

Probing of alpha, beta, and kappa-caseins variations in the *Gangatiri* cow milk with the use of polyacrylamide gel electrophoresis and High-Resolution Accurate Mass Spectroscopy

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SUPPLEMENTARY FILE

Fig. S1: Comparative demonstration of SDS-PAGE dissociation of whole caseins isolated from the two exotic cow breeds, Jersey and HF, and two indigenous breeds of cow, Sahiwal, and Gangatiri (Lanes 4-7). Lane first contains a protein ladder (Sigma Aldrich). Whole bovine caseins were fractionated into alpha, beta, and kappa casein bands.

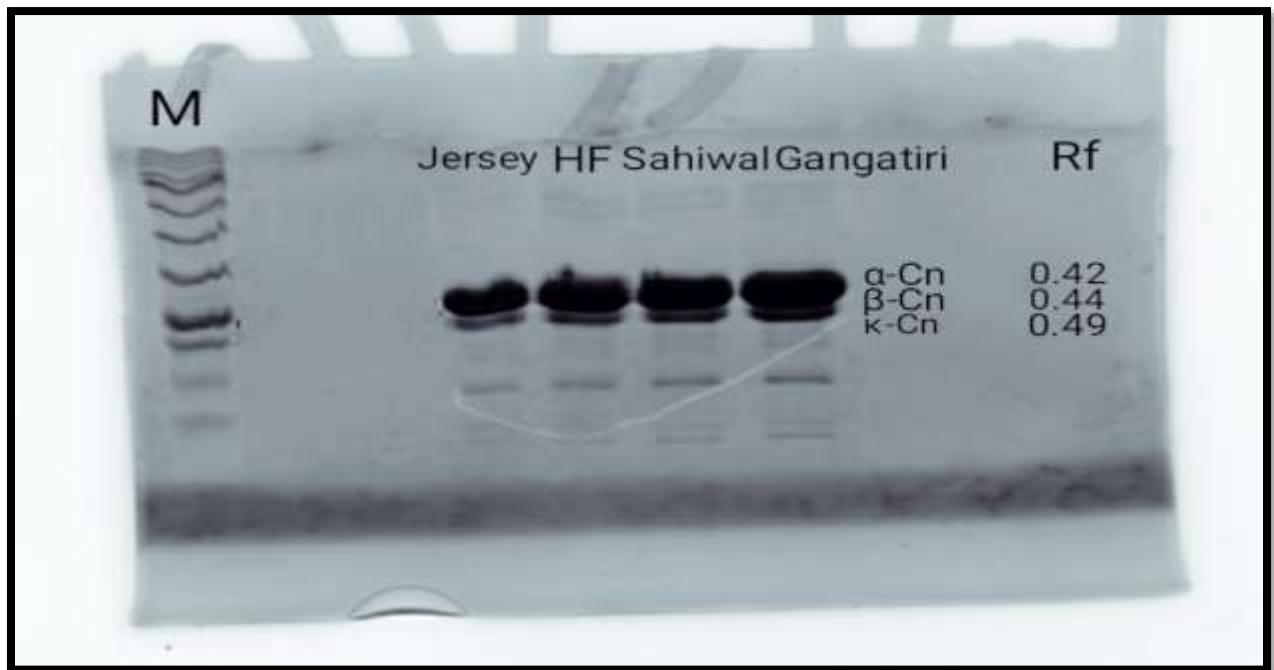


Fig. S2: The amino acid sequence of α s1-casein according to UniProt Custom reference proteome database (ID: P02662) as comparing data obtained from HRAMS analysis homologous to NCBI database depicts variant B.

1-Arg-Pro-Lys-His-Pro-Ile-Lys-His-Gln-Gly-Leu-Pro-Gln-Glu-Val-Leu-Asn-Glu-Asn-Leu-Leu-Arg-Phe-Phe-Val
26-**Ala**-Pro-Phe-Pro-Glu-Val-Phe-Gly-Lys-Glu-Lys-Val-Asn-Glu-Leu-Ser-Lys-Asp-Ile-Gly-SerP-Glu-SerP-Thr-
Glu

51–Asp-Gln-**Ala**-Met-Glu-Asp-Ile-Lys-Gln-Met-Glu-Ala-Glu-SerP-Ile-SerP-SerP-SerP-Glu-Glu-Ile-Val-Pro-Asn-SerP

76–Val-Glu-Gln-Lys-His-Ile-Gln-Lys-Glu-Asp-Val-Pro-Ser-Glu-Arg-Tyr-Leu-Gly-Tyr-Leu-Glu-Gln-Leu-Leu-Arg

101–Leu-Lys-Lys-Tyr-Lys-Val-Pro-Gln-Leu-Glu-Ile-Val-Pro-Asn-SerP-Ala-Glu-Glu-Arg-Leu-His-Ser-Met-Lys-Glu

126–Gly-Ile-His-Ala-Gln-Gln-Lys-Glu-Pro-Met-Ile-Gly-Val-Asn-Gln-Glu-Leu-Ala-Tyr-Phe-Tyr-Pro-Glu-Leu-Phe

151–Arg-Gln-Phe-Tyr-Gln-Leu-Asp-Ala-Tyr-Pro-Ser-Gly-Ala-Trp-Tyr-Tyr-Val-Pro-Leu-Gly-Thr-Gln-Tyr-Thr-Asp

176–Ala-Pro-Ser-Phe-Ser-Asp-Ile-Pro-Asn-Pro-Ile-Gly-Ser-Glu-Asn-Ser-**Glu**-Lys-Thr-Thr-Met-Pro-Leu-Trp

Fig. S3: The amino acid sequence of α s₂-casein according to UniProt Custom reference proteome database (ID: P02663) as comparing data obtained from HRAMS analysis homologous to NCBI database depicts variant A.

1–Lys-Asn-Thr-Met-Glu-His-Val-SerP-SerP-SerP-Glu-Glu-Ser-Ile-Ile-SerP-Gln-Glu-Thr-Tyr-Lys-Gln-Glu-Lys-Asn

26–Met-Ala-Ile-Asn-Pro-Ser-Lys-Glu-Asn-Leu-Cys-Ser-Thr-Phe-Cys-Lys-Glu-Val-Val-Arg-Asn-Ala-Asn-Glu-Glu

51–Glu-Tyr-Ser-Ile-Gly-SerP-SerP-SerP-Glu-Glu-SerP-Ala-Glu-Val-Ala-Thr-Glu-Glu-Val-Lys-Ile-Thr-Val-Asp-Asp

76–Lys-His-Tyr-Gln-Lys-Ala-Leu-Asn-Glu-Ile-Asn-Gln-Phe-Tyr-Gln-Lys-Phe-Pro-Gln-Tyr-Leu-Gln-Tyr-Leu-Tyr

101–Gln-Gly-Pro-Ile-Val-Leu-Asn-Pro-Trp-Asp-Gln-Val-Lys-Arg-Asn-Ala-Val-Pro-Ile-Thr-Pro-Thr-Leu-Asn-Arg

126–Glu-Gln-Leu-SerP-Thr-SerP-Glu-Glu-Asn-Ser-Lys-Lys-Thr-Val-Asp-Met-Glu-SerP-Thr-Glu-Val-Phe-Thr-Lys-Lys

151–Thr-Lys-Leu-Thr-Glu-Glu-Glu-Lys-Asn-Arg-Leu-Asn-Phe-Leu-Lys-Lys-Ile-Ser-Gln-Arg-Tyr-Gln-Lys-Phe-Ala

176–Leu-Pro-Gln-Tyr-Leu-Lys-Thr-Val-Tyr-Gln-His-Gln-Lys-Ala-Met-Lys-Pro-Trp-Ile-Gln-Pro-Lys-Thr-Lys-Val-Ile-Pro-Tyr-Val-Arg-Tyr-Leu

Fig. S4: The total amino acids chain of β -Cn according to UniProt Custom reference proteome database (ID: P02666) as correlating results obtained from HRAMS proteomics showing similarity to NCBI database depicts Proline (P) at position 67 showing A2 variant.

1–Arg-Glu-Leu-Glu-Glu-Leu-Asn-Val-Pro-Gly-Glu-Ile-Val-Glu-SerP-LeuSerP-SerP-SerP-Glu-Glu-Ser-Ile-Thr-Arg
26–Ile-Asn-Lys-Lys-Ile–Glu-Lys-Phe-Gln-SerP-Glu-Glu-Gln-Gln-Gln–Thr-Glu-Asp-Glu-Leu-Gln-Asp-Lys-Ile-His
51–Pro-Phe–Ala-Gln–Thr–Gln-Ser–Leu-Val-Tyr-Pro–Phe-Pro-Gly-Pro-Ile–**Pro**-Asn-Ser-Leu-Pro–Gln-Asn–Ile-Pro-
76–Pro-Leu-Thr–Gln-Thr-Pro-Val–Val-Val-Pro-Pro-Phe–Leu-Gln-Pro-Glu-Val-Met-Gly-Val-Ser-Lys-Val-Lys-Glu
101–Ala-Met-Ala-Pro-Lys-His-Lys-Glu-Met-Pro-Phe-Pro-Lys-Tyr-Pro-Val-Glu-Pro-Phe-Thr-Glu-Ser-Glu-Ser-Leu
126–Thr-Leu-Thr–Asp–Val-Glu–Asn-Leu-His-Leu–Pro-Leu-Pro-Leu-Leu–Gln-Ser-Trp–Met-His-Gln–Pro-His-Gln-
Pro
151–Leu-Pro-Pro-Thr-Val-Met–Phe-Pro-Pro-Gln-Ser–Val-Leu-Ser-Leu-Ser-Gln–Ser-Lys-Val-Leu-Pro-Val-Pro-Gln
176–Lys-Ala-Val-Pro-Tyr-Pro-Gln-Arg-Asp-Met-Pro-Ile-Gln-Ala-Phe-Leu-Leu-Tyr-Gln-Glu-Pro-Val-Leu-Gly-Pro
201–Val-Arg-Gly-Pro-Phe-Pro-Ile-Ile-Val

Fig. S5: The amino acid sequence of kappa-casein according to UniProt Custom reference proteome database (ID: P02668) as comparing data obtained from HPLC-MS analysis homologous to NCBI database depicts Threonine(T) at position 136 and Aspartic acid(D) at position 148.

1–Glu-Glu-Gln-Asn-Gln-Glu-Gln-Pro-Ile-Arg-Cys-Glu-Lys-Asp-Glu-Arg-Phe-Phe-Ser-Asp-Lys-Ile-Ala–Lys-Tyr
26–Ile-Pro-Ile-Gln-Tyr-Val-Leu-Ser–Arg-Tyr-Pro-Ser-Tyr-Gly-Leu–Asn-Tyr-Tyr-Gln-Gln-Lys–Pro-Val–Ala-Leu
51–Ile-Asn-Asn-Gln-Phe–Leu-Pro-Tyr-Pro–Tyr-Tyr-Ala-Lys-Pro-Ala-Ala-Val-Arg-Ser-Pro-Ala-Gln-Ile–Leu-Gln
76–Trp-Gln-Val-Leu-Ser-Asn-Thr-Val-Pro-Ala-Lys-Ser-Cys-Gln-Ala-Gln-Pro-Thr-Thr-Met-Ala-Arg-His-Pro-His
101–Pro-His-Leu-Ser–Phe-Met–Ala-Ile-Pro-Pro-Lys–Lys-Asn-Gln-Asp-Lys–Thr-Glu-Ile-Pro-Thr-Ile–Asn-Thr-Ile
126–Ala-Ser-Gly-Glu-Pro-Thr-Ser-Thr-Pro-Thr–**Thr**-Glu-Ala-Val-Glu-Ser-Thr-Val-Ala-Thr-Leu-Glu–**Asp**-SerP-Pro
151–Glu-Val-Ile-Glu-Ser-Pro-Pro-Glu-Ile-Asn-Thr-Val-Gln-Val-Thr-Ser-Thr-Ala-Val

Table S1 Result of detailed sequence database search in UniProt

S. No.	Protein Group IDs	Accession	Description	Sequence	Sum PEP Score	Coverage [%]	# Peptides	# PSMs	# Unique Peptides	# AAs	MW [kDa]	calc. pI	Score Sequest HT: Sequest HT	# Peptides (by Search Engine): MS Amanda	# Peptides (by Search Engine): Sequest HT	Entrez Gene ID	Gene Symbol	Chromosome	# Protein Pathway Groups
1	9	P02662	Alpha-S1-casein	MKLLITCLVAVALARPKHPKHQGLPQEVLE NLRFFVAFPEVFGKEKVNLSKDIGSESTEDQ AMEDIKQMEAESISSSEIIPNSVEIQKHQKED VPSERYLYLEQLLRKKYKVPQLEIVPNSAEER LHSMKEGIHAQQKEPMIGVNLQELAYPELFR QFYQLDAYPSGAWYYVPLGTDAPSFSDIP NPIGSENSEKTTMPLW	277	59	20	504	20	214	24.5	5.02	78106.1	16	20	282208	CSN151	6	2
2	25	P02663	Alpha-S2-casein	MKFFIFTCLLAVALAKNTMEHVSSSEESIISQET KQENMMAINPSKENLCTFCKEVRNANEEFY SIGSSSEAEVATEEVKITVDDKHQKALNEIN QFYQKFPQYLQLYQGPIVLPWDQVIRNAV PIPTLNREQLSTSSEENSKXTVDMESTEVFTKT KLTEEEKNRLNFKISQRYQKFPALPQYLVYVQ HQKAMKPIWQPKTKVIPYRYL	50.4	53	13	95	13	222	26	8.43	10445.3	12	13	282209	CSN152	6	0
3	29	P00760	Cationic trypsin	MKTFRLALLGAAVFPVDDDDKIVGGYTCGAN TVPYQVSLNSGYHFCGGSLSNQWVWSAAHCY KSGIQVRLGEDNINWVEGNEQFISAKSIVHPSY NSNTLNNDIMLIKLSAASLSNRVASISLPTSCA SAGTQCUSGWNKTSSTSYDVLKCLKAPILS DSSCKSAVPGQITSNMF CAGYLEGGRDSCQGD SGGPVVCSGKLQGVSWGSGCAQKNKPGVYTK VCNYYVSWIKQTIAN	235	73	22	966	22	246	25.8	8.07	1951.13	12	22	615026; 780933	LOC615026; PRSS1; LOC780933	4	4
4	59	P02666	Beta-casein	MKVLILACLVALALARELEELNVPGEIVESSSE ESITRINKKIEKQSEEQQTEDELQDKIHFFAQ TQSLVYFPFGPIPNLQNI PPLTQTPVWVPPFL QPEVMGVSKVEAMAPKHKEMPPKYPVEPFT ESQSLTLTDVENLHLPLQLQSWMHQPHQPLP PTVMFPPQSVLSLSQSKVLPVQKAVPYQRD MPIQAFLLYQEPVLPVIRGPPPIV	132	33	13	302	13	224	25.1	5.35	298.86	10	13	281099	CSN2	6	0
5	8	F1MC11	Keratin, type I cytoskeletal 14	MTTCSRQYTSSSSIKSSGGIGGGSSRISSVLAGGS CRAPSAYGGLSVSSRYSSGGVCGLG GYG GGF SSSSFGGALGSSFGGGYGGGLGAGFGGGFGG GVGGGFGGGFGVGDGLLAGSEKVTMQNLND RLASYLKVRALLEEADLEVKIRDWYQRORP AEIKDYSPYFKTIEDLRNKILTATVONANVVLQI DNARLAADDFRTKYETELNRLSVEADINGLRR VLDELTLARADLEMQIESKEELAYLRKNHEEE MNSLRGQVGGDVNEMD AAPGVOLSRILNE MRDQYKMAEKNRKDAEDWFFSKTEELNREV ATNSELVQSGKSEISELRRTLQNL EIELQSLM KASLENSLEETKGRYCMQLAQIQELISSVEEQLA QLRC EMEQQNQYKILLDVKTRLEQEIATYRRL LEGEDAHLS SQSSGQS SRDVSSSRQVR TKV VDVHDGKVVSTHEQVTRTKN	96	42	23	121	17	477	51.9	5.16	172.25	19	23	404111	KRT14	19	7
6	66	P02668	Kappa-casein	MMKSFLLWTILALTPFLGAQEQNQEQPIRCE KDERFFSDKIAKYPQVLSRYPSYGLNYYQKIP VALINNQLFPYPIYAKPAAVRSPACILQWQVLS NTVPAKSCQAQPTTMRHPHPLHSFMAIPPKK NQDKTEIPTINTIASGEPSTPTTEAVESVATLE DSPEVIESPPEINTVQVTSTAV	19	25	4	18	4	190	21.3	6.77	31.75	4	4	281728	CSN3	6	0