Bioactive peptides from milk: animal determinants and their implications in human health

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SUPPLEMENTARY FILE

Supplementary table S1
Activity, fragment, amino acid sequence, chemical mass and monoisotopic mass of different bioactive peptides reported in bovine α_{s1}-casein

Activity	Fragment	Sequence	Chemical mass	Monoisotopic mass
Angiotensin-converting-enzyme inhibitor	1-9	RPKHPIKHQ	1140.35	1139.65
	90-94	RYLGY	670.74	670.33
	90-93	RYLG	507.57	507.27
	136-139	LFRQ	562.66	562.31
	142-147	LAYFYP	772.87	772.37
	143-150	AYFYPELF	1049.17	1048.48
	143-149	AYFYPEL	901.99	901.41
	144-149	YFYPEL	830.91	830.37
	157-164	DAYPSGAW	865.88	865.35
	158-161	AYPS	436.45	436.18
	170-173	GTQY	467.46	467.19
	184-199	TTMPLW	747.90	747.35
Antibacterial	1-7	RPKHPIK	875.07	874.54
	10-14	GLPQE	542.58	542.26
	21-29	IKHQGLPQE	1049.18	1048.55
	30-37	VLNENLLR	970.13	969.55
	195-208	SDIPNPIGSENSEK	1486.55	1485.68
Antioxidative	93-97	GYLEQ	608.63	608.27
	98-100	LLR	400.51	400.27
	100-104	RLKKY	706.87	706.44
	146-150	YPELF	667.74	667.31
	154-157	YQLD	537.55	537.23
	154-156	YQL	422.46	422.21
	158-161	AYPS	436.45	436.18
	170-173	GTQY	467.46	467.19
Immunomodulation	1-23	RPKHPIKHQGLPQEVLNENLLRF	2764.22	2762.52
	194-199	TTMPLW	747.90	747.35
	92-94	LGY	351.38	351.17
Opioid	91-96	YLGYLE	756.83	756.36
	194-199	TTMPLW	747.90	747.35
Opioid antagonist	90-95	RYLGYL	783.90	783.42

Based on data from BIOPEP (2018)

Supplementary table S2
Activity, fragment, amino acid sequence, chemical mass and monoisotopic mass of different bioactive peptides reported in bovine α_{s2}-casein

Activity	Fragment	Sequence	Chemical mass	Monoisotopic mass
Angiotensin-converting-enzyme inhibitor	92-98	FPQYLQY	958.06	957.45
	165-170	LKKISQ	715.88	715.45
	170-174	RYQKF	740.84	740.38
	170-172	RYQ	465.49	465.22
	174-181	FALPQYLK	979.17	978.54
	189-192	AMPKPW	728.90	728.35
	189-192	AMKPW	631.78	631.30
	190-197	MKPWIQPK	1027.28	1026.55
	198-202	TKVIP	556.69	556.35
	203-208	PYVRYL	809.94	809.43
	204-206	VRY	436.49	436.23
Antibacterial	164-179	LKKISQRYQKFALPQY	2011.38	2010.12
	164-207	LKKISQRYQKFALPQYLKTVYQHQKAMKPWIQPKTKVIPYVRYL	5451.57	5448.06
	165-181	LKKISQRYQKFALPQYL	2124.54	2123.21
	165-181	LKKISQYYQKFAWPQYL	2204.57	2203.17
	172-207	QKFALPQYLKTVYQHQKAMKPWIQPKTKVIPYVRYL	4434.33	4431.45
	175-207	ALPQYLKTVYQHQKAMKPWIQPKTKVIPYVRYL	958.06	957.45
	180-207	LKTVYQHQKAMKPWIQPKTKVIPYVRYL	715.88	715.45
	181-207	KTVYQHQKAMKPWIQPKTKVIPYVRYL	740.84	740.38
	183-206	VYQHQKAMKPWIQPKTKVIPYVRY	465.49	465.22
	183-207	VYQHQKAMKPWIQPKTKVIPYVRYL	979.17	978.54
	184-208	VDQHQKAMKPWTQPKTNAIPYVRYL	728.90	728.35
	203-208	PYVRYL	631.78	631.30
Antioxidative	89-93	YQKFP	1027.28	1026.55
	170-172	RYQ	556.69	556.35
	174–181	FALPQYLK	809.94	809.43
	179-182	YLKT	436.49	436.23
	182-185	TVYQ	2011.38	2010.12
Immunomodulation	1-32	KNTMEHVSSSEESIISQETYKQEKNMAINPSK	2204.57	2203.17

Based on data from BIOPEP (2018)

Supplementary table S3
Activity, fragment, amino acid sequence, chemical mass and monoisotopic mass of different bioactive peptides reported in bovine β-casein

Activity	Fragment	Sequence	Chemical mass	Monoisotopic mass
Angiotensin-converting-enzyme inhibitor	59-64	VYPFPG	678.77	678.32
	59-61	VYP	377.42	377.18
	60-66	YPFPGPIP	887.03	886.44
	60-66	YPFPGPI	789.91	789.39
	60-68	YPFPGPIPN	1001.13	1000.49
	63-67	PGPIP	479.56	479.26
	80-90	TPVVVPPFLQP	1193.44	1192.67
	104-120	PKHKEMPFPPKYPVEPFT	2169.56	2168.09
	108-113	EMPFPK	747.90	747.35
	120-126	TESQSLT	764.78	764.34
	125-131	LTLTDVE	789.87	789.40
	140-143	LQSW	532.58	532.25
	169-174	KVLPVP	651.83	651.42
	169-175	KVLPVPQ	779.97	779.48
	177-183	AVPYPQR	829.94	829.43
	177-179	AVP	285.33	285.16
	179-181	PYP	375.40	375.17
	180-197	YPQRDMPIQ	1147.30	1146.54
	189-192	AFLL	462.58	462.27
	191-197	LLYQQPV	860.00	859.47
	193-198	YQEPVL	747.83	747.37
	193-198	YQQPVL	746.84	746.38
	193-202	YQEPVLQPVR	1228.40	1227.65
	202-209	RGPFPIIV	898.10	897.53
	203-209	GPFPIIV	741.92	741.43
	205-209	FPIIV	587.75	587.36
Antibacterial	184-210	QELLLNPTHQYPVTQPLAPVHNPISV	2906.32	2904.53
	169-176	KVLPVPQK	908.14	907.57
	170-176	VLPVPQK	779.97	779.48
	177-183	AVPYPQR	829.94	829.43
	183-188	RDMPIQ	758.88	758.36
Immunomodulation	1-28	RELEELNVPGEIVESLSSSEESITRINK	3158.47	3156.60
	1-28	LLYQEPVLGPVRGPFPIIV	2107.55	2106.21
	63-68	PGPIPN	593.67	593.30
	91-93	GVM	305.38	305.13
	191-193	LLY	407.49	407.23

Based on data from BIOPEP (2018)

Supplementary table S4
Activity, fragment, amino acid sequence, chemical mass and monoisotopic mass of different bioactive peptides reported in bovine κ-casein

Activity	Fragment	Sequence	Chemical mass	Monoisotopic mass
Angiotensin-converting-enzyme inhibitor	12-17	EKDERF	822.86	822.37
	18–24	FSDKIAK	807.93	807.44
	13-17	KDERF	693.74	693.33
	21-23	YVP	377.42	377.18
	22-24	IAK	330.41	330.22
	25-30	YIPIQY	795.91	795.40
	28-30	IQY	422.46	422.21
	33-37	SRYPS	608.64	608.28
	34-37	RYPS	521.56	521.25
	34-36	RPY	434.48	434.22
	42-47	YYQQKP	825.89	825.39
	51-53	VAV	287.35	287.17
	56-60	LPYPY	651.73	651.31
	61-65	YAKPA	548.62	548.28
	61-64	YAKP	477.54	477.25
	67-68	VR	273.32	273.17
	76-80	PNSHP	550.56	550.24
	78-80	SHP	339.34	339.14
	94-97	IAIP	412.52	412.26
	94-98	IAIPP	509.63	509.31
	95-98	AIPP	396.47	396.23
	96-101	ARHPHP	713.79	713.36
	97-101	RHPHP	642.71	642.32
	120-122	PAP	283.31	283.14
	185-190	VTSTAV	576.64	576.30
Antibacterial	18-24	FSDKIAK	807.93	807.44
	28-30	IQY	422.46	422.21
	30-32	YVL	393.46	393.22
	42-49	YYQQKPVA	996.11	995.49
	64-75	PAAVRSPAQILQ	1250.45	1249.70
	118-121	EIPT	458.50	458.23
	139-146	VESTVATL	818.91	818.43
	141-146	STVATL	590.66	590.32
	162-169	VQVTSTAV	803.90	803.43
	25-30	YIPIQY	795.91	795.40
Antioxidative	25-30	FYQL	569.64	569.27
	28-30	IQY	822.86	822.37

	30-32	YVL	807.93	807.44
	31-37	VLSRYPS	693.74	693.33
	33-37	SRYPS	377.42	377.18
	34-37	RYPS	330.41	330.22
	38-41	YGLN	795.91	795.40
	61-65	YAKPA	422.46	422.21
	61-64	YAKP	608.64	608.28
	96-101	ARHPHP	521.56	521.25
	97-101	RHPHP	434.48	434.22
	124-130	TIASGEP	825.89	825.39
Antithrombotic	106-116	MAIPPKKNQDK	287.35	287.17
	106-111	MAIPPK	651.73	651.31
	106-112	MAIPPKK	548.62	548.28
	109-111	PPK	477.54	477.25
	112-116	KNQDK	273.32	273.17
	113-116	NQDK	550.56	550.24
Immunomodulation	25-34	YIPIQYVLSR	339.34	339.14
Opioid antagonist	33-38	SRYPSY	396.47	396.23
Opioid	35-41	YPSYGLN	713.79	713.36

Based on data from BIOPEP (2018)

Supplementary table S5
Activity, fragment, amino acid sequence, chemical mass and monoisotopic mass of different bioactive peptides reported in bovine lactoferrin

Activity	Fragment	Sequence	Chemical mass	Monoisotopic mass
Antibacterial	1-8	APRKNVRW	1026.20	1025.57
	1-11	APRKNVRWCTI	1360.46	1359.71
	1-16	APRKNVRWCTISQPEW	1988.12	1986.97
	1-42	APRKNVRWCTISQPEWFKCRRWQWRMKKLGAPSITCVRRAFA	5200.73	5197.65
	17-42	FKCRRWQWRMKKLGAPSITCVRRAFA	3230.62	3228.68
	17-47	FKCRRWQWRMKKLGAPSITCVRRAFALECIR	3862.24	3859.99
	17-43	FKCRRWQWRMKKLGAPSITCVRRAFAL	3343.78	3341.76
	17-30	FKCRRWQWRMKKLG	1940.23	1939.01
	17-43	FKCRRWQWRMKKLGAPSITCVRRAFAL	3343.78	3341.76
	17-48	FKCRRWQWRMKKLGAPSITCVRRAFALECIRA	3933.32	3931.03
	19-37	CRRWQWRMKKLGAPSITCV	2353.55	2352.17
	43-48	LECIRA	720.70	720.35
	45-48	CIRA	478.43	478.22
Immunomodulation	79-93	AGIYGTKESPQTHYY	1714.82	1713.78
Antiviral	222-230	ADRDQYELL	1122.18	1121.52
	264-269	EDLIWK	802.91	802.41

Based on data from BIOPEP (2018)

Supplementary table S6
Quantitative Trait Loci (QTL) and candidate genes related to milk protein composition in cattle

QTL trait	Candidate gene	QTL information			Total
		Chromosome	Peak location (cM)	Span (cM)	
Milk alpha-casein content (MACAS)	PAEP	11	120.1	94.70-94.90	4
	CSN1S1	6	99.32	99.32-99.32	1
Milk beta-casein content (MBCAS)	PAEP	11	120.1	94.70-94.90	2
	CSN1S2	6	99.46	99.46-99.46	2
	CSN1S2	6	99.45	99.45-99.45	1
	STATH	6	99.41	99.41-99.41	1
Milk casein content (MTCAS)	PAEP	11	120.1	94.70-94.90	1
	CSN3	6	-	-	1
	GH1*	20	-	-	1
	CCL2*	19	-	-	1
	CSN2	6	96.66	96.66-96.66	1
	CSN3	6	-	-	1
	FABP4*	14	-	-	1
	STAT5A*	19	-	-	1
Milk kappa-casein content (MKCAS)	PAEP	11	120.1	94.70-94.90	3
	CSN3	6	37.32	20.57-54.07	1
	CSN3	6	99.59	99.59-99.59	9
Milk casein percentage (MCASP)	CCL2	19	-	-	2
	CSN1S1	6	96.63	96.63-96.63	2
	CSN2	6	96.66	96.66-96.66	5
	CSN3	6	-	-	1
	CSN3	6	-	-	1
	DGKG	1	-	-	1
	ETS2	1	147.43	147.43-147.43	1
	GH1	20	-	-	1
	GHR	20	-	-	1
	LPIN1	11	120.1	94.70-94.90	2
	LPL	8	-	-	1
	NR1H3	15	-	-	1
	OLR1	5	-	-	1
	POU1F1	1	-	-	1
	PPARGC1A	6	-	-	3
	PRLR	20	-	-	1
	SCD	26	-	-	1
	TLR4	8	119.5	119.50-119.50	1
Milk alpha-S1-casein percentage (MAS1CP)	CSN2	6	90.5	49.52-49.72	1
	CSN3	6	90.7	49.72-49.92	1

	PAEP	11	120.1	94.70-94.90	2
Milk alpha-S2-casein percentage (MAS2CP)	CSN2	6	90.5	49.52-49.72	1
	CSN3	6	90.7	49.72-49.92	1
	PAEP	11	120.1	94.70-94.90	2
Milk beta-casein percentage (MBCASP)	CSN2	6	90.5	49.52-49.72	1
	CSN3	6	90.7	49.72-49.92	1
	PAEP	11	120.1	94.70-94.90	1
Milk alpha-casein to beta-casein ratio	CSN1S1	6	99.32	99.32-99.32	1
Milk casein index (MCASIND)	PAEP	11	120.1	94.70-94.90	1
	CSN2	6	90.5	49.52-49.72	1
	CSN3	6	90.7	49.72-49.92	1

*__Associated Genes: CCL2;__ chemokine (C-C motif) ligand 2, __CSN1S1;__ Casein alpha s1__, CSN2;__ Casein beta__, CSN3;__ Casein kappa__, DGKG;__ diacylglycerol kinase gamma, __ETS2;__ ETS proto-oncogene 2, transcription factor, __FABP4;__, fatty acid binding protein 4- adipocyte, __GH1__; growth hormone, __GHR;__ growth hormone receptor, __LPIN1__; lipin 1, __LPL__; lipoprotein lipase, __NR1H3;__ nuclear receptor subfamily 1 group H member 3, __OLR1__; oxidized low density lipoprotein receptor 1, __PAEP;__ Progestagen-associated endometrial protein, __POU1F1;__ POU class 1 homeobox 1, __PPARGC1A;__ PPARG coactivator 1 alpha, __PRLR__; prolactin receptor, __SCD;__ Stearoyl-CoA desaturase (delta-9-desaturase)__, STAT5A;__ signal transducer and activator of transcription 5A, __STATH;__ Statherin, __TLR4;__ toll like receptor 4.

Based on data from NRSP-8 (2018) & NCBI (2018)

Supplementary table S7
Genetic variants of main milk proteins in different ruminants

Gene	Cow	Goat	Sheep	Buffalo
CSN1S1	A, B, C, D, E, F, G, H	A^1, B^1, B^2, B^3, B^4, C, E, F, G, H, I, L, M, N, 0^1, 0^2	A, B, C, D, E, F, G, H	A, B
CSN1S2	A, B, C, D	A, B, C, E, F, 0, 0´	A, B, C, D	-
CSN2	A^1, A^2, A^3, B, C, D, E, F, G, H^1, H^2, I	A,B,C,O,0^1	A, B	A^1, A^2,
CSN3	A, A^1, B, C, E, F^1, F^2, G^1, G^2, H, I, J	C, D, E, F, G, H, I, J, K, L, M	T, C	B, X^1, X^2
LALBA	A, B	A, B	A, B	-
MBLG	A, B, C, D, E, F, G, W, H, I, J, X	A, B	A, B	-
PAEP	A, B	-	A, B, C	B

CSN1S1; Casein alpha s1**, CSN1S2;** Casein alpha-S2**, CSN2;** Casein beta**, CSN3;** Casein kappa**, LALBA;** lactalbumin-alpha**, MBLG**, beta-lactoglobulin **PAEP;** Progestagen-associated endometrial protein. Adapted from Borkova & Snaselov, 2005; Kučerová *et al.* 2006; Barłowska *et al.* 2012; Giambra *et al.* 2014; Vacca *et al.* 2014 & Ramesha *et al.* 2016

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