



MILK: the well-known(?) food

Prof G Osthoff

SASDT congress 2002

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UFS·UV
NATURAL AND
AGRICULTURAL SCIENCES
NATUUR- EN
LANDBOUWETENSAPPE

CONTENTS

Background about milk

General composition

Saccharides

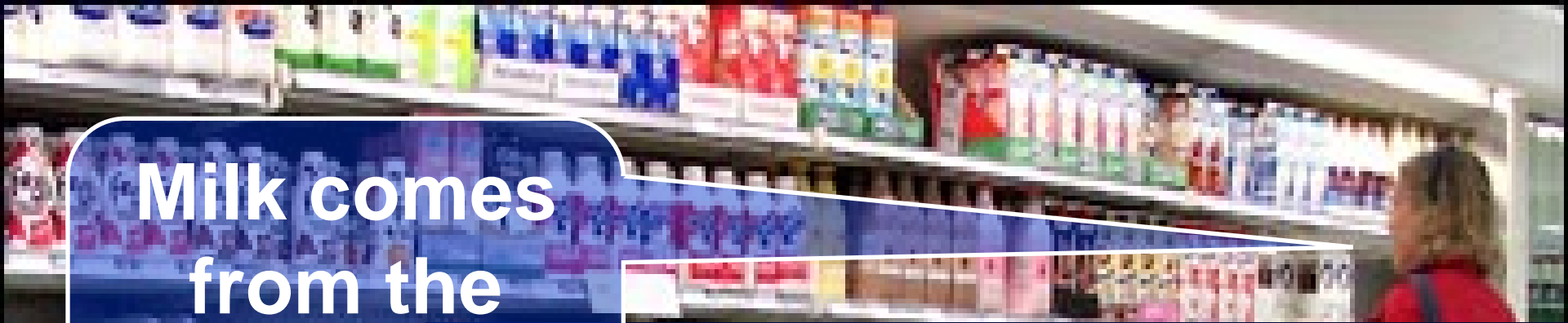
Lipids/Fats

Proteins

(Lessons for Technology & Nutrition)

Future

Milk comes
from the
supermarket



Milk comes from a cow?

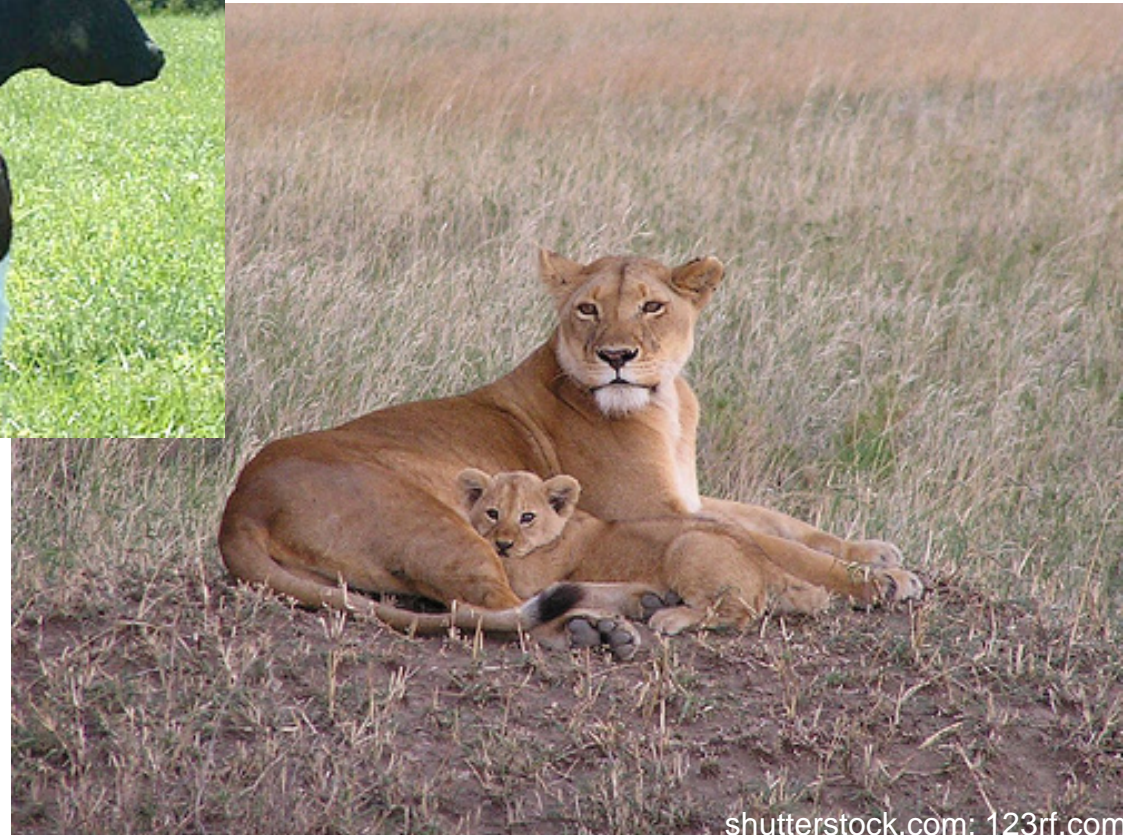


randomactsoflindz.blogspot.com



What the consumer of milk and dairy products has forgotten, is that milk is the first food to be utilized by ***young*** mammals.....

.... and that milk is custom-designed for each species.



corbis
Mankind is an opportunist and has found ways of easy access to food by the practice of agriculture.

Plants and animals are employed (exploited?)



corbisimages.com; flickr.com



The cow is the best milk producer. Other animals are also employed.

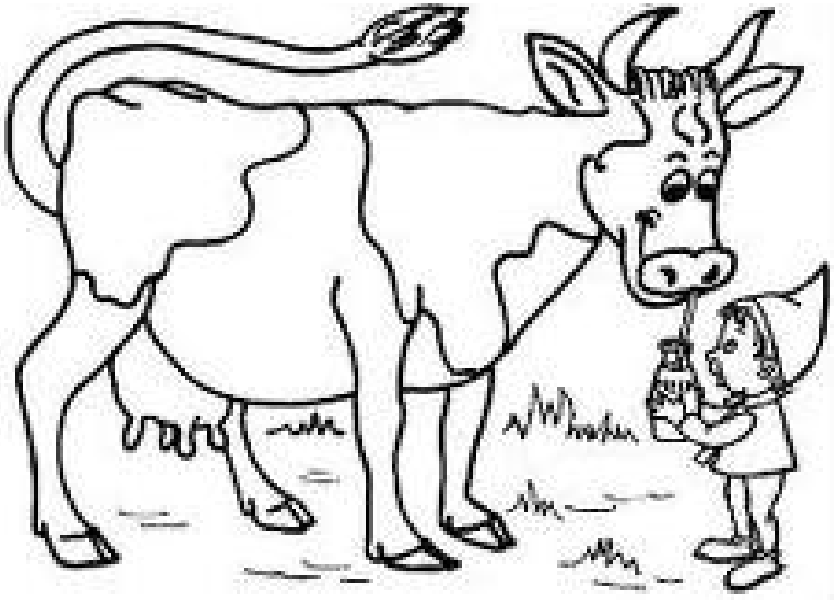


Cattle have not adapted to the most extreme conditions.



en.kllproject.lv ; gulfnews.com; healthbanquet.com

The consumption of the milk as grownup is not natural. Neither is the consumption of milk across species.



Malnutrition or diseases: allergy to milk and lactose intolerance



Allergies are the result of an immune response to the foreign proteins in the milk.

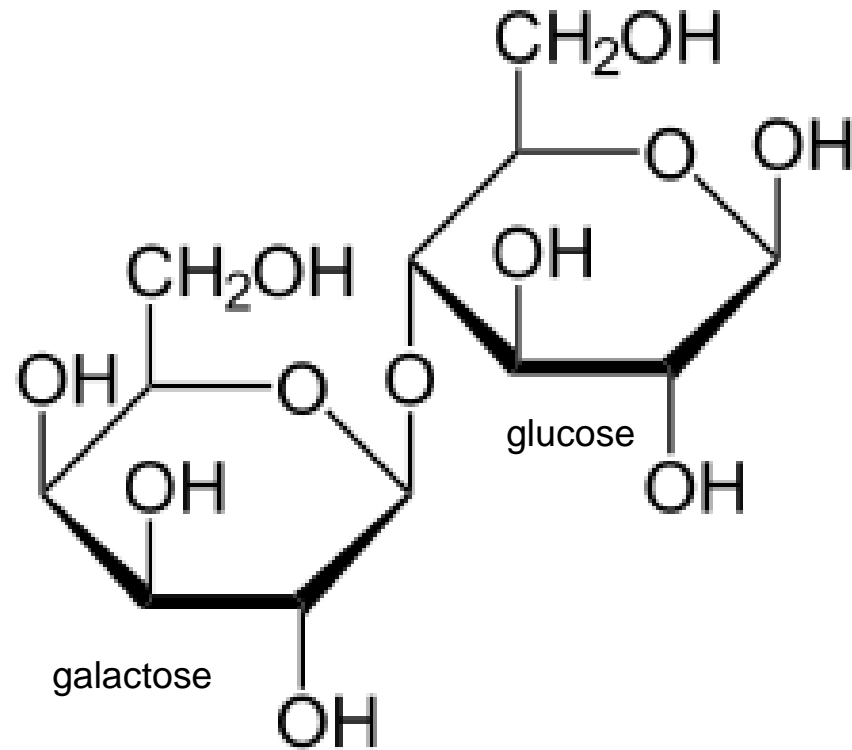
Switch from cow's milk to goat's milk.



Lactose intolerance:
inability of adult humans
to digest lactose
(milk sugar).

This is natural;
grownups lose the ability
to digest lactose.

Symptoms:
stomach cramps and
diarrhoea.

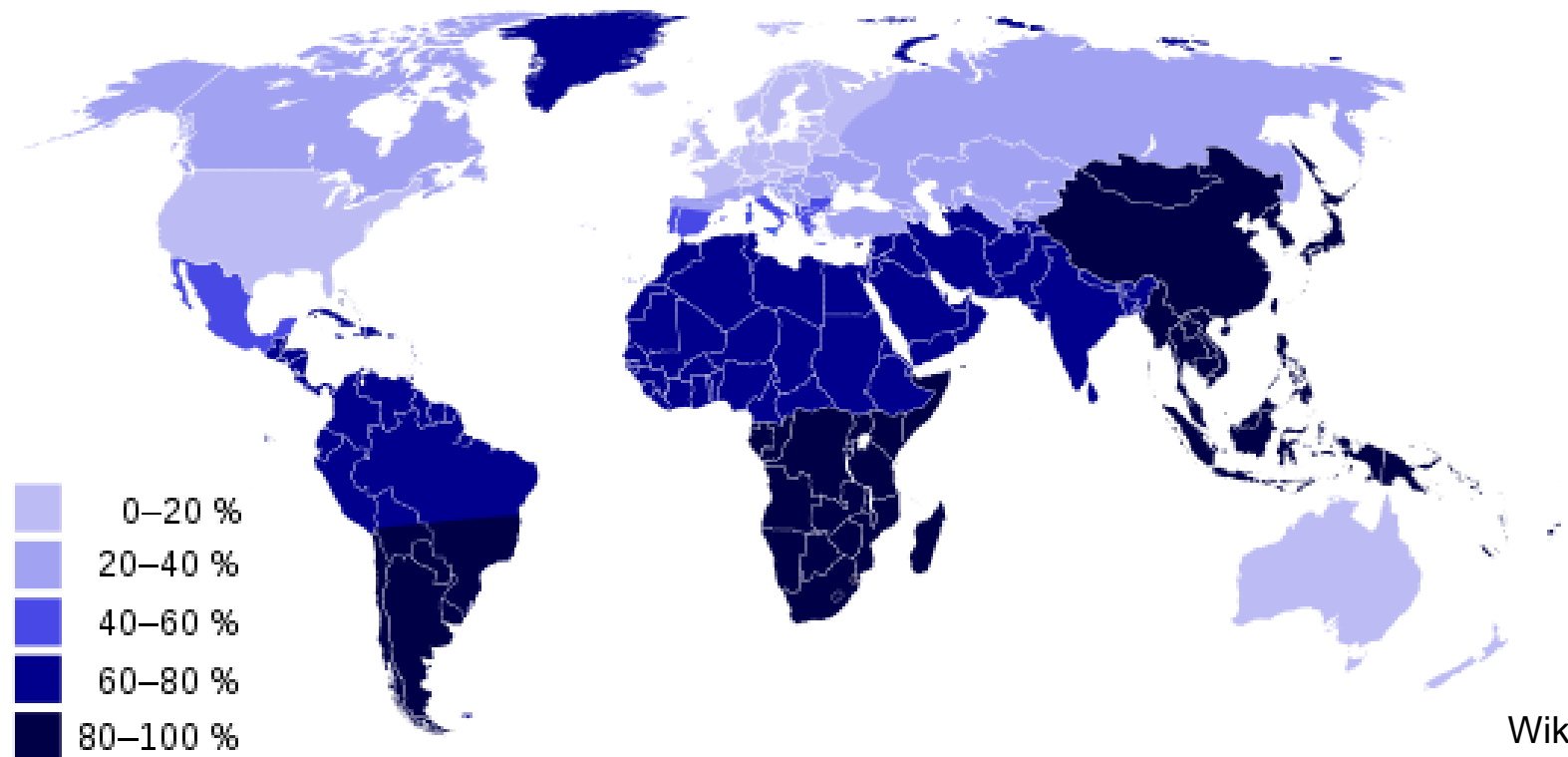


Lactose intolerance mainly found in the warmer climates of the world.

Milk not stored fresh - *fermented*.

This human population never adapted to digesting lactose in adulthood.

An early passive development of dairy technology.

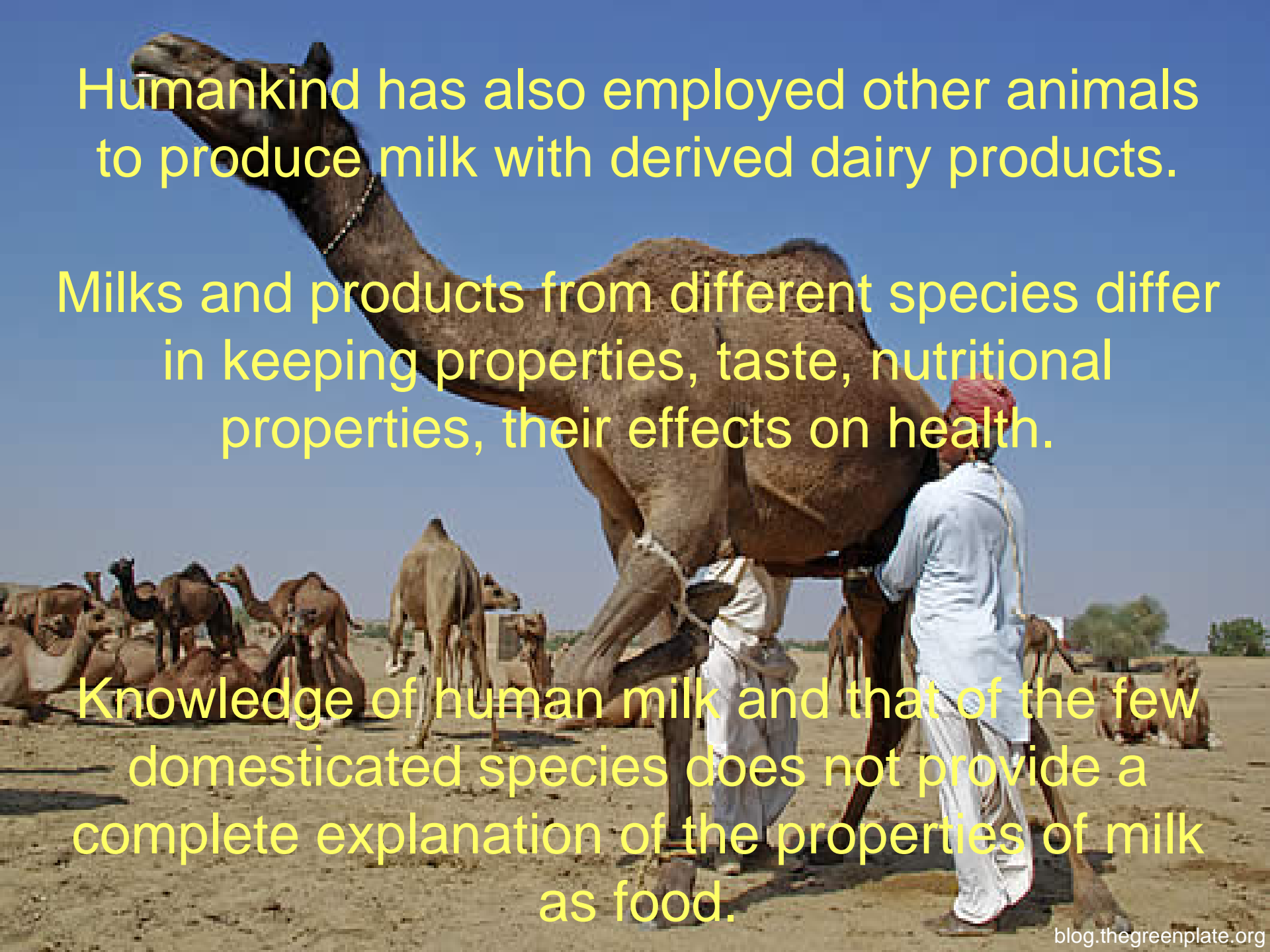


Lactose in milk has spurred dairy technology: Lactose fermentation by micro-organisms.

Cheese

Lactose free milk

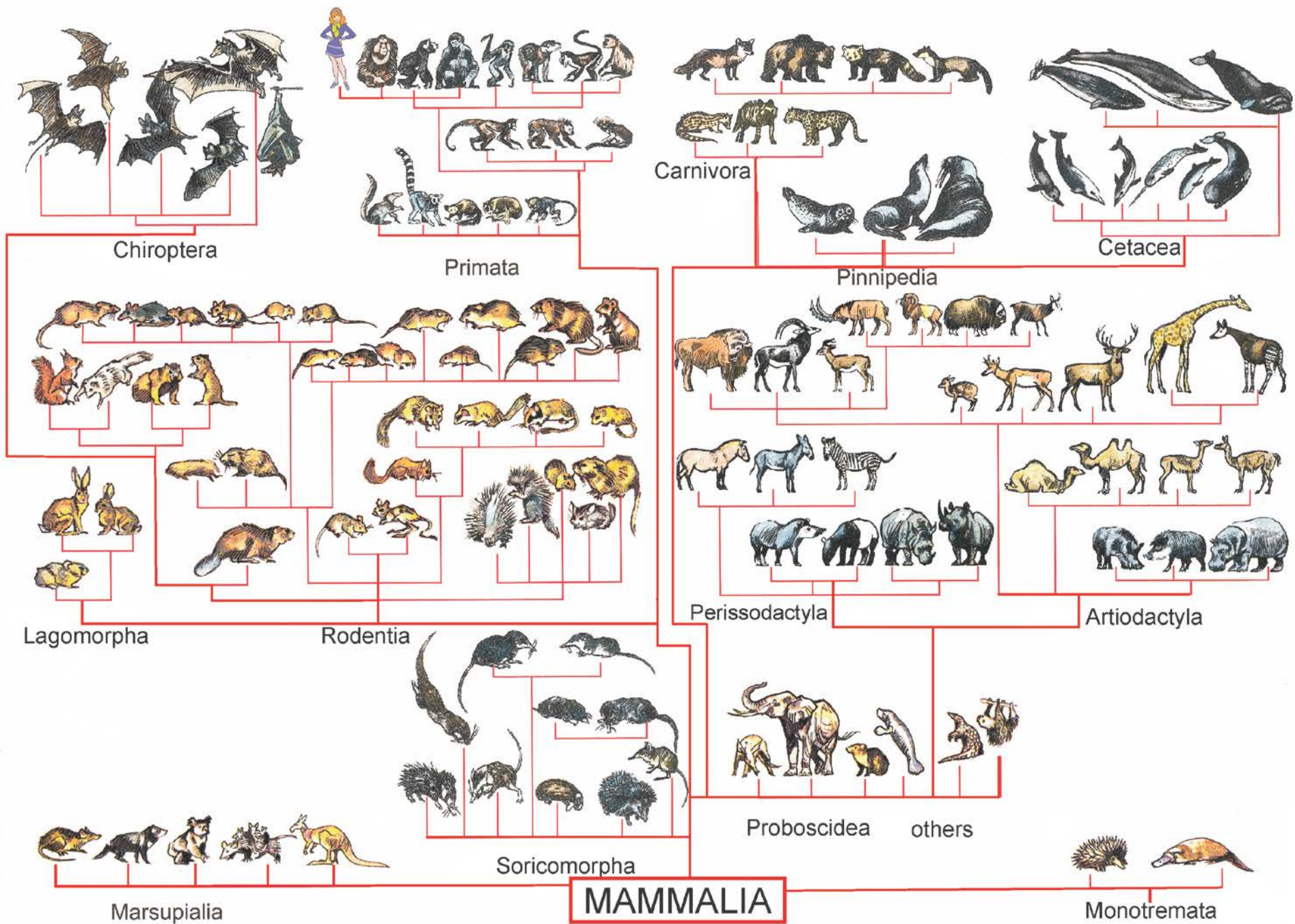


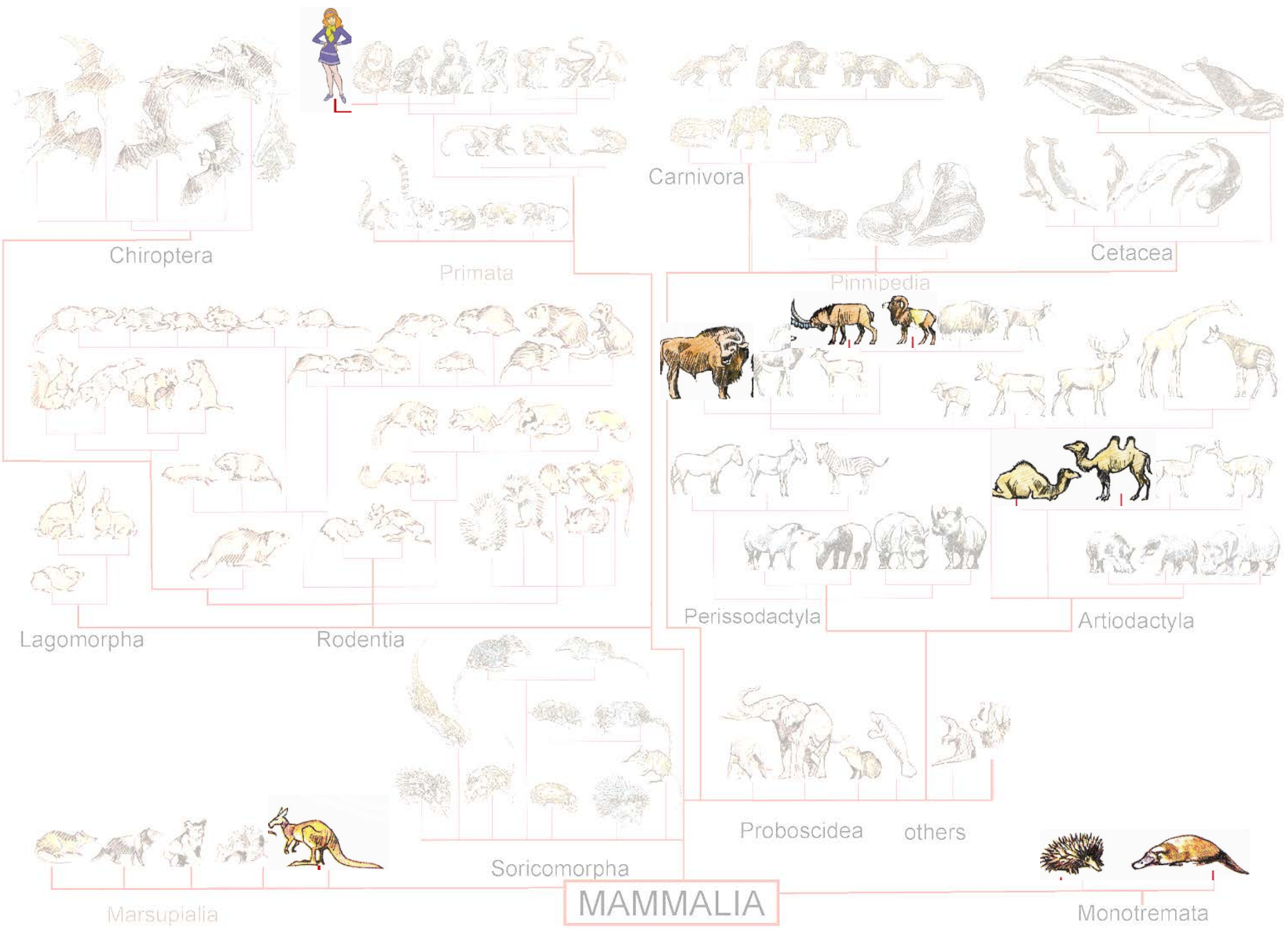
A photograph of a man in a white shirt and red turban milking a brown camel in a desert setting. The camel is standing on a sandy ground, and the man is leaning over it, holding a milk bucket. In the background, other camels are visible, some standing and some lying down. The sky is clear and blue.

Humankind has also employed other animals to produce milk with derived dairy products.

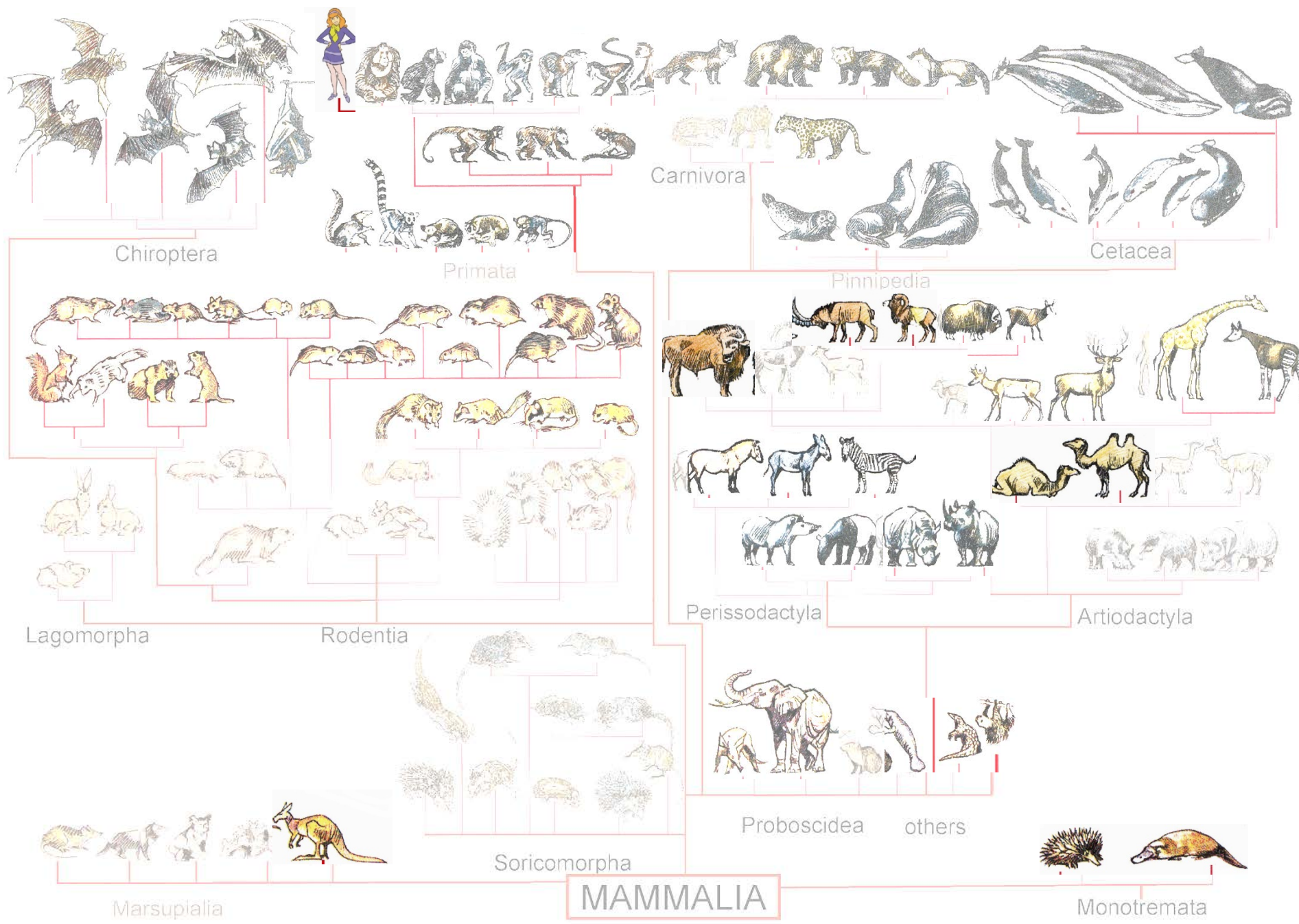
Milks and products from different species differ in keeping properties, taste, nutritional properties, their effects on health.

Knowledge of human milk and that of the few domesticated species does not provide a complete explanation of the properties of milk as food.

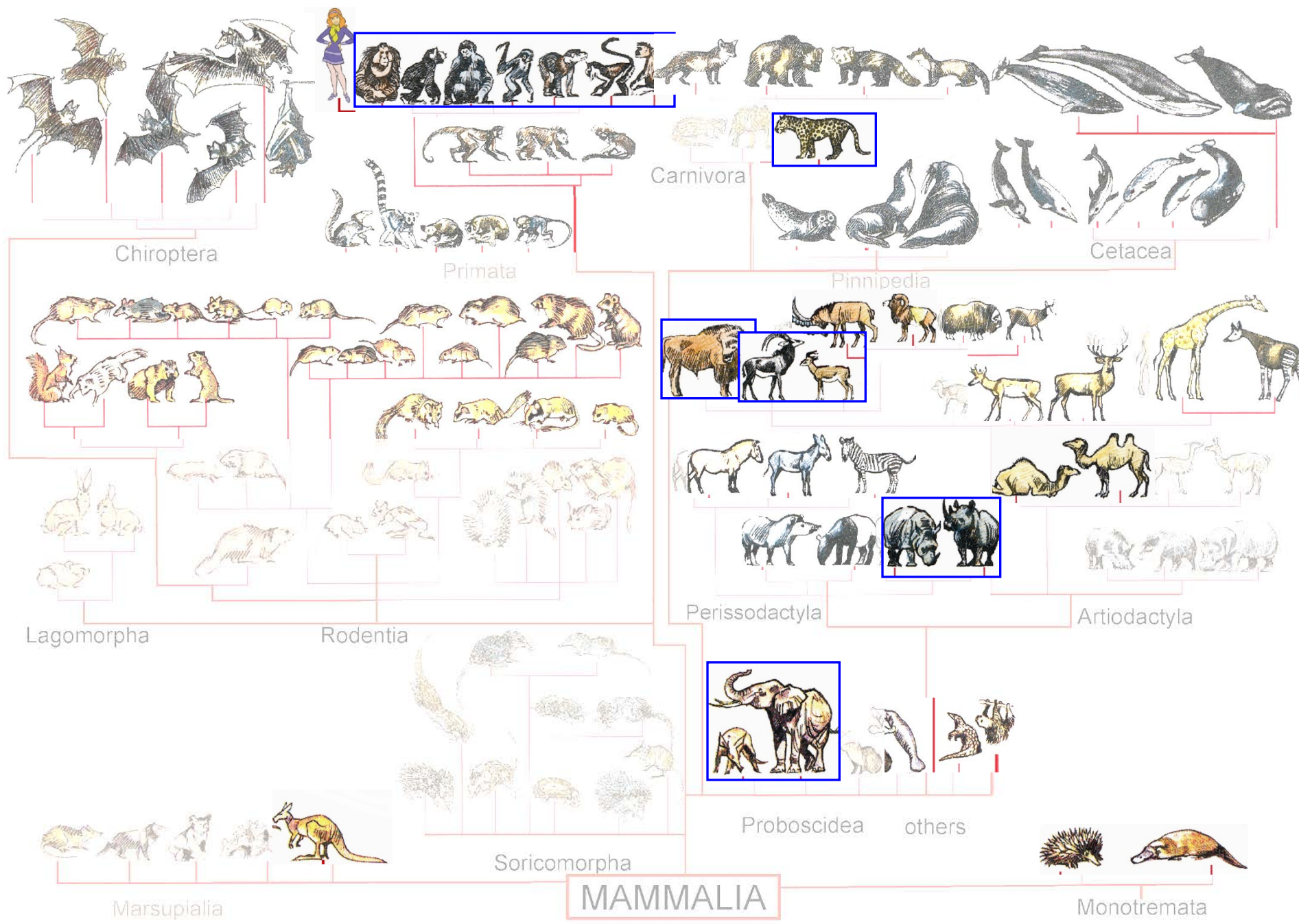




MAMMALIA



MAMMALIA



MAMMALIA

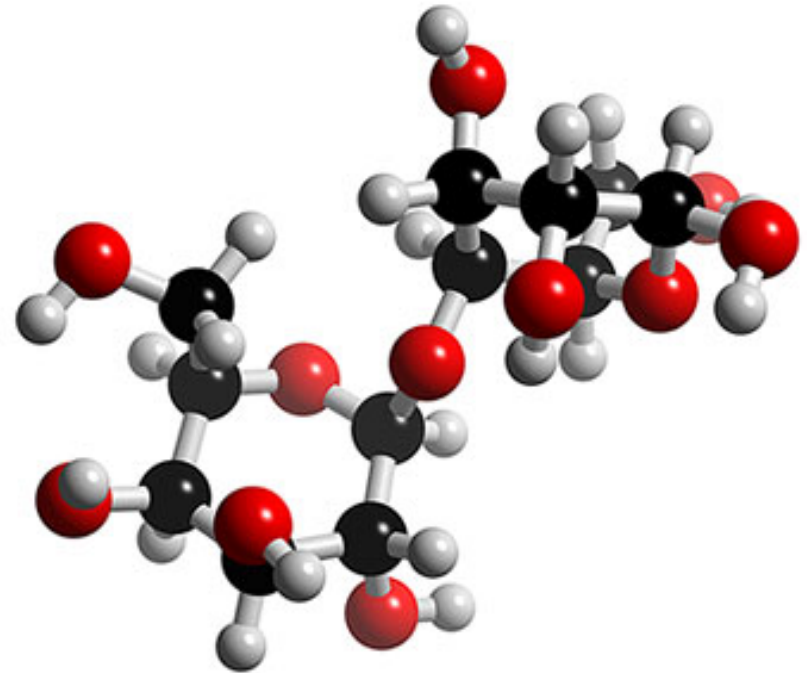
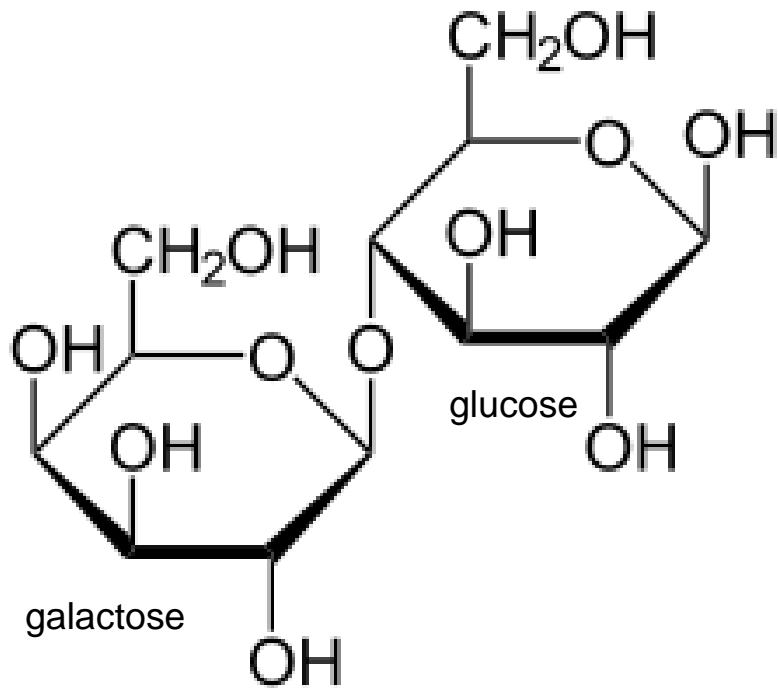
Each species provides nutrients and bio-reactive components in the correct amount and form; the result of adaptation to environmental opportunities and physiological constraints.

	Primata	Bovidae		Felidae		Proboscidae	Perissodactyla
Nutrients (%)	Human	Cow	Springbok	Cheetah	Serval	African Elephant	White Rhino
Fat	4	3.9	14.5	6.5	15.36	8.7	0.7
Protein	0.8	3.2	7.4	9.9	14.3	5.2	1.6
Casein	0.2	2.6	6.0	3.4	11.8	3.2	0.3
Whey	0.6	0.6	1.4	6.5	4.1	2.0	1.3
NPN	2.2	0.05	0.07	0.1	0.5	0.07	0.03
Saccharide	8.5	4.6	4.3	4.0	0.7	4.1	7.6
Lactose	7.3	4.6	4.3	4.0	0.7	1.5	7.5
Oligosacch.	1.2	0.1	0.3	0.02	0	2.7	0.05

(Osthoff et al. 2005;2006; 2007, 2008; 2009)

SACCHARIDES

Lactose



Saccharide content of mammalian milk

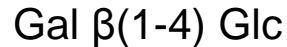


Species	Lactose (%)	Oligo-saccharides (%)
<u>Monotremes</u> (No α -Lactalbumin)	≈ 0	3.7
<u>Marsupials</u> (No α -Lactalbumin)	1-2	4.6-12.0
Seal (No α -Lactalbumin)	0	0
Sea lions (Low α -Lactalbumin)	0.5	0.3
Cow	4.0	0.1
Sable antelope	4.1	0.4
<u>Eutherian</u> (High α -Lactalbumin)		
Human	7.3	1.2
Gorilla	6.2	0.7
African elephant	0.7-5.3	1.5-2.7

(Iverson & Oftedal, 1995; Messer & Urashima 2002; Osthoff et al, 2007; 2008; 2009)

Selection of oligosaccharides in mammalian milk

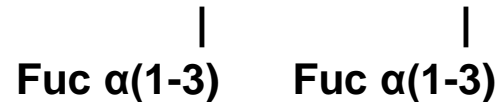
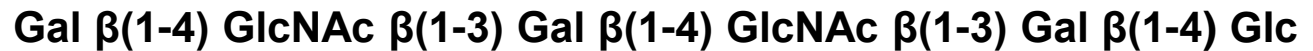
Lactose



2'-Fucosyllactose



DF-para-LNnHexaose

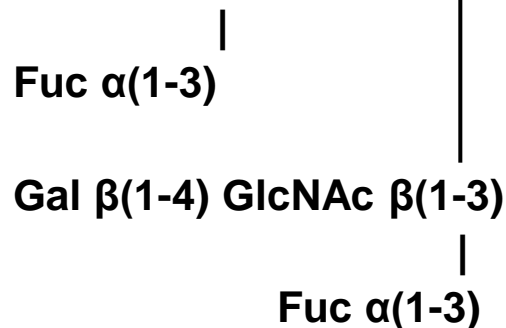


DF-para-LNnNonaose



(Osthoff et al. 2008)

DF-LNH



250+ structures known
12 structural groups
Different chain types

(Urashima et al. 2001)

Selection of oligosaccharides in mammalian milk

Note the Type II bond **Gal β (1-4) GlcNAc**

Lactose Gal β (1-4) Glc

2'-Fucosyllactose

Fuc α (1-2) Gal β (1-4) Glc

DF-para-LNnHexaose

Gal β (1-4) GlcNAc β (1-3) Gal β (1-4) GlcNAc β (1-3) Gal β (1-4) Glc

 | |
Fuc α (1-3) Fuc α (1-3)

DF-para-LNnNonaose (Only in elephant)

(Osthoff et al. 2008)

Gal α (1-3) Gal β (1-4) GlcNAc β (1-3) Gal β (1-4) GlcNAc β (1-3) Gal β (1-4) Glc

 | |
Fuc α (1-3) Fuc α (1-3)

DF-LNH

Gal β (1-4) GlcNAc β (1-6) Gal β (1-4) Glc

 | |
Fuc α (1-3) Gal β (1-4) GlcNAc β (1-3)
 |
 Fuc α (1-3)

(Urashima et al. 2001)

Selection of oligosaccharides in human milk

Note the Type II bond **Gal β(1-4) GlcNAc (in all species)**

Note the Type I bond **Gal β(1-3) GlcNAc (predominant in primates)**

Lactose Gal β(1-4) Glc

Lacto-N-tetraose

Gal β(1-3) GlcNAc β(1-3) Gal β(1-4) Glc

Lacto-N-hexaose

Gal β(1-4) GlcNAc β(1-6) Gal β(1-4) Glc

 |
Gal β(1-3) GlcNAc β(1-3)

Lacto-N-Decaose

Gal β(1-4) GlcNAc β(1-6)

 |
Gal β(1-4) GlcNAc β(1-6) Gal β(1-4) Glc

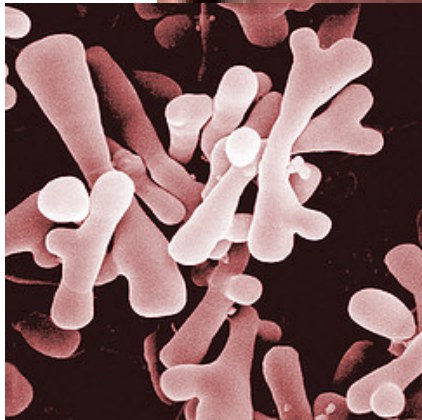
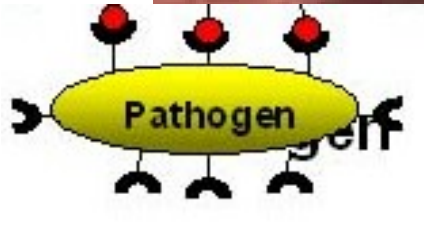
 |
Gal β(1-3) GlcNAc β(1-3)

 |
Gal β(1-3) GlcNAc β(1-3)

(Urashima et al. 2007)

Oligosaccharides and intestinal micro-organisms

Oligosaccharides



Bifidobacteria
& Lactobacilli

Pathogens

Predominant anaerobic microorganisms in the human colon

Microbial group	Range in log counts (g dry wt⁻¹)
<i>Bacteroides</i>	9.2-13.5
<i>Eubacteria</i>	5.0-13.3
<i>Bifidobacteria</i>	4.9-13.4
<i>Clostridia</i>	3.3-13.1
<i>Lactobacilli</i>	3.6-12.5
<i>Ruminococci</i>	4.6-12.8
<i>Peptostreptococci</i>	3.8-12.6
<i>Peptococci</i>	5.1-12.9
<i>Streptococci</i>	7.0-12.3
<i>Methanobrevibacter</i>	7.0-10.3
<i>Desulfovibrios</i>	5.2-10.9
<i>Lactococci, Enterococci,</i>	<i>Pediococci, Leuconostoc</i>

(Musatto & Mancilha, 2007)

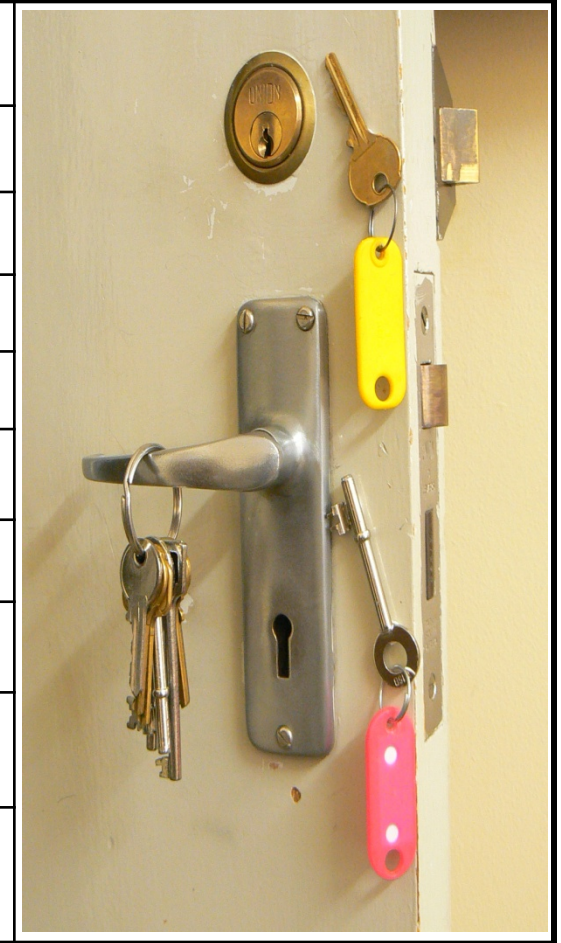
Saccharide bonds of human and plant oligosaccharides

Human oligosaccharides	Saccharide bond
Fucosyllactose	α 1-2, β 1-4, α 1-3
Lacto-N-tetraose	β 1-4, β 1-3
Sialyllactose	β 1-4, α 2-3, α 2-6
Sialylacto-N-tetraose	β 1-4, α 2-3, α 2-6, β 1-3
Plant oligosaccharides	
Fructo-oligosaccharides	β 1-2
Galacto-oligosaccharides	β 1-4, α 1-6
Transgalacto- oligosaccharides	β 1-4, β 1-6
Soybean oligosaccharides	α 1-2, α 1-6

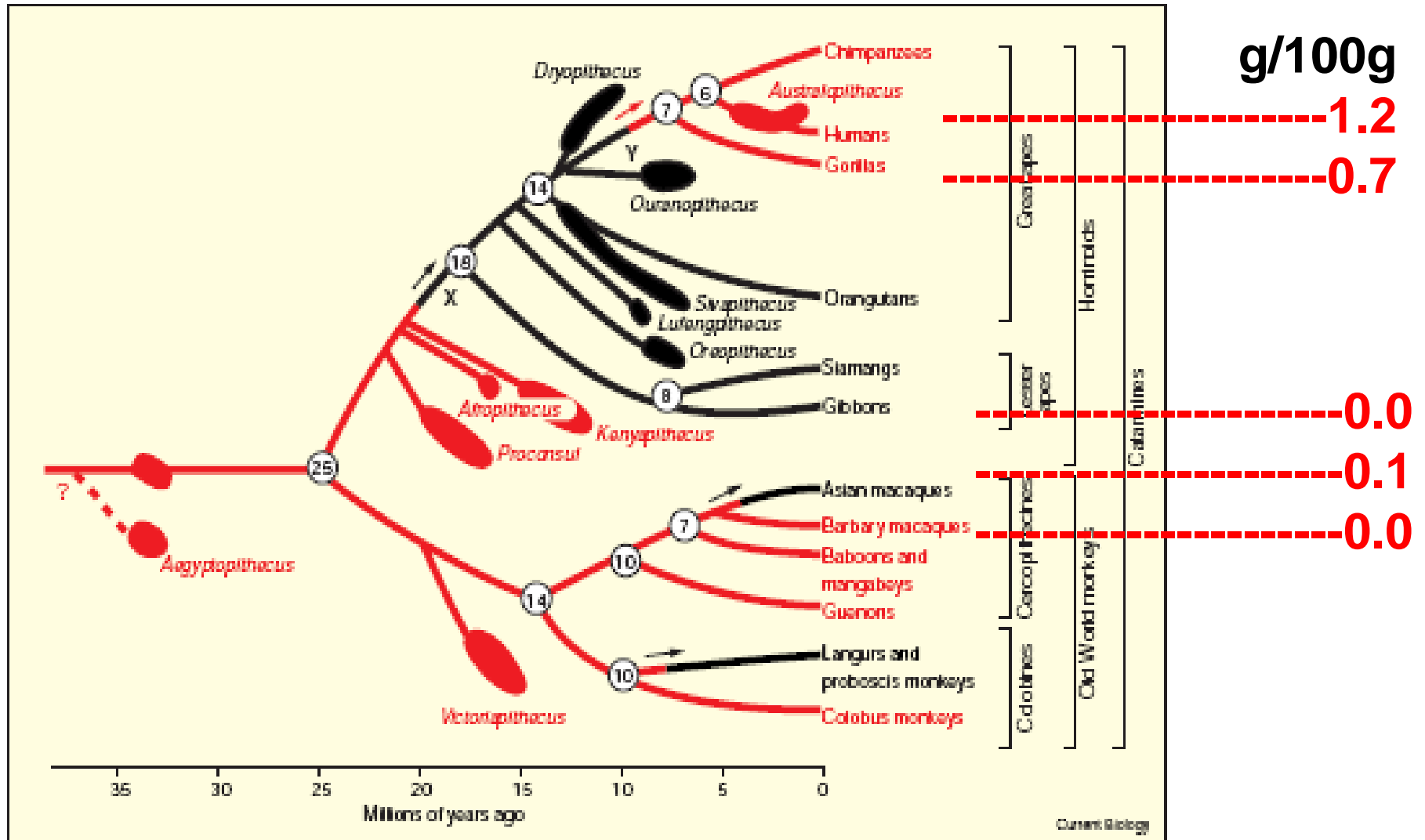
(Crittenden et al. 1996; Urashima et al. 2001;2007)

Saccharide bonds of human and plant oligosaccharides

Human oligosaccharides	Saccharide bond
Fucosyllactose	α 1-2, β 1-4, α 1-3
Lacto-N-tetraose	β 1-4, β 1-3
Sialyllactose	β 1-4, α 2-3, α 2-6
Sialyllacto-N-tetraose	β 1-4, α 2-3, α 2-6, β 1-3
Plant oligosaccharides	
Fructo-oligosaccharides	β 1-2
Galacto-oligosaccharides	β 1-4, α 1-6
Transgalacto- oligosaccharides	β 1-4, β 1-6
Soybean oligosaccharides	α 1-2, α 1-6



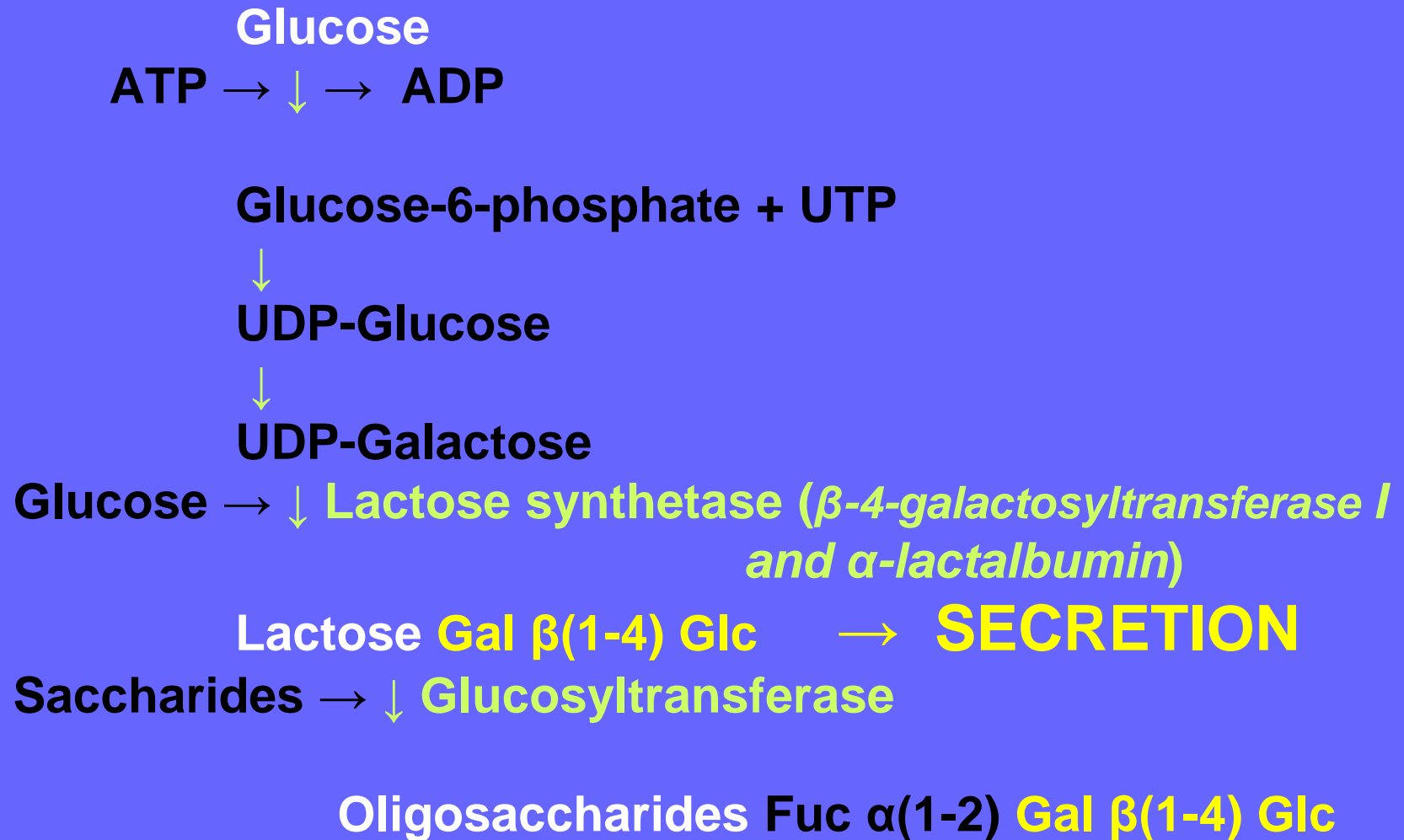
Phylogenetic relationship of oligosaccharide content in primate milk



(Stewart & Disotell, 1998)

(Osthoff et al. 2010)

Synthesis of lactose and oligosaccharides



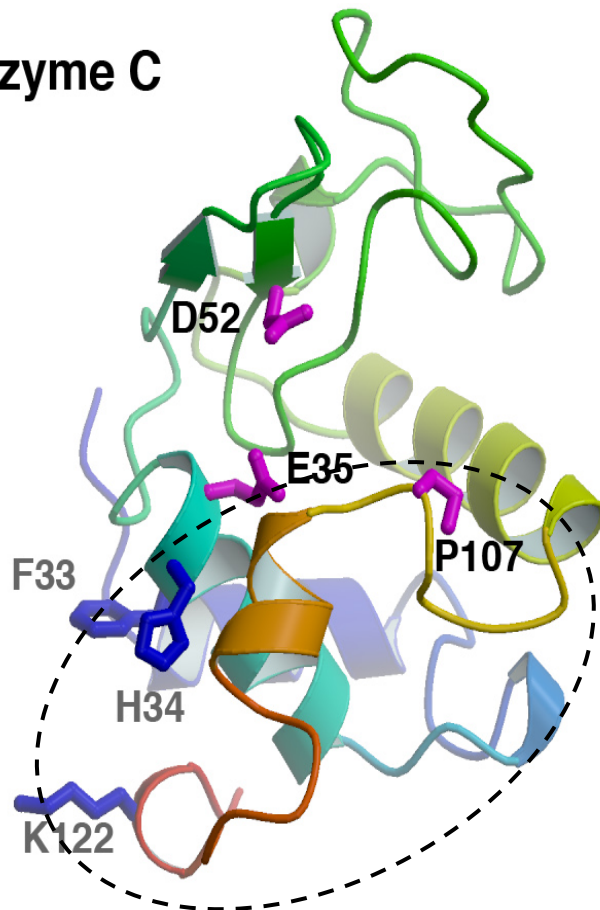
Alignment of the amino acid sequences of platypus and echidna α -lactalbumin and bovine lysozyme

	1				50
Echidna	KVFEKCELSQ	MLKANGLDGF	QGITLEEWIC	IAFHESGFDS	RALNYY--NG
Platypus	RIFQICELSR	VLKENLGGF	HGVSLEEWLC	VIFHESGYDS	QALNYY--NG
Cow	KVFERCELAR	TLKKLGLDGK	YGVSLANWLC	LTKWESSYNT	KATNYPSSSE
	51				100
Echidna	SSSHGLFQIN	RQYWCDGQDK	ASTEPSVNAC	QISCDKLRDD	DIEDDIKCVK
Platypus	SSSHGLFQIN	QPYWCDDXDS	ESTEPSVNAC	QIPCSKLLDD	DILDDIECAK
Cow	STDYGIFQIN	SKWWCN---D	GKTPNAVDGC	HVSCSELMEN	DIAKAVACAK
	101				150
Echidna	KILKESQGIT	AWEAWQPFCIA	D-LDQWK--C	--	
Platypus	KIVKEPKGIT	AWEAWQPFCNS	D-LDQWK--C	--	
Cow	HIVSE-QGIT	AWVAWKSHCRD	HDVSSYVEGC	TL	(Messer et al. 1998)

Comparison of the 3-D structures of α -lactalbumin and lysozyme C

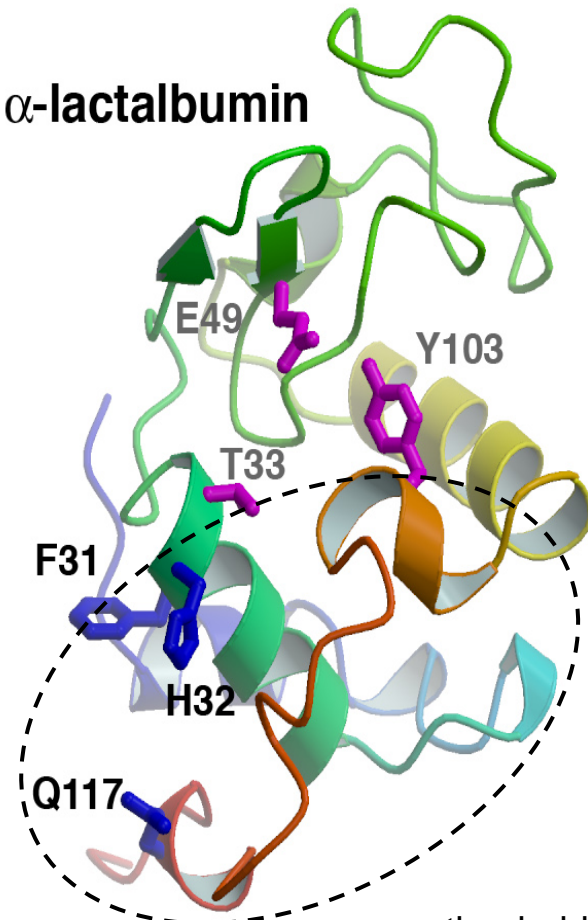
A.

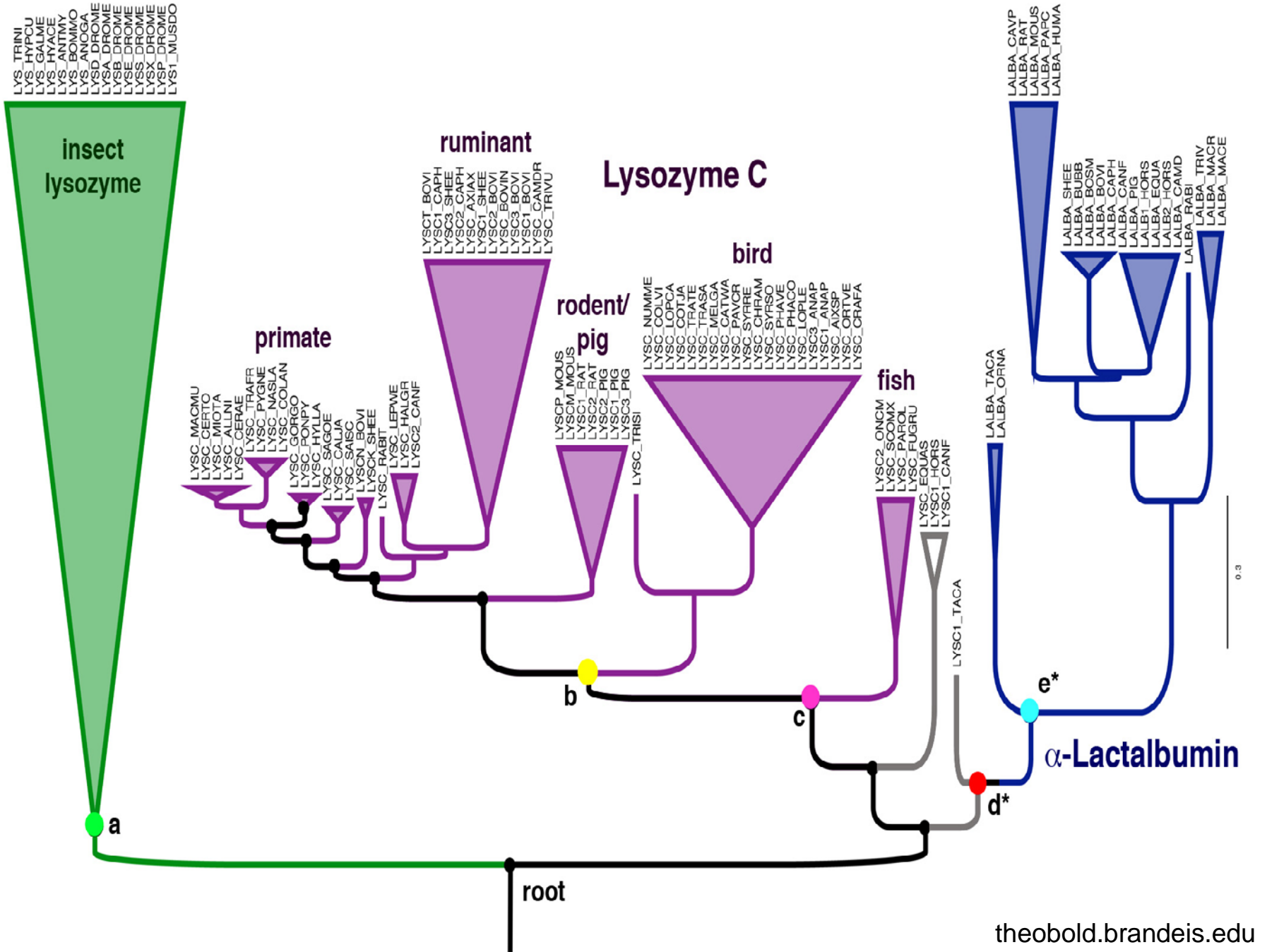
lysozyme C



B.

α -lactalbumin



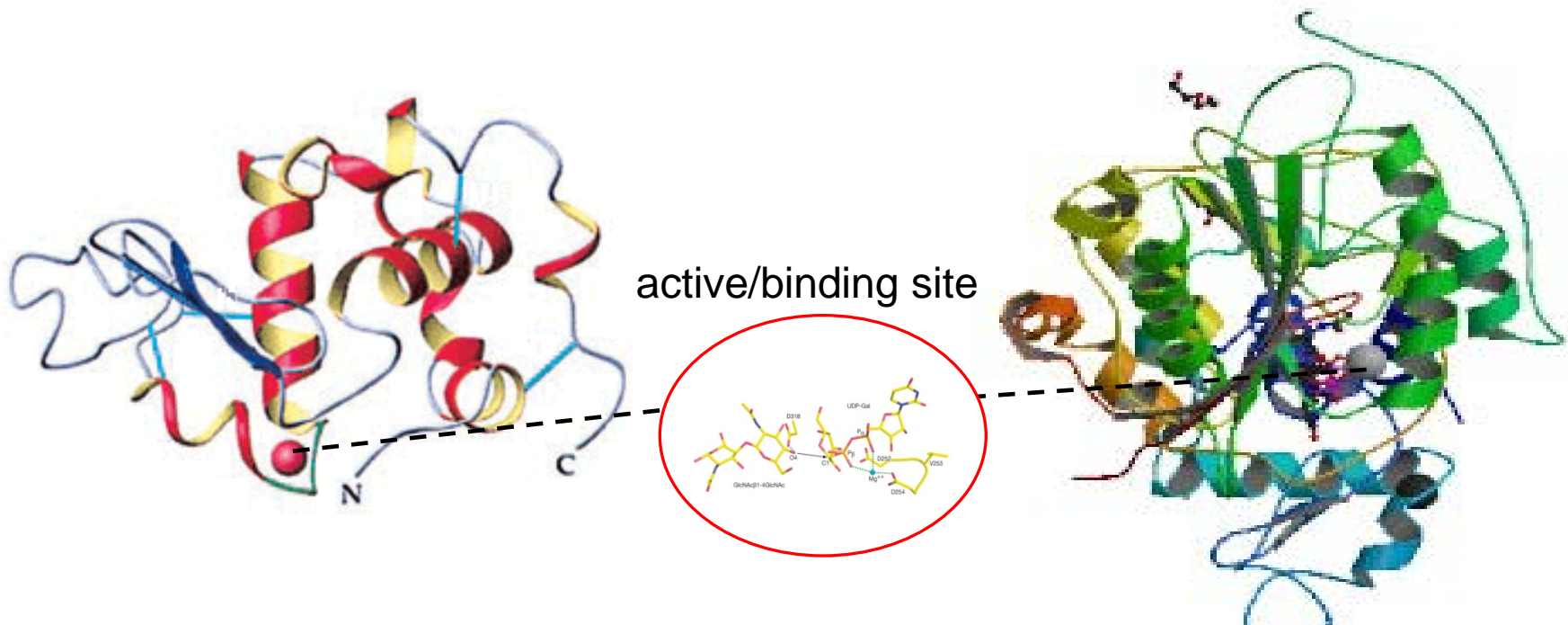


IDEA!?

Comparative study of lactose release- and oligosaccharide synthesis enzymes:

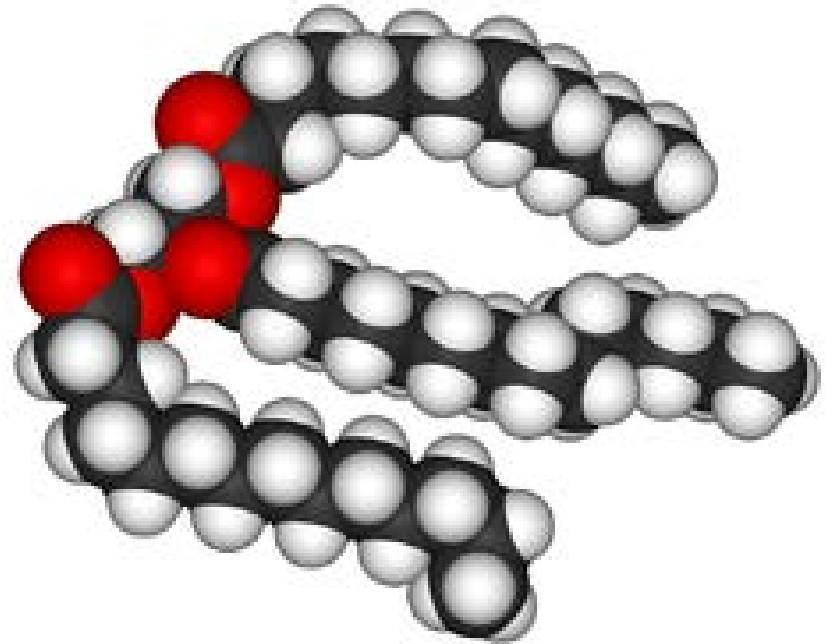
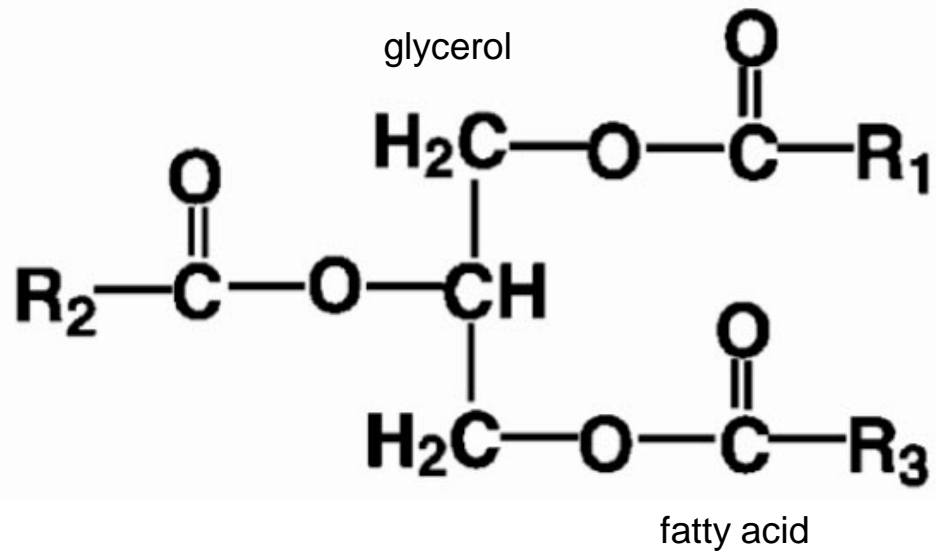
α -Lactalbumin

Glucosyltransferase



(Ramakrishnan et al. 2005)

LIPIDS



Sources of fatty acids

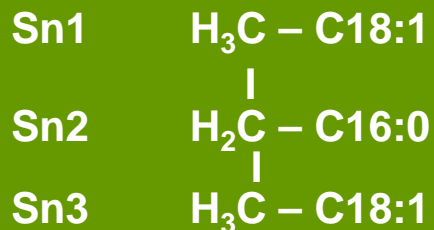
- (1) de novo synthesis
- (2) the diet
- (3) modification by desaturation and elongation
(essential: long chain + unsaturated)
(20:1 – 24:1)

HUMAN MILK

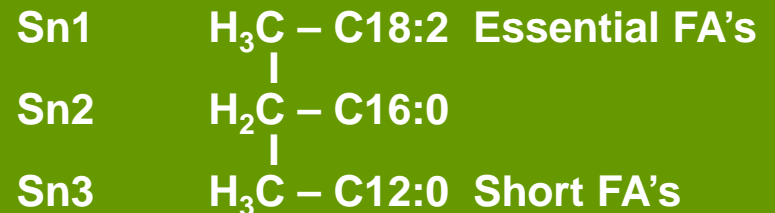
Table 3. Distribution of fatty acids at sn-positions of human milk fat

Fatty acid (Mol %)	Sn-1	Sn-2	Sn-3
10:0	0.2	0.2	1.8
12:0	1.3	2.1	6.1
14:0	3.2	7.3	7.1
16:0	16.1	58.2	6.2
16:1	3.6	4.7	7.3
18:0	15.0	3.3	2.0
18:1	46.1	12.7	49.7
18:2	11.0	7.3	2.0
18:3	0.4	0.6	1.6
20:1	1.5	0.7	0.5

Typical fat molecule



Secondary fat molecules



(Christie 1983)

Digestion in 3 phases

1. Pre-duodenal lipase acts on sn-3



2. Pre-duodenal pancreatic lipases act on sn-1 & sn-3



Antimicrobial
Increase absorption of FA's

3. Breast milk lipase act on sn-1, sn-2, sn-3 Bile salt stimulated in duodenum

(Lien et al 1997; Lien & Lucas 1997)

Fatty acids at sn-positions: human and cow's milk fat

Fatty acid (Mol%)	HUMAN			COW		
	Sn-1	Sn-2	Sn-3	Sn-1	Sn-2	Sn-3
C4:0				-	-	5.47
C6:0				4-	0.9	12.9
C8:0				1.4	0.7	3.6
10:0	0.2	0.2	1.8	1.9	3.0	6.2
12:0	1.3	2.1	6.1	4.9	6.2	0.6
14:0	3.2	7.3	7.1	9.7	17.5	6.4
16:0	16.1	58.2	6.2	34.0	32.3	5.4
16.1	3.6	4.7	7.3	2.8	3.6	1.4
18:0	15.0	3.3	2.0	10.3	9.5	1.2
18.1	46.1	12.7	49.7	30.0	18.9	23.1
18.2	11.0	7.3	2.0	1.7	3.5	2.3
18.3	0.4	0.6	1.6			
20:1	1.5	0.7	0.5			

(Christie 1983)

FA's not in correct sn-position. May lead to deficiencies.
Limiting is the essential FA's

Human		Typical Cow's fat	
Sn1	H ₃ C – C18:1 Essential FA's	Sn1	H ₃ C – C16:0/C18:1
Sn2	H ₂ C – C16:0	Sn2	H ₂ C – C16:0
Sn3	H ₃ C – C18:1 Short FA's	Sn3	H ₃ C – C18:1/C6:0

FAT SOURCES FROM PLANT MATERIAL IN FORMULAS

Corn oil

Safflower oil

Coconut oil

High oleic sunflower oil

Soy oil

Palm oil

Free fatty acids (essential)

Single cell oil

**FA's not in correct sn-position. May lead to deficiencies.
Limiting is the essential FA's**

HUMAN

Sn1 H₃C – C18:1 Essential FA's

Sn2 H₂C – C16:0

Sn3 H₃C – C18:1 Short FA's

TYPICAL PLANT FAT

Sn1 H₃C – C18:1

Sn2 H₂C – C18:2 Essential FA's

Sn3 H₃C – C18:1

Lesson:

If FA's not in correct sn-position: May lead to deficiencies

Fat absorption is very efficient

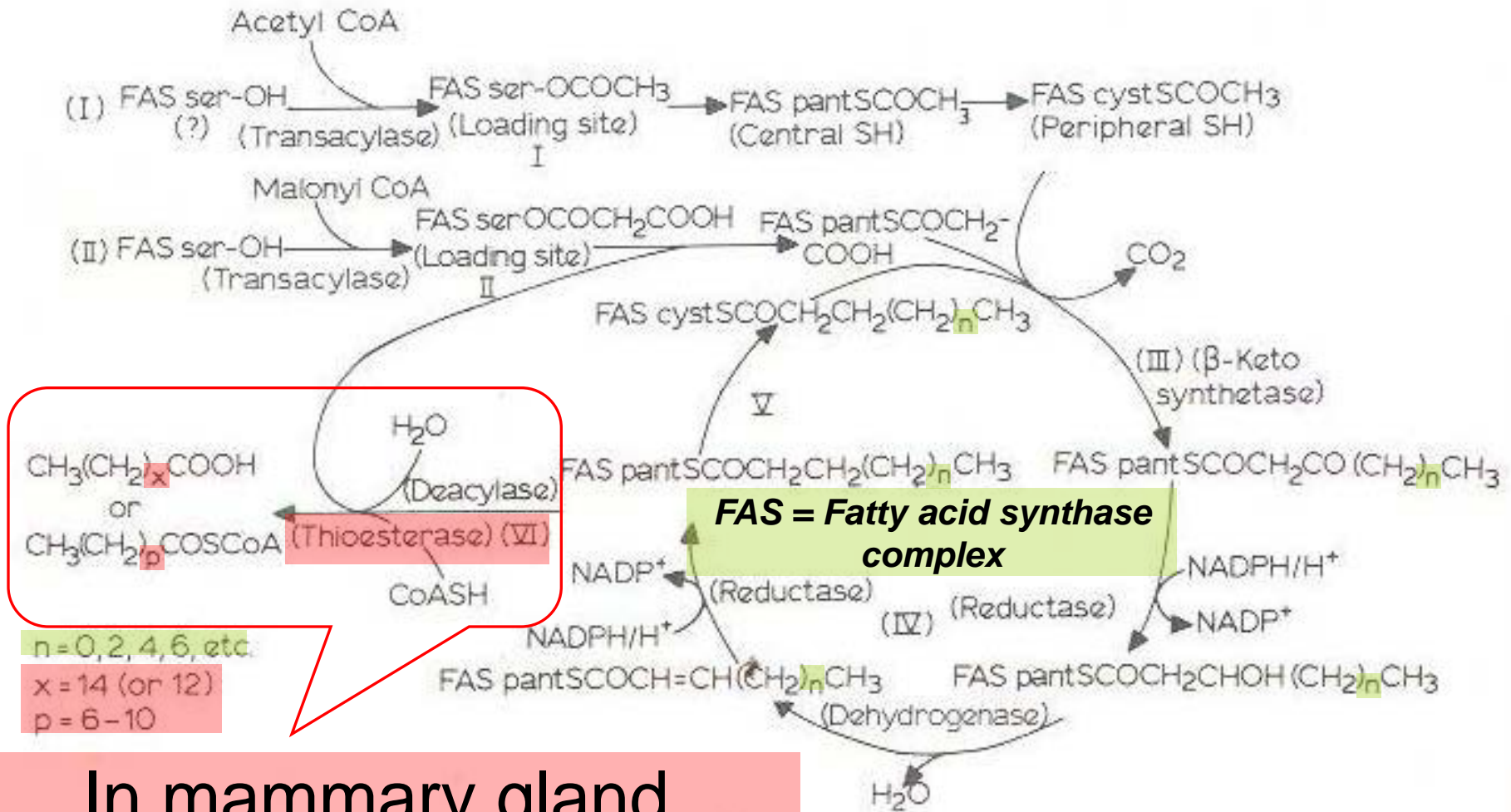
Essential FA's can be added

Fatty acid composition (% of total FA's) of the fat fraction of milks

Fatty acid	Human	Cow	Springbok	Blue W Beest	White Rhino	Elephant	Cheetah
C4:0	0	3.3	0.7	0.2	0	0	0
C6:0	0	1.6	0.7	2.0	0	0	0
C8:0	0.4	1.3	0.5	5.6	3.0	2.9	0
C10:0	1.0	3.0	0.9	20.7	25.5	35.3	0
C11:0						1.2	0
C12:0	4.4	3.1	1.3	9.0	16.5	26.9	0.1
C13:0			0.1			0.3	2.7
C14:0	6.3	9.5	13.4	20.6	9.6	0.5	
C15:0			1.3	1.2	0.4	0.2	0.3
C16:0	22.0	26.3	21.3	21.5	15.8	9.5	21.0
C16:1c9	3.3	2.3	1.7	0.6	1.2	1.0	5.8
C17:0			1.6	1.2	0.5	0.3	0.4
C17:1c10			0.2			0.3	0.4
C18:0	8.1	14.6	17.2	5.5	8.9	1.4	4.5
C18:1c9	31.3	29.8	25.1	8.1	8.6	11.3	32.4
C18:c7						2.1	
C18:2c9,12(n-6)	10.8	2.5	3.0	1.6	3.7	0.9	15.3
C18:3c9,12,15(n-3)	0.8	2.5	1.2	0.6	2.5	0.8	10.1
C18:3c6,9,12 (n-6)	0.2		0.1			-	0.1
C20:0				0.6	0.4	0.1	0.3
C20:2c11,14 (n-6)	0.3		0.1			0.1	
C20:3c8,11,14 (n-6)	0.3					0.2	
C20:3c11,14,17 (n-3)						0.1	1.1
C20:4c,5,8,11,14 (n-6)	0.4						
C20:5c5,8,11,14,17 (n-3)	0.1						
C22:6c4,7,10,13,16,19(n-3)	0.2						

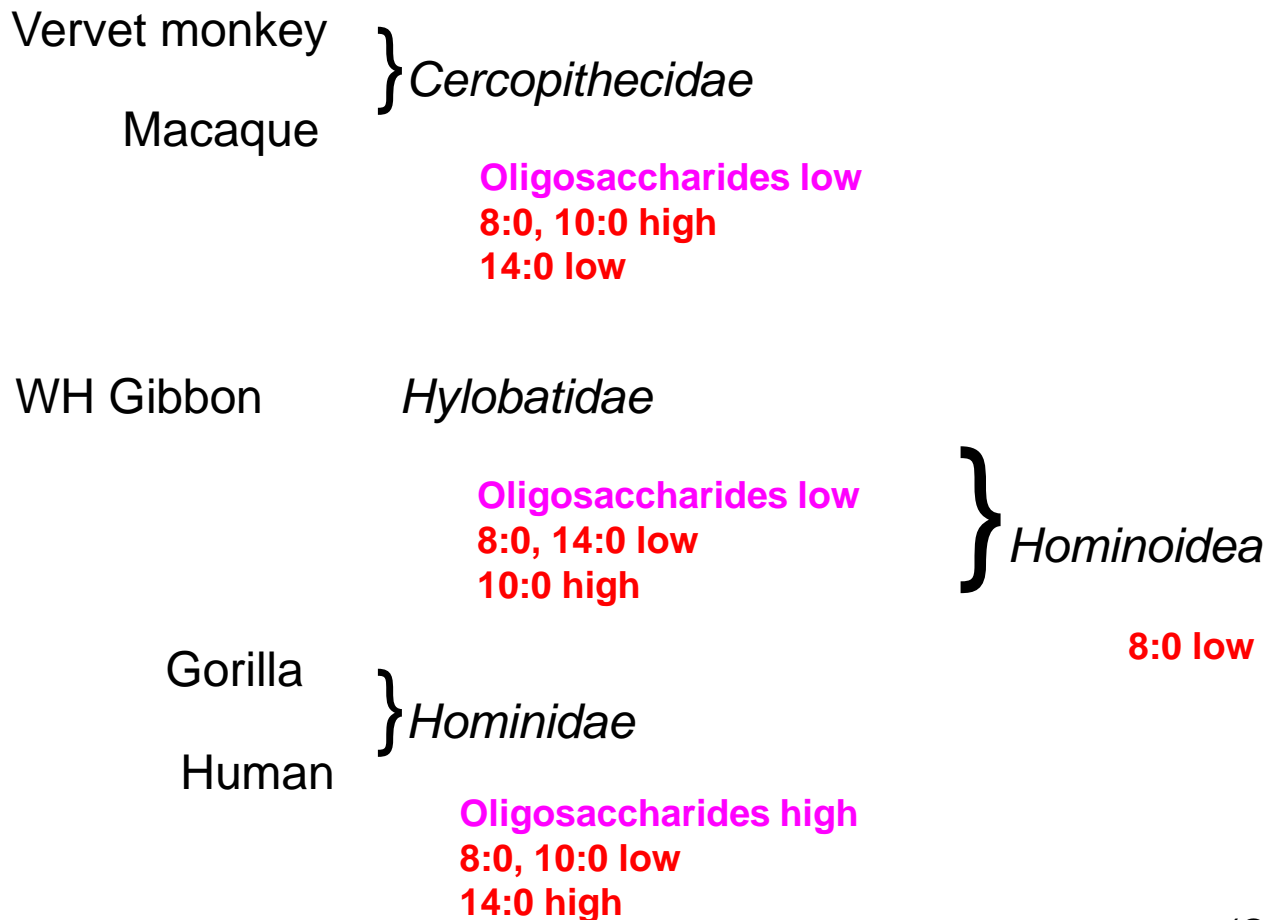
Osthoff et al. 2005;2006; 2007, 2008; 2009)

Fatty acid synthesis



In mammary gland
Thioesterase determines length
C8 – C14

Phylogenetic relationship of fatty acid composition in primate milk



Metabolic disorder

- **Defective acylCoA dehydrogenase**
- Defective short chain acylCoA dehydrogenase
- Defective medium chain acylCoA dehydrogenase
- Defective long chain acylCoA dehydrogenase
- Defective very long chain acylCoA dehydrogenase (Adrenoleukodystrophy)

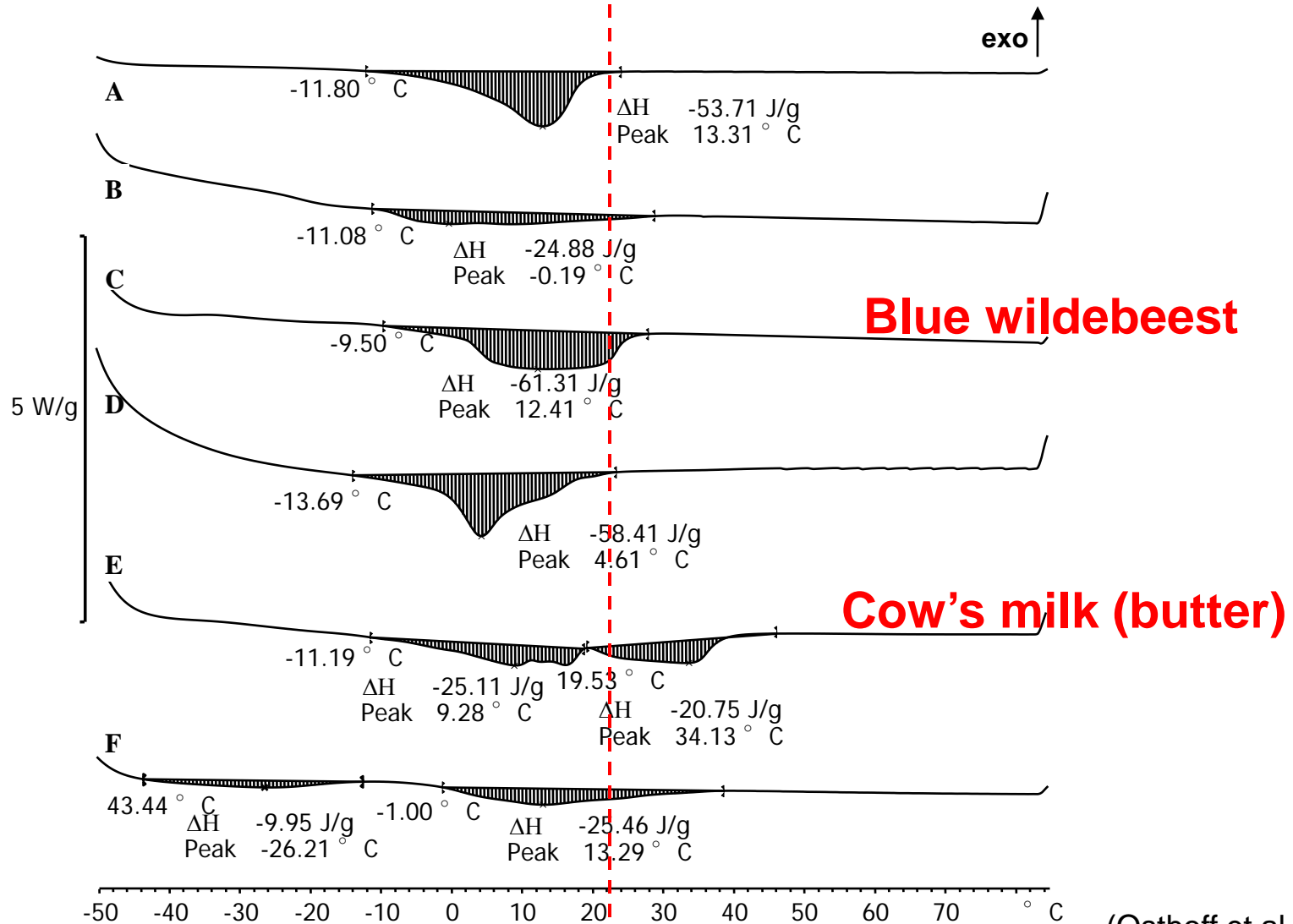
Fatty acid composition (% of total FA's) of the fat fraction of milks. Cattle grazed on same vegetation type

	Boran	Nguni	Tuli	Bonsmara	Drakensb	Afrik.	Signif.
	n = 5	n = 9	n = 10	n=6	n=6	n=6	
C4:0	0.70 ± 0.16 ^{ab}	0.49 ± 0.32 ^a	0.41 ± 0.34 ^a	0.96 ± 0.09 ^b	0.95 ± 0.13 ^b	0.97 ± 0.10 ^b	p < 0.001
C6:0	0.99 ± 0.22 ^{ab}	0.75 ± 0.39 ^a	0.73 ± 0.35 ^a	1.33 ± 0.15 ^b	1.41 ± 0.27 ^b	1.23 ± 0.19 ^b	p < 0.001
C8:0	0.71 ± 0.20 ^{ab}	0.62 ± 0.29 ^a	0.67 ± 0.12 ^a	1.07 ± 0.15 ^{bc}	1.28 ± 0.20 ^c	1.00 ± 0.17 ^{bc}	p < 0.001
C10:0	1.68 ± 0.62 ^{ab}	1.62 ± 0.52 ^{ab}	1.56 ± 0.33 ^a	2.79 ± 0.68 ^{cd}	3.43 ± 0.57 ^d	2.41 ± 0.44 ^{bc}	p < 0.001
C12:0	2.26 ± 0.73 ^{ab}	2.11 ± 0.63 ^a	2.06 ± 0.41 ^a	3.55 ± 0.75 ^{cd}	4.33 ± 0.53 ^d	3.04 ± 0.43 ^{bc}	p < 0.001
C14:0	10.01 ± 1.08 ^{ac}	9.43 ± 2.15 ^{ab}	8.60 ± 1.09 ^a	12.72 ± 1.68 ^{cd}	14.50 ± 0.97 ^d	11.50 ± 1.34 ^{bc}	p < 0.001
C18:1c9	26.96 ± 2.84 ^{bc}	27.77 ± 7.11 ^c	30.39 ± 4.50 ^c	20.03 ± 3.44 ^{ab}	14.84 ± 1.53 ^a	20.27 ± 3.23 ^{ab}	p < 0.001
C18:1c7	0.29 ± 0.09 ^{ab}	0.26 ± 0.12 ^{ab}	0.42 ± 0.19 ^b	0.22 ± 0.07 ^{ab}	0.20 ± 0.10 ^a	0.29 ± 0.14 ^{ab}	p < 0.05
Saturated FA's	63.99 ± 4.33 ^{ac}	63.72 ± 8.58 ^{ab}	60.88 ± 4.72 ^a	72.80 ± 4.30 ^{cd}	78.41 ± 2.32 ^d	71.87 ± 3.72 ^{bcd}	p < 0.001
Mono Unsat FA's	33.22 ± 3.81 ^{bcd}	33.22 ± 7.69 ^{cd}	36.47 ± 5.06 ^d	24.38 ± 3.84 ^{ab}	19.91 ± 2.12 ^a	25.67 ± 3.62 ^{ac}	p < 0.001
Total Omega-3 FA's	0.38 ± 0.14 ^{ab}	0.60 ± 0.35 ^b	0.45 ± 0.20 ^{ab}	0.76 ± 0.11 ^b	0.23 ± 0.18 ^a	0.54 ± 0.09 ^{ab}	p < 0.01

(Myburgh et al. in preparation)

Differential Scanning Calorimetry thermograms of milk fat from various mammals.

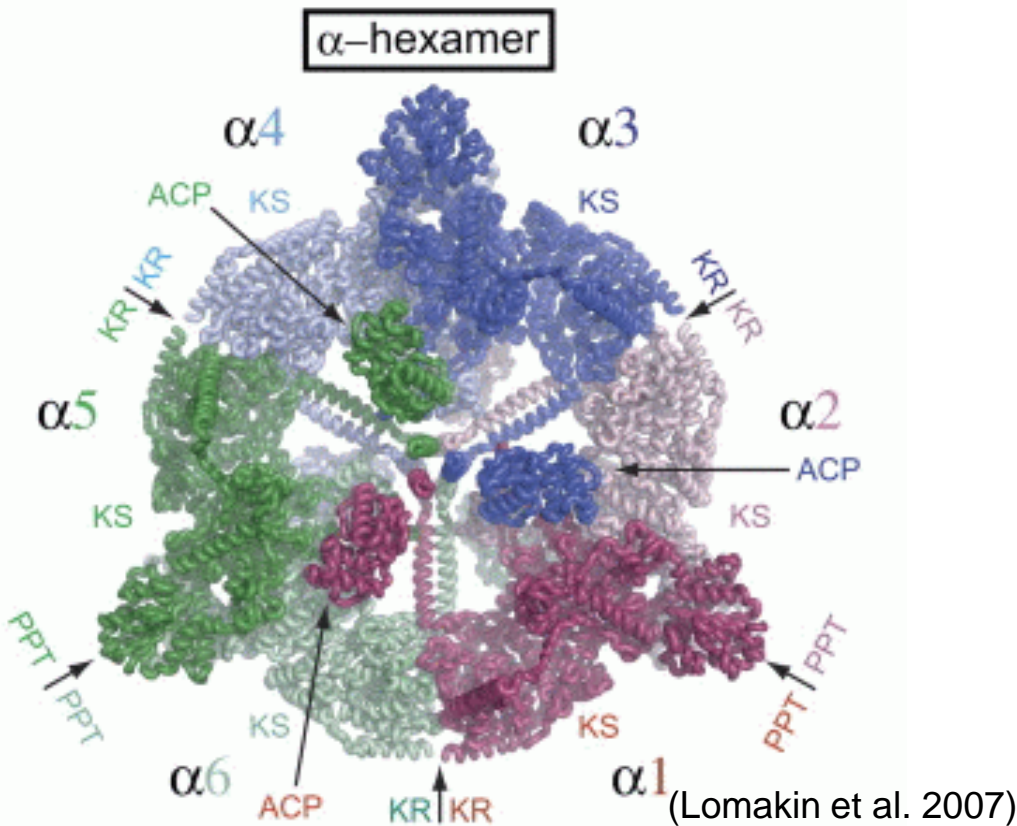
African elephant (A), cheetah (B), blue wildebeest (C), vervet monkey (D), domestic cow (E) and bottlenose dolphin (F)



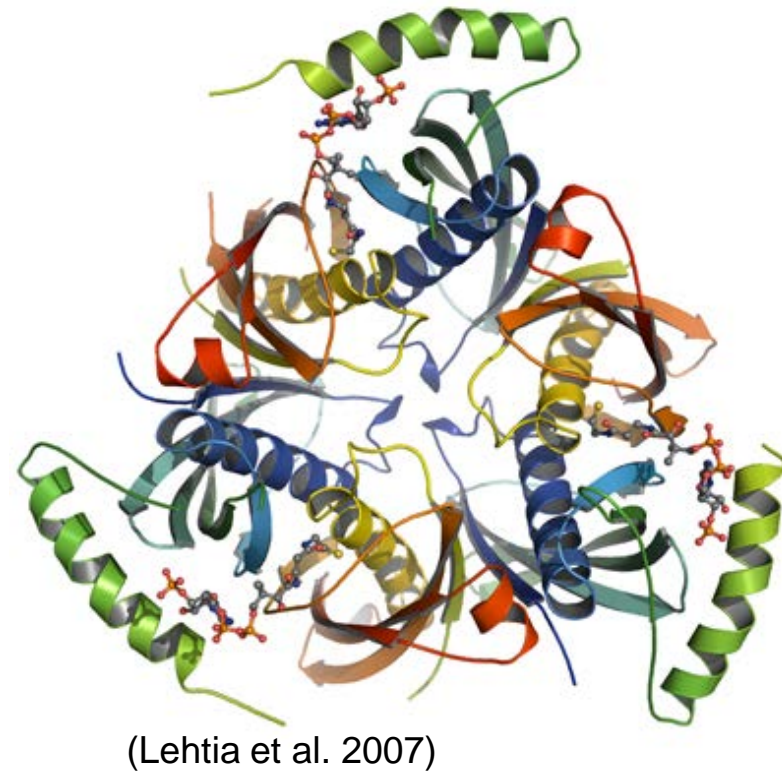
IDEA!?

Comparative study of fatty acid synthesis enzymes :

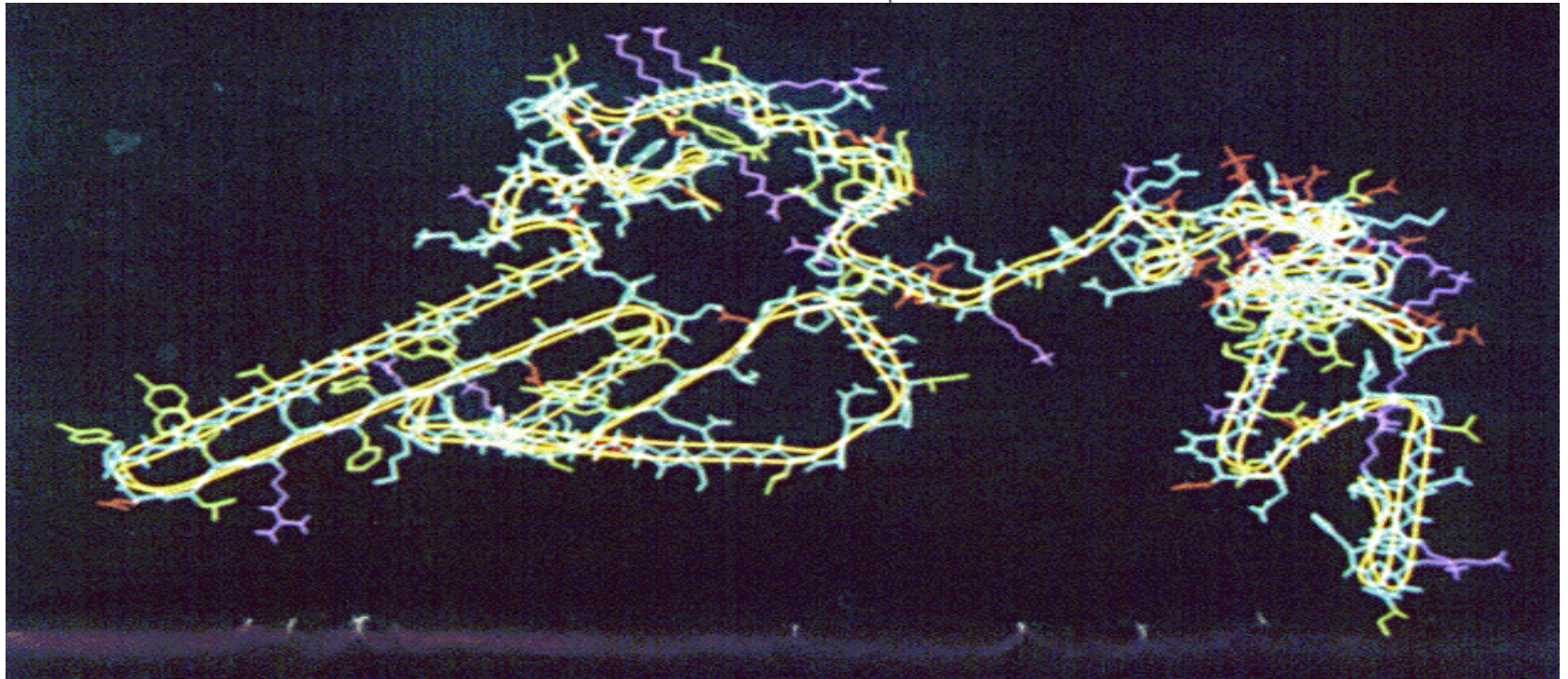
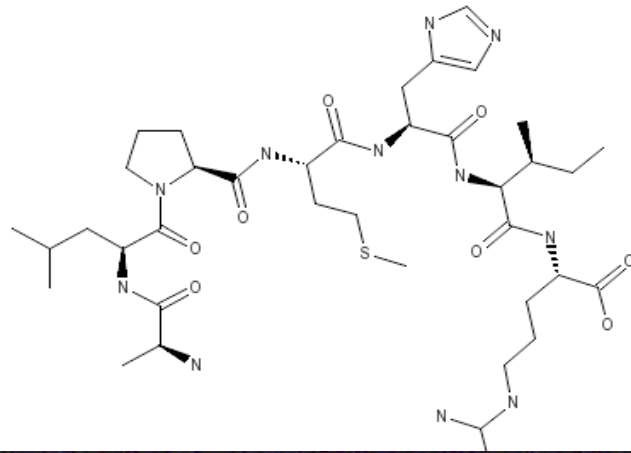
Fatty acid synthase



Thioesterase
[trimer]



PROTEINS



Milk proteins

Caseins (2.6%)

- α s1-casein
- β -casein
- κ -casein
- α s2-casein

Posttranslational modifications

*Glycosylation
phosphorylation
disulphide bond
proteolysis
genetic
variants*

Whey (0.7%)

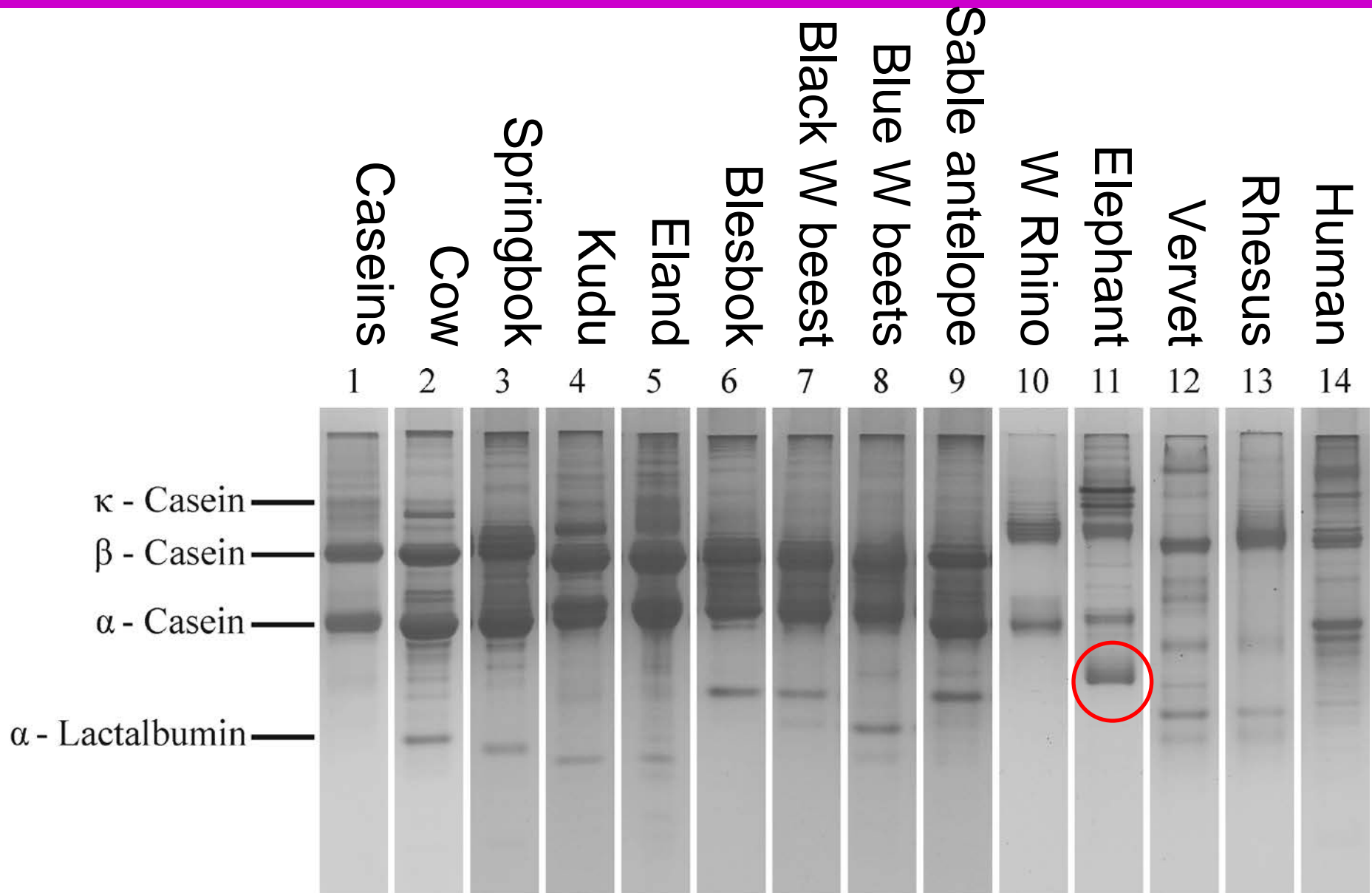
- β -lactoglobulin
- α -lactalbumin
- serum albumin
- immunoglobulins

Minor proteins

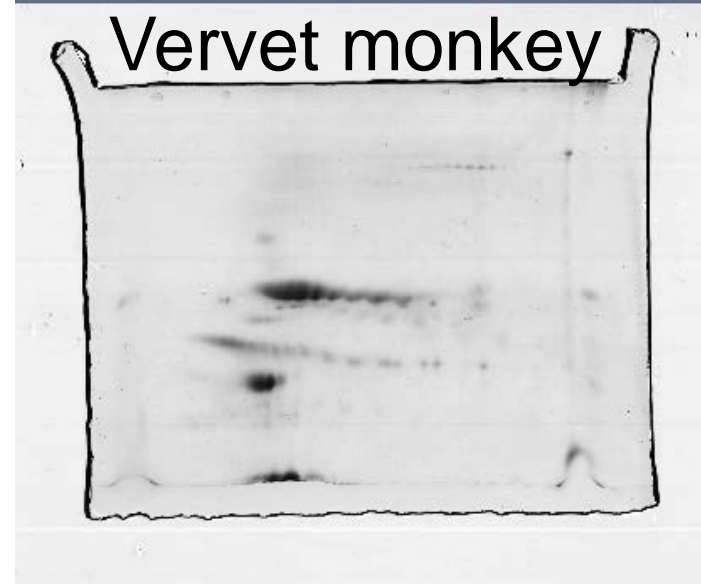
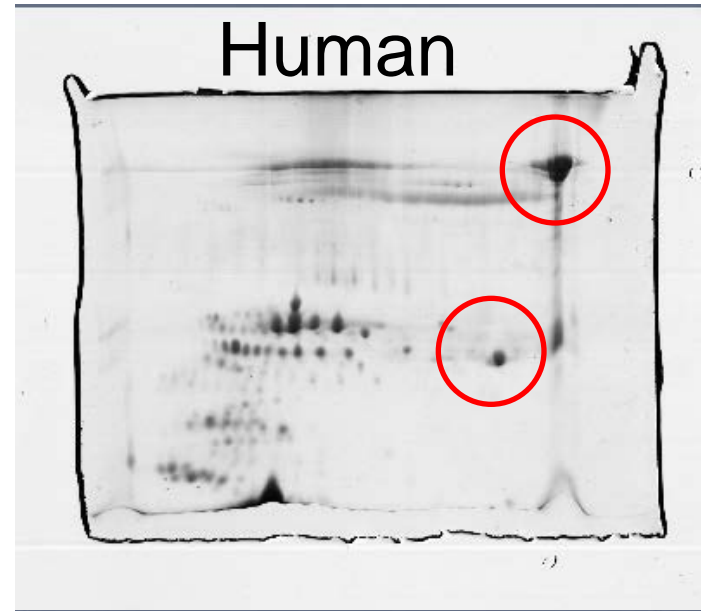
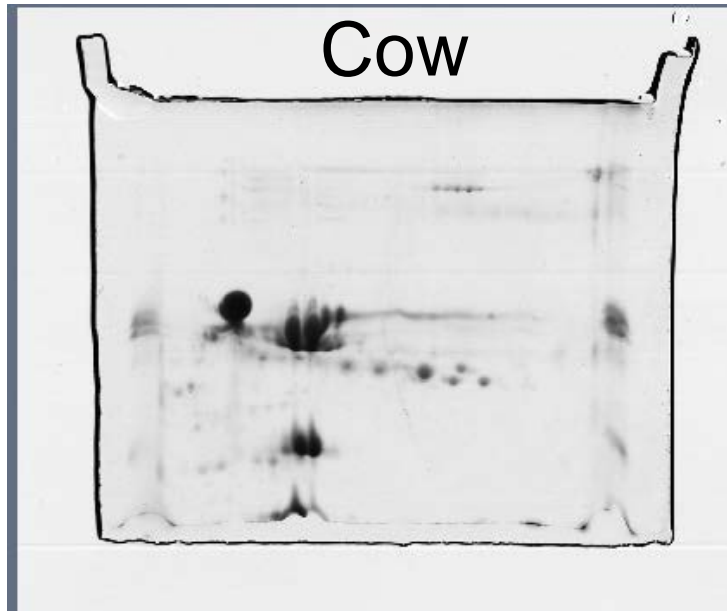
lipocalin-type prostaglandin d synthase, fatty acid binding protein, b2-microglobulin, complement C4, clusterin, a1-antritrypsin, lysozyme C, prealbumin, serotransferrin, fructose-bisphosphate aldolase A

Etc. etc. etc. etc. etc.....unknown?!

Milk proteins by electrophoresis



Proteins by 2-D electrophoresis



IDEA!?

Comparative proteome and protein modelling:

Enzymes in fatty acid synthesis:

Fatty acid synthetase

Thioesterase

Enzymes in lactose release and oligosaccharide synthesis:

α -Lactalbumin

Glucosyltransferase

FUTURE?

Explain the biochemical reason why milk of some mammals contains high amounts of oligosaccharides.

Explain the biochemical reason of the synthesis of different fatty acid chain lengths amongst mammals.

DISTANT FUTURE?

Genetic manipulation of commercial dairy animals to produce custom milk:

Oligosaccharide enriched

Custom Fat

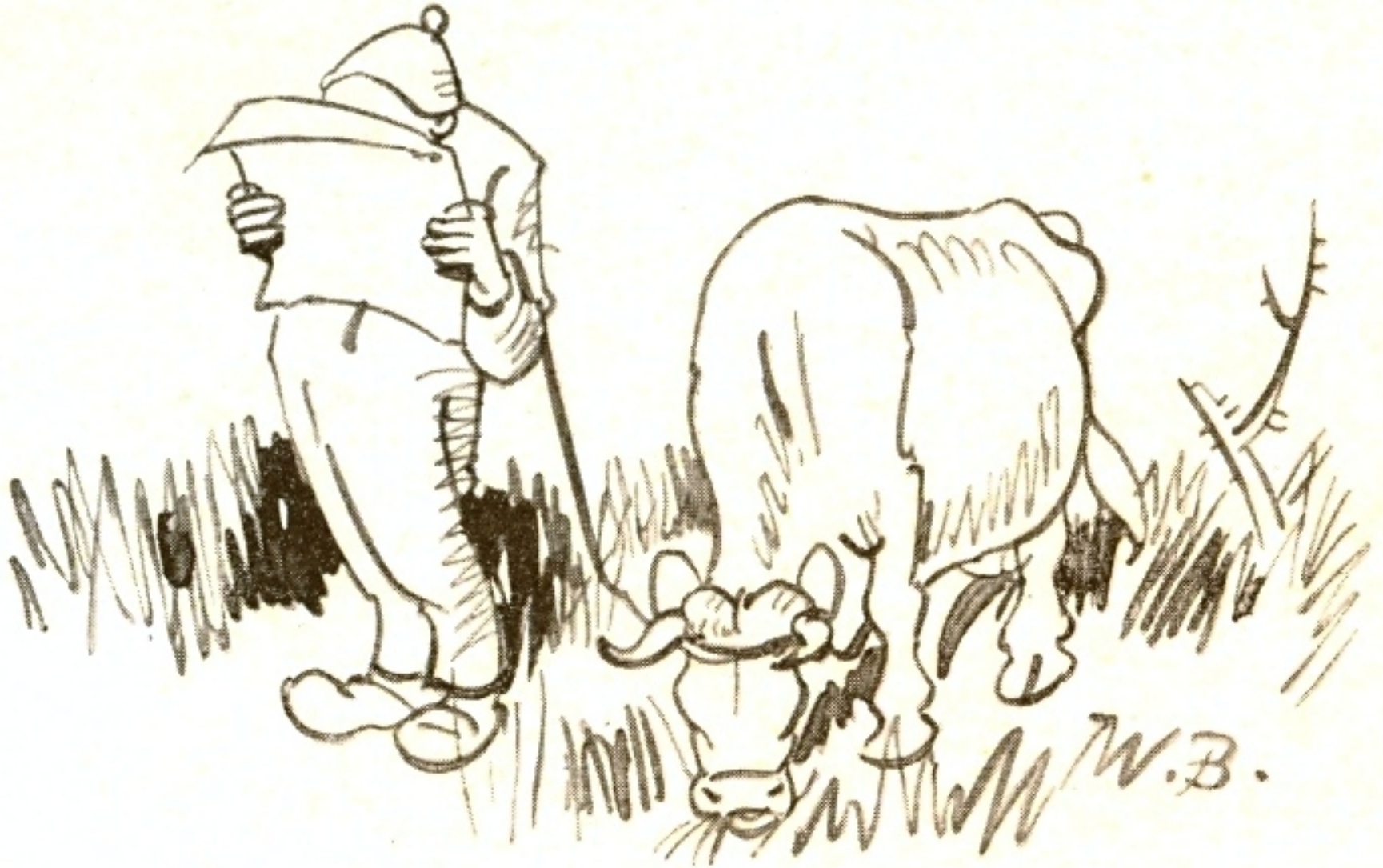
soft butter

improved digestibility

(for metabolically impaired)

Studying

milk...



Danke/Dankie/Thank you/Ke a leboha

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Therese & Merlind

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My students

Desiré Harris

The late Anita vd Westhuizen

Joan Nel, Stephen Collet

UFS



... surprising results



Danke/Dankie/Thank you/Ke a leboha