The Science of Proteins in Milk (including A1 vs A2 Milk)

Dr. Sabrina Greenwood
Department of Animal & Veterinary Sciences

Milk protein

- Whey proteins
  - →Lactalbumin (4%)
  - →Lactoglobulin (12%)
  - →Other (~4%)
- Casein
  - →αs1 (32%)
  - →αs2 (8%)
  - →β (32%)
  - →κ (8%)

Milk bioactive proteins and peptides

- "Bioactive" indicates that the compound (protein) can act on the body of the consumer to exert a biological effect

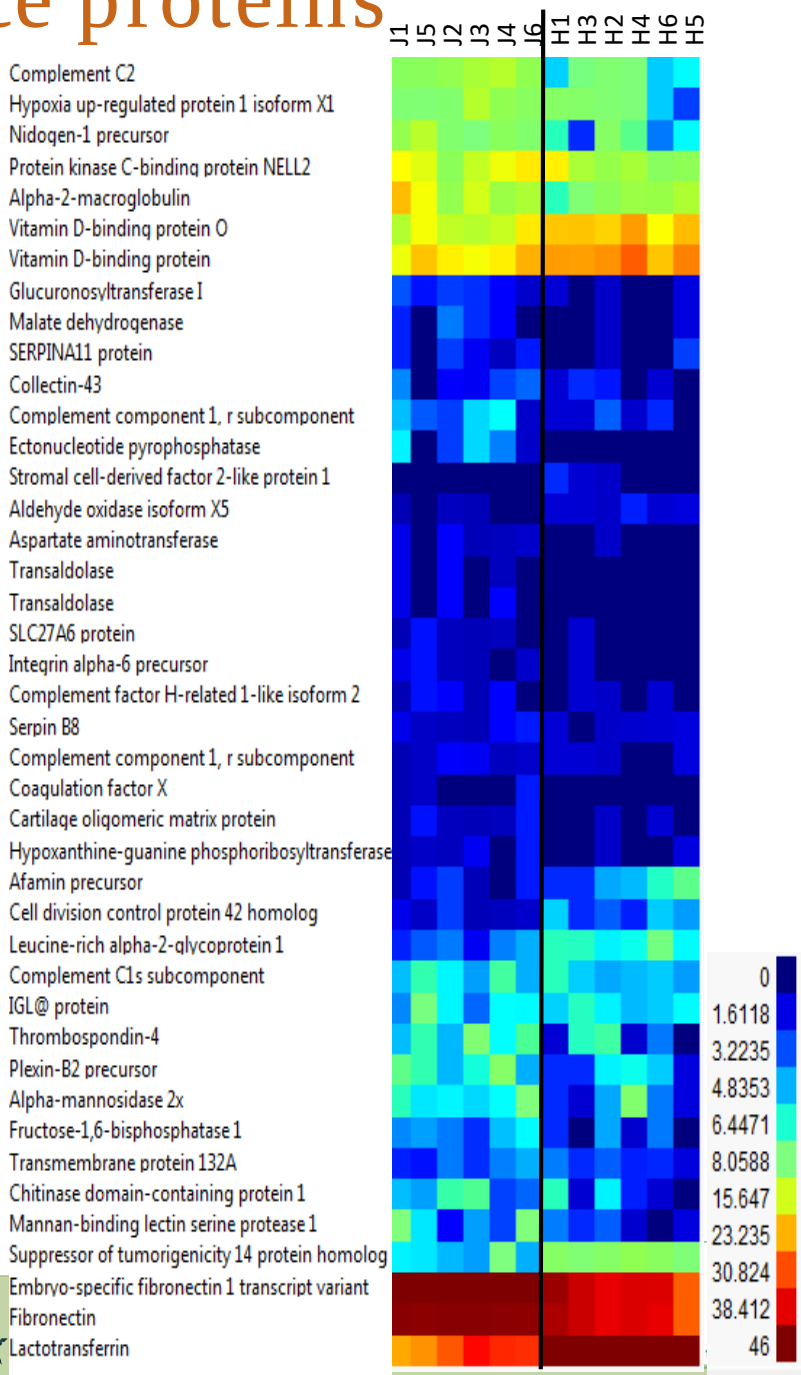
- Some milk proteins (example: lactoferrin) are considered bioactive as the complete protein

Highlighting low-abundance proteins

- Identification of 935 low-abundance proteins in Jersey and Holstein skim milk samples
- Only 43 (4%) low abundance proteins exist at different abundances between breeds
  - 35 of these found higher in Jersey

- Proteins higher in Jerseys:
  - Complement proteins C1 and C2
  - Chitinase domain-containing protein 1
  - Ectonucleotide pyrophosphatase

- Bioactive proteins higher in Holsteins:
  - Lactoferrin



Tacoma et al., 2015. J. Proteomics

Table 1. Minor proteins identified in bovine milk fat globule membrane since 2005 (partial list)

Protein name	Function[1]
Cathelicidin 1	Antimicrobial peptide
Calgranulins B and C	Antimicrobial peptide; associated with S100A8 and implicated in inflammatory response
Peptidyl-prolyl cis-trans isomerase A (cyclophilin A)	Catalyzes *cis-trans* isomerisation of proline imidic peptide bond
14–3-3 proteins γ and zeta chain and β/α	Cell signaling
A kinase anchor protein 4	Cell signaling
Glucose regulated protein 58kD protein disulfide isomerase-associated 3	Cell signaling
GTP binding proteins α 14 subunit, GL2, α-11 subunit and Rit 1	Cell signaling
GTP binding regulatory proteins G(I)/G(S)/G(T), subunit β-2 and Gs α	Cell signaling
Rap 1A and 1B	Cell signaling
Rho GDP-dissociation inhibitor 1	Cell signaling
STAT5 MGF Signal transducer and activator of transcription 5B	Cell signaling
Transforming protein Rho A	Cell signaling
Aldehyde dehydrogenase mitochondrial (NAD) 2 precursor	Enzyme
ATP synthase, H^+ transporting, mitochondrial F1 complex, α subunit	Enzyme
5′ nucleotidase	Fat transport/metabolism
Acetyl-CoA carboxylase 1	Fat transport/metabolism
Apolipoprotein E	Fat transport/metabolism
Fatty acid synthase	Fat transport/metabolism
Fatty acid-binding protein, brain type	Fat transport/metabolism
Long-chain-fatty-acid CoA ligase 1	Fat transport/metabolism
Retinal short-chain dehydrogenase/reductase	Fat transport/metabolism
Serum amyloid A3 isoform	Fat transport/metabolism
Excitatory amino acid transporter 3	General transport
Sodium and chloride dependent creatine transporter 1	General transport
Solute carrier family 5 and family 34 (sodium phosphate), member 2	General transport
CD9 antigen	Immune function
Ig D heavy chain, G1 heavy chain and M heavy chain	Immune function
Toll-like receptors 2 and 4	Immune function
Macrophage scavenger receptor types I and II	Mediate the binding, internalization, and processing of negatively charged macromolecules
ADP ribosylation factor 1	Membrane/protein trafficking
Rab 27b, 3A, 3B and 3C	Membrane/protein trafficking
SAR1B protein	Membrane/protein trafficking
Translocon associated protein subunit α	Membrane/protein trafficking
Peptidyl-prolyl isomerase (Cyclophilin I)	Protein synthesis, binding, or folding
Profilin I	Protein synthesis, binding, or folding
Elongation factor 1	Protein synthesis, binding, or folding
Heat shock 90kDa protein	Protein synthesis, binding, or folding
Tight junction protein 1	Protein synthesis, binding, or folding
Annexins 1 and A2	Structure
ARP3 actin-related protein 3	Structure
Mucin 1, glycoprotein 2 (MUC1)	Unclear but possibly antiadhesion or immunoprotective

Milk bioactive proteins and peptides

- "Bioactive" indicates that the compound (protein) can act on the body of the consumer to exert a biological effect

- Some milk proteins (example: lactoferrin) are considered bioactive as the complete protein
- Digestion of milk proteins (example β-casein) by human digestive enzymes yields protein fragments (called peptides)
  - Many of the different peptides are known to be bioactive

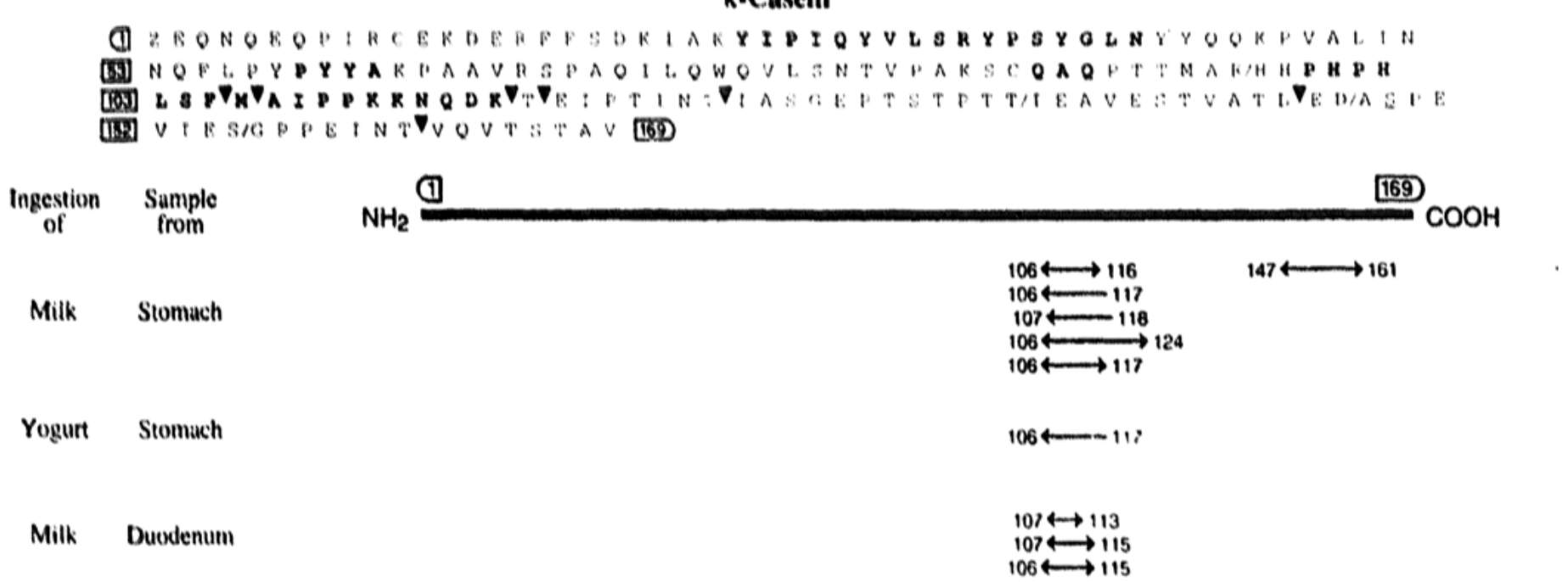


Fig 3. Amino acid sequences and cleavage sites of of κ-casein (▼) observed in human gastric or duodenal samples. Amino acids in bold type: biologically active peptides [2]. Numbers are those of the residues. Entire peptide (↔). N-terminal sequence of the peptide (←). N-terminal amino acid (encircled 1) and C-terminal amino acid (encircled 169).

Chabance et al. 1998. Biochimie. 80: 155-165.

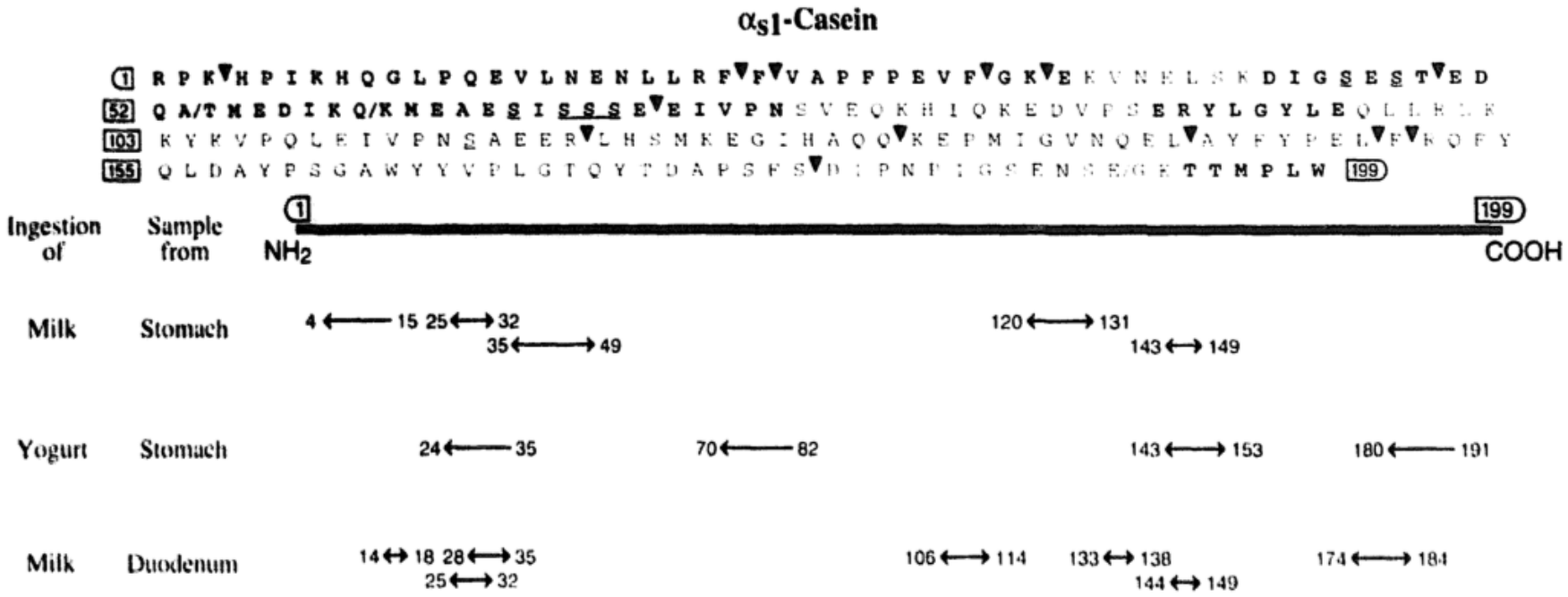


Fig 4. Amino acid sequences and cleavages sites of α_{s1}-casein (▼) observed in human gastric or duodenal samples. Amino acids in bold type, biologically active peptides [2]. Numbers are those of the residues. Entire peptide (↔). N-terminal sequence of the peptide (←). N-terminal amino acid (encircled 1) and C-terminal amino acid (encircled 199).

Chabance et al. 1998. Biochimie. 80: 155-165.

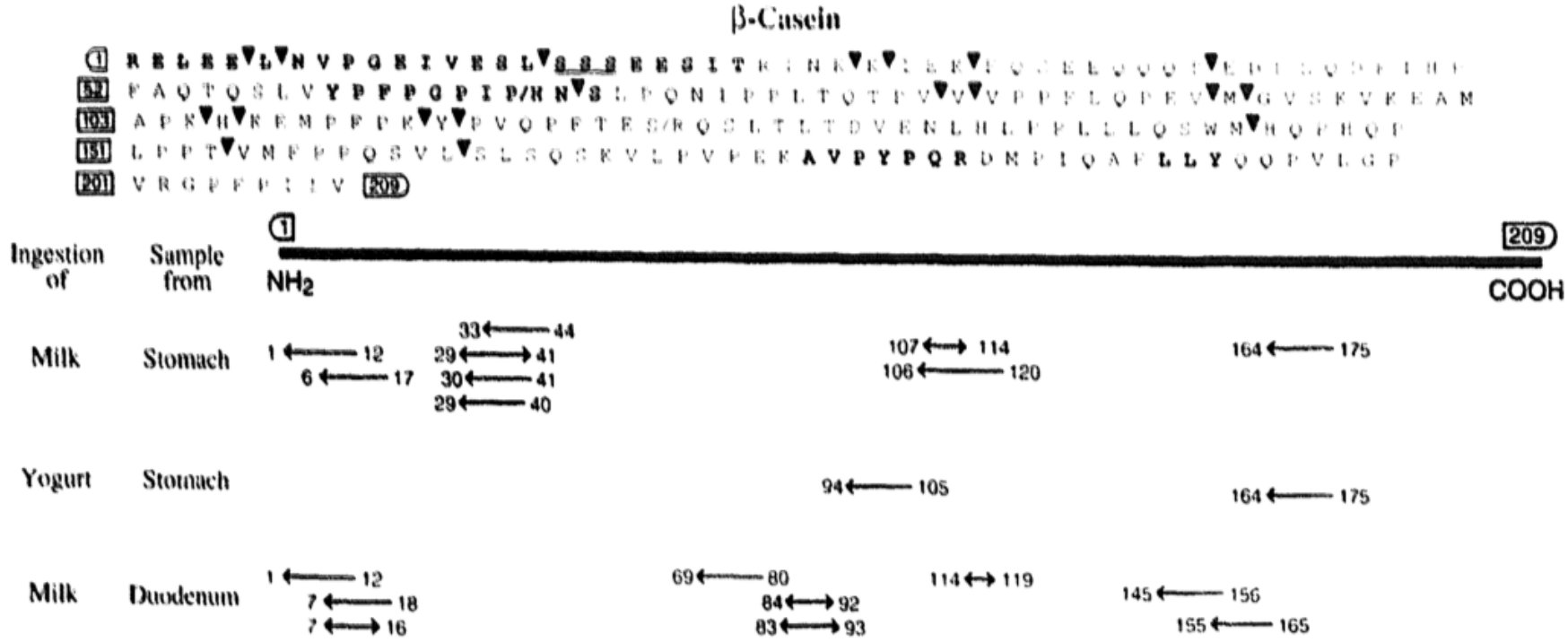


Fig 5. Amino acid sequences and cleavages sites of β-casein (▼) observed from gastric or duodenal samples. Bold amino acids: biologically active peptides [2]. The numbers refer to the residues numbers. Entire peptide (↔). N-terminal sequence of the peptide (←). N-terminal amino acid (encircled 1) and C-terminal amino acid (encircled 209).

Chabance et al. 1998. Biochimie. 80: 155-165.

Table 2. Examples of physiologically active milk peptides.

Sample number	Peptide sequence[1]	Name	AA[2] segment	Physiological classification	Release protease	Reference
1	FFVAP	α_{s1}-Casokinin-5	α_{s1}-CN (f 23–27)	ACE inhibitor	Proline endopeptidase	46
2	AVPYPQR	β-Casokinin-7	β-CN (f 177–183)	ACE inhibitor	Trypsin	46
3	YGLF	α-Lactorphin	α-LA (f 50–53)	ACE inhibitor and opioid agonist	Synthetic peptide	59
4	ALPMHIR	β-Lactorphin	β-LG (f 142–148)	ACE inhibitor	Trypsin	60
5	KVLPVPQ	Antihypertensive peptide	β-CN (f 169–174)	Antihypertensive peptide	Lactobacillus CP790 protease & synthetic peptide	44
6	MAIPPKKNQDK	Casoplatelin	κ-CN (f 106–116)	Antithrombotic	Trypsin & synthetic peptide	30
7	KDQDK	Thrombin inhibitory peptide	κ-CN glyco-macropeptide (f 112–116)	Antithrombotic	Trypsin	71
8	KRDS	Thrombin inhibitory peptide	Lactotransferrin (f 39–42)	Antithrombotic	Pepsin	70
9	QMEAES*IS*S*S*EEIVPNS*VEQK	Caseinophospho-peptide	α_{s1}-CN (f 59–79)	Calcium binding and transport	Trypsin	77
10	LLY	Immunopeptide	β-CN (f 191–193)	Immunostimulatory (+)	Synthetic	57
11	FKCRRWQWRMKKLGAPSITCVRRAF	Lactoferricin B	Lactoferrin (f 17–41)	Immunomodulatory (+) and antimicrobial	Pepsin	2, 58
12	YQQPVLGPVR	β-Casokinin-10	β-CN (f 193–202)	Immunomodulatory (+/–) & ACE Inhibitor	Synthetic	55
13	RYLGYLE	α-Casein exorphin	α_{s1}-CN (f 90–96)	Opioid agonist	Pepsin	43
14	YGFQNA	Serorphin	BSA (f 399–404)	Opioid agonist	Pepsin	84
15	YLLF·NH_2	β-Lactorphin (amide)	β-LG (f 102–105)	Opioid agonist = ACE Inhibitor	Synthetic or Trypsin	59
16	YIPIQYVLSR	Casoxin C	κ-CN (f 25–34)	Opioid antagonist	Trypsin	14
17	[YVPF PPF]	Casoxin D	α_{s1}-CN (f 158–164)	Opioid antagonist	Pepsin-chymotrypsin	96
18	YLGSGY-OCH_3	Lactoferroxin A	Lactoferrin (f 318–323)	Opioid antagonist	Pepsin	93

[1]The one-letter amino acid codes were used; S* = Phosphoserine.

[2]Amino acid.

Clare and Swaisgood. 2000. J. Dairy Sci. 83: 1187-1195.

Identification of bioactive peptide activity

- Bioactive peptide activity (Clare and Swaisgood, 2000; Silva and Malcata, 2005):
  - Antimicrobial peptides
  - Antithrombotic peptides
  - Antihypertensive peptides
  - Opioid peptides
  - Immunomodulating peptides
  - Caseinophosphopeptides
  - Miscellaneous peptides
    - Casomorphins slow gastric motility and emptying (Froetschel, 1996)
    - Caseinomacropeptide inhibits gastric secretions (Yvon et al., 1994)

Variation of Casein and whey fractions

- Genetics
- Environment
- Nutritional and management means

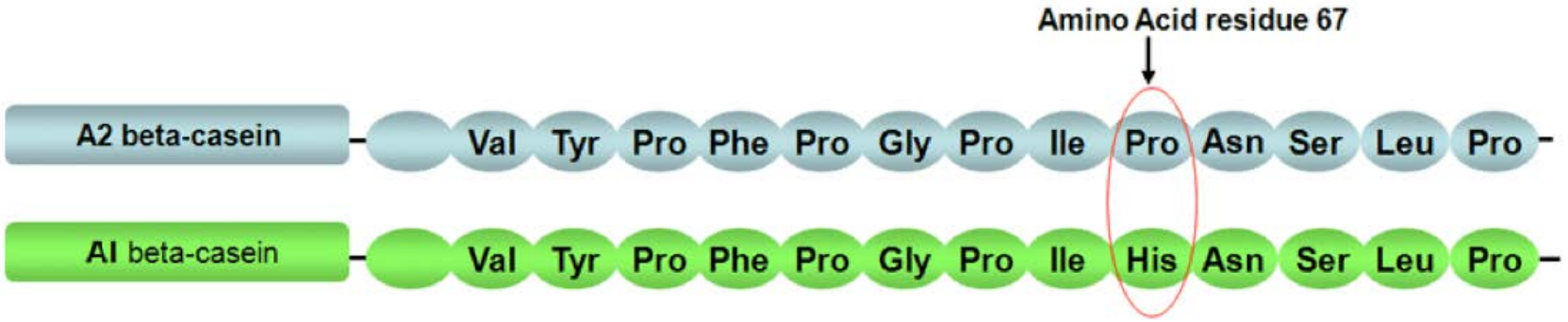
Nutritional and management means

Table 6. High abundance milk proteins from cows fed the two treatment diets

	Diet		[1]SE	*P*-value	
	Control	Treatment		Treatment	Period
Casein (mg/mL)	**RDP**	**RUP**			
β-Casein	16.34	15.81	0.29	0.06	0.03
κ-Casein	5.61	5.39	0.08	0.04	0.01
Total α-Casein	15.76	16.35	0.33	0.06	0.22
α-s1	13.78	13.31	0.45	0.22	0.16
α-s2	1.93	1.93	0.07	0.95	0.20
Total Caseins	37.80	36.32	0.48	<0.01	0.04
Whey (mg/mL)					
α-Lactalbumin (α-LA)	1.48	1.50	0.09	0.66	0.26
β-Lactoglobulin variant A (β-LGA)	2.52	2.50	0.19	0.91	0.99
β-Lactoglobulin variant B (β-LGB)	1.60	1.64	0.11	0.72	0.52
Total α-LA, β-LGA, β-LGB	5.50	5.75	0.37	0.52	0.61

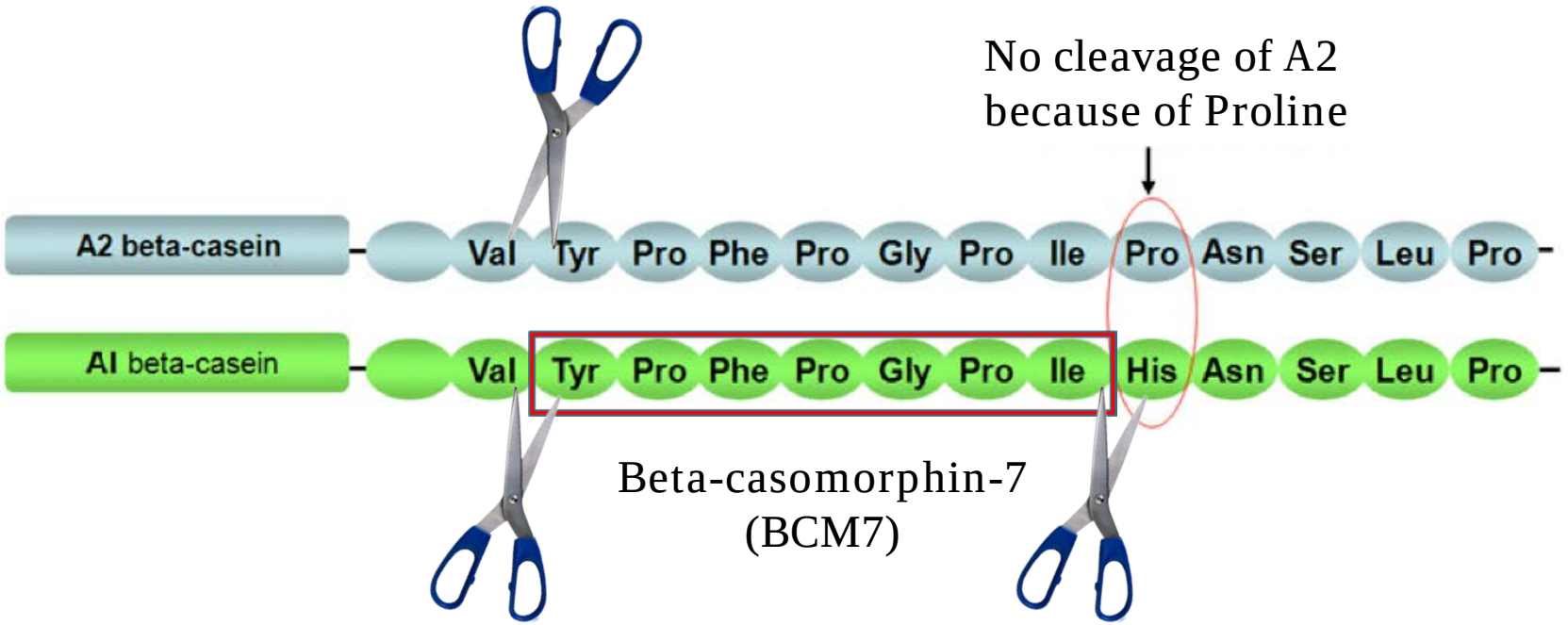
Tacoma et al., 2015. Manuscript in preparation.

An example of genetic variation: A1 vs. A2 milk



A2 Corporation

An example of genetic variation: A1 vs. A2 milk



Previous claims against A1 milk

- Claims suggested that A1 milk caused autism, ischaemic heart disease, schizophrenia, and type 1 diabetes mellitus
- National reports released:
  - **European Food Safety Authority (2009)**: "Based on the present review of available scientific literature, a cause-effect relationship between BCM7 and etiology or cause of any suggested non-communicable diseases cannot be established."
  - **Report to New Zealand Food Safety Authority (2004):** "I do not believe there is sufficient evidence to warrant the government agencies taking further specific public health actions such as changing dietary recommendations, requiring labelling of products containing A1 β-casein, or encouraging changes in the dairy herd composition in order to promote and protect the health of the population. There is a requirement to monitor the health claims being made for A2 milk to ensure that they comply with existing regulations"

Current research

- Current research suggests BCM7 actually improves learning, memory and psychomotor development, decreases pain sensitivity, exerts anxiolytic and antihypertensive effects and reduces renal interstitial fibrosis caused by diabetes (Zhang et al. Peptides, 2012)

a2 corporation current claim:

"Ordinary cows' milk contains A1 and A2 proteins, and for some, A1 may cause side effects such as bloating, nausea, gas and diarrhea. a2 Milk* comes from specially handpicked dairy cows that produce only A2, so it may be gentler on your stomach"

Summary

- Thousands of proteins present in milk
- Milk proteins do not just provide amino acids, they also provide bioactive proteins and peptides
- Many positive health benefits of bioactives currently identified
- Some proteins with negative attributes may be present but at present the good seems to far outway the bad