTTCTTGTGCGCGGACCCGCCAGTGACTGGGATGGTCGAGGGTTGCGTCTGGAGAGAGTTTCCCGAGTCACACCTCGGAGA 696

697  GAAATCTGGGCAAGGATTTGCTCTGCAGAGGCAGAAGCAAGCAGGTGGATGGATAAGATAACATGGCCTTGTTCCTGGTGCGCTTGCT 783
697  CTTTAGACCCGTCTAACAGAGACGTCTCCGTCTTCGTTGCTCCACTACCTATTTCATTTGTACCGGAACAAGGACCACCGGAACGA 783

784  CGCATTCTCTGAGCTTGGGCTCAGGATGCCACCATCGACTCTGTCACTGCTCTAAAGGAGTCTTCTCTGCCAGGA 860
784  GCATAAGGACTCGAACCCGAGTCTACGGTGGTAGCTGAGACAGTGACGAGATTACCTCAGAAAGGAGACGGTCTCTCC 860

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Figure 2. Primer pairs flanking a region (855 bp) of FSHR gene in goat. The forward primer begins from the nucleotide number four, while the reverse primer begins from the nucleotide number 858 in the opposite direction.

Table 2. Alignments of the forward primer of the FSHR fragment in camel. Selected accession numbers are camel specie only, while the other accession number (not selected) is Pteropus specie (species of fox).

Descriptions							
Sequences producing significant alignments:							
Select: All None Selected: 4							
Alignments Download GenBank Graphics Distance tree of results							
	Description	Max score	Total score	Query cover	E value	Ident	Accession
☒	PREDICTED: Camelus ferus follicle stimulating hormone receptor (FSHR), transcript	42.1	42.1	100%	0.034	100%	XM_006185787.1
☒	PREDICTED: Camelus ferus follicle stimulating hormone receptor (FSHR), transcript	42.1	42.1	100%	0.034	100%	XM_006185786.1
☒	PREDICTED: Camelus ferus follicle stimulating hormone receptor (FSHR), transcript	42.1	42.1	100%	0.034	100%	XM_006185785.1
☒	Camelus bactrianus follicle stimulating hormone receptor (FSHR) gene, partial cds	42.1	42.1	100%	0.034	100%	GU990799.1
☐	PREDICTED: Pteropus alecto follicle stimulating hormone receptor (FSHR), transcrip	38.2	38.2	90%	0.53	100%	XM_006909606.1

Table 3. Alignments of the forward primer of the FSHR fragment in goat. Selected accession number is goat specie only, while the next accession number (not selected) is *Albugo laibachii* (species of oomycete).

Descriptions							
Sequences producing significant alignments:							
Select: All None Selected:1							
Alignments Download GenBank Graphics Distance tree of results							
	Description	Max score	Total score	Query cover	E value	Ident	Accession
☒	Capra hircus follicle-stimulating hormone receptor (FSHR) gene, promoter region anti	40.1	40.1	100%	0.15	100%	AY765375.1
☐	Albugo laibachii Nc14, genomic contig CONTIG_797_NC14_v4_5976_220	36.2	36.2	90%	2.3	100%	FR824725.1
☐	Selaginella moellendorffii hypothetical protein, mRNA	36.2	36.2	90%	2.3	100%	XM_002974807.1

PCR amplification. PCR was performed in a reaction volume of 25 μ L using 25 ng of genomic DNA of each specie, 25 pmol of each primer, 10X Taq DNA polymerase buffer including $MgCl_2$, 0.2 mM dNTPs and 5 unit/ μ L Taq DNA polymerase (Promega). Thermal cycling (MyGene Series Peltier Thermal Cycler) was carried out by initial denaturation at 94°C for 4 min, followed by 35 cycles each at 94°C for 1 min, annealing temperature at 51 (goat) and 58 (camel) for 1 min, polymerization temperature at 72°C for 1 min and final extension at 72°C for 10 min, then the samples were held at 4°C. The amplified DNA fragments were separated on 2.5% agarose gel, stained with ethidium bromide, visualized on a UV Transilluminator and photographed by Gel Documentation system (Alpha Imager M1220, Documentation and Analysis System, Canada).

Results and discussion

In this study, genomic DNA from meat (red meat) and milk (leukocytes and epithelial mammary cells) was extracted to amplify specific segments or regions of FSHR gene encoding ovulation in two different species camel and goat. The amplification of species-specific DNA segments of the FSHR gene yielded PCR product with size of 300 bp in length in camel's meat and milk. While, in goat's meat and milk the PCR product was 855 bp (Figure 3A and B). To avoid the fraudulent and to assure consumers of accurate labeling, it was necessary to invent new precise and fast techniques for differentiation and detection of adulteration of meat and milk species-specific. Where, the common fraudulent practice found in the meat or milk products line is the use of a less costly type of meat or milk in substitution or mixing of more expensive or authenticated ones. In the current study, PCR technique was developed to identify and detect the adulteration of camel's and goat's meat and milk products. Where, two primer pairs (forward and

reverse) were specifically designed to generate two specific fragments in length of FSHR gene in both camel (300 bp) and goat (855 bp).

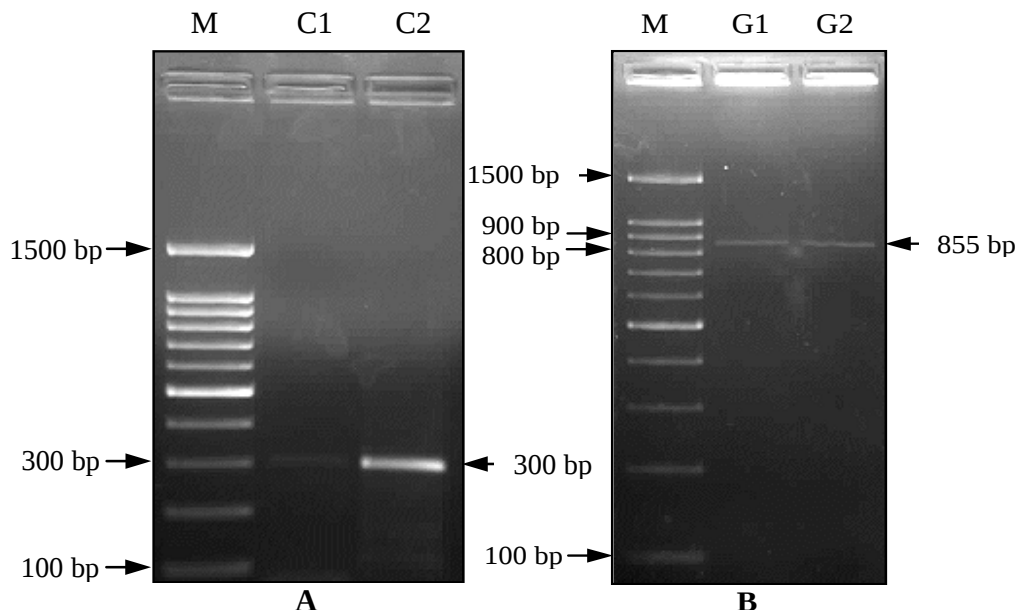


Figure 3. PCR products generated by species-specific designed primers in both camel (A) and goat (B). Lanes C1 and C2 are camel's milk and meat fragment size (300 bp), respectively. Lanes G1 and G2 are goat's milk and meat fragment size (855 bp), respectively. Lane M is a molecular weight marker.

Numerous studies have been previously carried for identification species-specific meat and milk, weather using immunological, electrophoretic and chromatographic methods (Addeo *et al.* 1990; Chianese *et al.* 1990; Moio *et al.* 1990; Molina *et al.* 1999) or molecular genetic methods such as PCR and PCR-RFLP techniques (Baradakci and Skibinski 1994; Meyer *et al.* 1995; Meyer *et al.* 1996; Hopwood *et al.* 1999; Partis *et al.* 2000; Abdel-Rahman and Ahmed 2007; Ahmed *et al.* 2007; Abdel-Rahman *et al.* 2009). In our previous studies, species-specific PCR and PCR-RFLP techniques were used to identify species meat and milk. Where, genomic DNA from buffalo's, cattle's and sheep's meat and milk and from cat's, dog's, donkey's, horse's and pig's meat was extracted to amplify the gene encoding species-specific repeat (SSR). The results of PCR amplification were 603 bp in length in buffalo and cattle, 374 bp in sheep, 672 bp in cat, 808 bp in dog, 221 bp in donkey and horse, and ≤ 100 bp in pig. To differentiate between buffalo's and cattle's meat and milk, as well donkey's and horse's meat, cytochrome-*b* gene was amplified (359 bp) and digested with restriction enzymes. By *TaqI* restriction enzyme, two different fragments (191 bp and 168 bp) were generated in buffalo, whereas no fragments were obtained in cattle. With *AluI*

restriction enzyme, three different patterns were generated in horse (189 bp, 96 bp and 74 bp), while in donkey no digestion was obtained (*Abdel-Rahman and Ahmed 2007; Ahmed et al. 2007; Abdel-Rahman et al. 2009*). To avoid the similarity of the PCR products between the previous mentioned species and the two species under study, species-specific fragments in length 300 bp and 855 bp of FSHR gene were amplified in both camel and goat, respectively. However, the author's previous and present results of PCR product sizes for identification of species-specific meat and milk products are concluded in Table 4.

Table 4. PCR products of the species-specific meat and milk products descending ordered according to the fragment sizes.

Species	Primer sequence 5' - 3'	Annealing temperature (°C)	PCR product size (bp)
Goat	CGACAAGGCAAAACGGACAC TCCTGGCAGAGGAAGACTCCA	51	855
Dog	GGAGTATGCTTGATTCTACAG AGAAGTGGAAATGAATGCC	52	808
Cat	CTCATTCATCGATCTACCCA GTGAGTGTTAAACTAGTACTAGAAGA	52	672
Buffalo/Cattle	AAGCTTGTGACAGATAGAACGAT CAAGCTGTCTAGAATTCAGGGA	60	603
Sheep	GTTAGGTGTAATTAGCCTCGCGAGAA AAGCATGACATTGCTGCTAAGTTC	62	374
Camel	ACTGGAATCTATCTGCTGCTC GCTGCTGATGCCAAAGAGG	58	300
Donkey/Horse	TTCTGCTCTGGGTGTGCTACTT CTACTTCAGCCAGATCAGGC	55	221
Pig	GGAGCGTGGCCCAATGCA ATTGAATCCACTGCATTCAATC	57	≤100

Conclusion

PCR technique was developed for rapid and sensitive identification of camel's, and goat's meat and milk using designed species-specific primers. PCR amplified fragment size was 300 bp in camel's meat and milk, while the fragment size in goat's meat and milk was 855 bp. The proposed PCR assay represents a quick and sensitive method applicable to the detection and authentication of meat and milk species-specific.

Brzo i osjetljivo određivanje kamiljeg i kozjeg mesa i mleka korišćenjem specifičnih genetičkih markera za vrstu

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Rezime

Za brzo i osjetljivo određivanje kamiljeg i kozjeg mesa i mleka, regioni folikula specifični za vrstu (SSR) koji stimulišu hormon receptore (FSHR) gena i u kamile i koze su pojačani pomoću PCR tehnike. DNK je ekstrahovana iz male količine mišića (0.05 gm) i veoma malo svežeg mleka (100 µl) za amplifikaciju specifičnih DNK sekvenci FSHR gena i u kamile i koze korišćenjem dizajniranih prajmera parova specifičnih za vrstu. Veličina amplifikovanog PCR fragmenta je bila 300 bp mesa i mleka kamile, dok je veličina fragmenata u kozjem mesu mleku bio 855 bp. Upotreba regiona FSHR gena specifičnih za vrstu omogućava direktnu i brzu detekciju autentifikacije kamiljeg i kozjeg mesa i mleka.

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