INAUGURAL LECTURE
PROF G OSTHOFF

MILK: THE WELL-KNOWN (?) FOOD

TUESDAY 8 MARCH
19:00

1) Milk: the well-known(?) food Prof G Osthoff

An inaugural lecture is an honour, but also a challenge. This is because the audience consists of experts as well as laymen on the topic. The lecture must therefore be superficial as well have depth. I hope that my lecture satisfies both. I wish you a hearty welcome.

My werk behels die chemiese aspekte van voedsel, met ‘n fokus op suiwelkunde. Ek is betrokke by navorsing van kaasprosessering sowel as melkevolusie. Ek het besluit om my lesing op laasgenoemde te laat fokus, aangesien dit ’n holistiese benadering verg met ’n uiteindelike raakvlak met daaglikse voedselitems. Ek wil wys hoe belangrik dit is dat die kennis van melk van suiweldiere nie alle aspekte van melk as voedsel verduidelik nie. Ek wil graag praat oor ’n paar aspekte wat deur my navorsing geraak word en waarvoor ek ’n toepassing in suiwelkunde in die toekoms voorspel.

<u>2) CONTENTS</u>

I am involved in chemical aspects of food, with a focus on dairy science and technology. I am involved in the research of cheese processing as well as milk evolution. For my talk I chose to touch on the latter because it requires a holistic approach with an eventual application on daily food items. I wish to show how important it is that the knowledge of milk from dairy animals alone does not provide all the explanations of milk as food. I wish to speak about a few aspects touched by my research and for which I envisage future application in dairy technology.

I will provide a brief background of milk, its utilization and certain nutritional problems that are experienced.
Then I will turn to the general composition of milk, focusing on comparison between species.
I will specifically concentrate on two of the three macro-nutrients, sugars and fats, and their role in nutrition and technology. I will make use of information obtained by interspecies comparison. I will also have to touch on proteins, because some of them play a role in the properties of the sugars and fats.
Finally I will conclude with ideas and vision for future research.

<u>3) Introduction</u>
When the words “milk” or “dairy” are mentioned, the picture that immediately springs to mind is a product on the shelf of a supermarket.

4) Very few people associate their first thoughts with milk winning from its producer, the cow.

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

6) ….and that it is custom-designed for each species.

7) However, mankind is an opportunist and has found ways of easy access to food by the practice of agriculture, where plants as well as animals were employed or rather exploited.

8) While the cow is the best known of the milk producers, other animals are also employed. The selection of some of these animals was perhaps forced due to adaptability to environmental conditions.

9) In spite of breeding selection, cattle seem not to have adapted to the most extreme conditions such as high altitudes with sub-freezing temperatures, deserts and marshes.

10) The consumption of the milk as adult is not natural. Neither is the consumption of milk across species. This practice of mankind may often have consequences, when signs of malnutrition or diseases are noticed.

11) Two commonly occurring problems that might occur are allergy to milk and lactose intolerance. Allergies are normally the result of an immune response of the consumer to the foreign proteins found in the milk.

12) In some cases it might help to switch from one milk source to another, such as switching from cow's milk to goat's milk.

13) Lactose intolerance is the inability of adult humans to digest lactose, the milk sugar. This is natural, as adults loose that ability to digest lactose. The symptoms of the condition are stomach cramps and diarrhea.

14) It is interesting to note that this problem is mainly found in the warmer climates of the world. This could be an indication of early passive development of dairy technology. In these regions milk could not be stored in its fresh form, but in a fermented form, in which case the lactose was pre-digested by microorganisms, and the human population never adapted to digesting lactose in adulthood.

15) It is basically the lactose in milk that has spurred dairy technology. Its fermentation has resulted in the development of yoghurts and all the cheeses that we know.
And the intolerance to lactose has spurred a further technological solution: Lactose-free milk is currently produced by pre-digestion of lactose with enzymes.

16) As mentioned, humankind has also employed other animals to produce milk with derived dairy products. It was soon realized that the milks and products

from different species differed in quality aspects such as keeping properties and taste. It was also realized that the nutritional properties differed as well as their effects on health. One example is the mentioned allergy against cow's milk proteins which may be solved by the consumption of goat's milk. The nutritional benefits and technological processing of milk aroused an interest in more information, and it was realized that the information gained from human milk and that of the few domesticated species does not provide a complete explanation of the properties of milk as food.

17) Of the 250 species of which milk has been studied, …

18) …only the milk of humans and a few domesticated dairy animals has been studied in detail. Valuable information and understanding of milk as food was gained by research of the milk of humans and cow, and also the monotremes and marsupials.

19) Information from some non dairy species provided a better understanding of milk as food in general. Apart from the proximate/general composition, very little detailed information is known for others.

20) My own work also provided additional knowledge, specifically from elephants, rhinoceros, wild ruminants, the cat family and primates. Where appropriate, I will refer to the data in my lecture.

21) Although the milks of all mammals contain the same components: water, salts, vitamins, fat, carbohydrates, and proteins, it does not imply that all milks are the same.

General composition.

When the milk composition of species are compared (Table), it may be concluded that, in general, the milks of some primates, horses and rhinoceroses are dilute, low fat-milks, while others may produce high-fat and energy-dense milks. Deviations from these general compositions are observed, and may be ascribed to adaptation to special needs, such as to the environment or special dietary requirements. Amongst the Bovidae (the ruminants), the total dry matter of the milks of eg. the springbok is above 20%, compared to the approximately 13% of other members. This may possibly be as adaptation to arid environments. Amongst the Felidae (the cats), the total dry matter may vary; of serval milk it is 29%, while that of the cheetah is 17%. In these cases an adaptation to environment cannot be considered as reason, because these two cats co-exist in the same regions. At this stage I would also like to draw your attention on the composition of lactose and oligosaccharides amongst different species, because I will elaborate on this later. It is therefore clear that each milk provides nutrients and bio-reactive components in the correct amount and form, which is the result of adaptation to environmental opportunities as well as physiological constraints.

Let us now take a look at the individual nutrients and their roles in nutrition and also in technological exploitations.

22) SACCHARIDES

While lactose is the major carbohydrate in milk, longer chain oligosaccharides are observed in abundance in some mammalian families and species, including humans. The main purpose of the lactose is nutrition to provide energy.

23) For many years the existence of the oligosaccharides was not known nor was their role. Comparison with milk from other species provided a large part of the explanation. The milks of monotremes and marsupials contain no or little lactose, but have a high content of oligosaccharides. While the milk of most eutherian mammals contain mainly lactose and low amounts of oligosaccharides, some, such as the seal, also contain no or little lactose. Others, such as humans and elephants, contain relatively high amounts of both. I wish to draw your attention to the high amounts of both the lactose and the oligosaccharides in elephant milk, as well as the higher amount of oligosaccharides in milk from sable antelope, compared to other ruminants such as the cow. I want you to remember this information on the sable antelope, because I will refer to it later.

24) What is the role of the oligosaccharides? They consist of 3-10 sugar units and their structures may be linear or branched. Approximately 250 different structures have been identified based on the composition of building blocks, their arrangement and branching. Oligosaccharides in the milk of monotremes and marsupials are not very complex, but 12 structural groups are found in the milks of eutherian mammals. Some structures are unique in certain species or groups.
POINT: This specific oligosaccharide structure is unique to the milk of elephants, Asian as well as African.

25) Different chain types of oligosaccharides are also observed between human and other eutherian species: Type II chain (Gal(β1-4)GlcNAc) is found in most species, but is the only type in species such as the great apes.

26) The type I chain (Gal(β1-3)GlcNAc) is more prominent in human milk. I want you to remember the (β1-4) and the (β1-3) for later reference.

27) The oligosaccharides are not digestible by humans, however, they are digestible by the bacteria in the intestine, specifically the colon. The presence of these colon bacteria is very important, because they keep pathogenic bacteria at bay. Apart from antibodies in milk, the oligosaccharides also have a protective role. There is also evidence that the oligosaccharides bind to the pathogenic bacteria, which in turn prevents them to settle in the intestine. For this function, the oligosaccharides are also called pre-biotics, which is a large research field of

its own. Oligosaccharides therefore contribute to the reason why breast nurtured babies are more healthy than bottle-fed babies. Oligosaccharides have already found an application in the foods available in the market, such as baby foods, breakfast foods, yoghurts, and energy drinks.

28) There are several species of bacteria living in the intestine, the best known are lactobacilli and bifidobacteria. What is surprising, is that each of these bacteria seems to prefer to utilize an oligosaccharide with a specific structure, which has its consequences on the commercial use of pre-biotics.

29) Because there is a shortage of Oligosaccharides of dairy origin, plant saccharides are also used as pre-biotics, however, with varying and limited success. The reason might be because they have a different structure. The type I chain (Gal(β1-3)GlcNAc) and type II chain (Gal(β1-4)GlcNAc) bonds are not readily found in plant saccharides. Plant saccharides are mainly polymers of glucose and xylose.

30) The effect is that the colon bacteria cannot utilize the oligosaccharides of plant origin so that protection of the host is limited. This can be explained by a lock-and-key example, where certain locks can only be opened by matching keys.

31) From my own research a phylogenetic effect was observed amongst the primates, concerning the amounts of oligosaccharides found in the milk. When the data is incorporated into a taxonomic tree, it can be seen that human milk contains the most oligosaccharides, while the amount decreases with increasing distance in the taxonomic relationship. Since the sable antelope was shown to contain higher amounts of oligosaccharides, the question might be asked whether there is a similar phylogenetic relationship amongst the Bovidae family, which includes the domestic cow?

32) The explanation of the high amounts of lactose and/or oligosaccharides in milk lies in the synthesis of the milk sugars. During the synthesis, two proteins work in conjunction; β-4-galactosyltransferase and α-lactalbumin. The presence and absence of high amounts of lactose can be explained by the presence or absence of the α-lactalbumin. This protein facilitates the secretion of lactose. This protein is absent in the monotremes and marsupials, so that elongation to form longer oligosaccharides is favoured. The milk of the monotremes and marsupials, however, contains the evolutionary predecessor of α-lactalbumin, lysozyme, which is a well known protein in all animals, specifically associated with eggs of birds and reptiles.

33) The evolutionary development can be visualized by comparison of the amino acid sequences…..,

34) ……as well as their 3-D structures. The structure of lysozyme does not have the correct conformation or shape, specifically the lactose binding site, to

act out the function of secreting lactose. It would be of interest to determine the variation of structure and activity of these proteins from different species.

35) Here is a diagram which shows the evolutionary change from lysozyme to α-lactalbumin of a couple of species. While the amino acid sequences of these are known, the 3-D structures and kinetic properties are not..

36) The health protective function of the oligosaccharides and the commercial application, directed me to a topic for future research. The reason why the milk of some mammals, humans, great apes and elephants, contain high amounts of lactose as well as oligosaccharides lies in the saccharide synthesis involving the proteins β-4-galactosyltransferase and α-lactalbumin. I am of the opinion that it is the activity and affinity to lactose that plays a role. It is the structure and activity of these proteins that should be studied by specifically comparing the proteins from different mammals.

37) LIPIDS/FAT

The second macro nutrient in milk that I wish to discuss is the fat. A fat molecule consists of a backbone glycerol molecule to which three fatty acids are bound. These three fatty acids may differ in length and saturation.

38) The fatty acids of milk fat are obtained from three sources: (1) de novo synthesis, (2) the diet, and (3) modification by desaturation and elongation. This leads to relatively clear differences in fat composition between mammalian families. While specific fatty acids have been found to be essential in a diet in order to promote development of the nervous system, it is still not completely clear why the milk of certain species would rather contain long chain than medium chain length fatty acids. The positioning of fatty acids on each of the three sn-positions differs between plant fats and animal fats, and also between fats from different species.

39) In human milk the distribution of the fatty acids over the three sn-positions is such that most of the18:1 is found on sn-positions 1 and 3, while sn-2 is mainly occupied by 16:0. The essential fatty acids, the very long unsaturated molecules, are mainly found on position 1 and 3. Typical milk fat molecules would have the structures as indicated.

40) This has some consequences for the nurtured infant. The digestive system of babies is not completely developed, and the lipases, the enzymes that digest fat, have a specific preference of digestion. Pre-duodenal lipase specifically removes fatty acids from sn-position 3. This is followed by digestion

by the pre-duodenal pancreatic lipase, which removes fatty acids from sn-positions 1 and 3. This means that the essential fatty acids are immediately available. A large amount of 16:0 containing monoacylglycerides are then left, which play a protective role in the intestine against mico-organisms. Final digestion of the fat is then carried out by the milk lipase, which comes with the mother's milk.

41) In cow's milk fat, the fatty acid composition is different. It contains high amounts of short chain fatty acids, which come from the rumen bacteria, and the content of essential long chain unsaturated fatty acids is almost zero. The typical fat molecules therefore have a different structure, resulting in different digestion.

42) In baby milk formulations the milk fat is replaced by plant fats. In plant fats the essential fatty acids are located on sn-position 2, with the consequence that they are not as readily released and the resulting monoacylglyceride does not act its protective role against micro-organisms.

43) If we expand the comparison of the fatty acid composition of different species to other animals, specifically the non-ruminants, it is not surprising to observe that the short chain fatty acids are only found in ruminants. These fatty acids are produced by the ruminant bacteria. The ruminant milks also contain high amounts of 18:0. The milk fats of carnivorous cats also contain high amounts of essential fatty acids, similar to human milk. Interesting is the high amounts of short to medium chain, 8:0-12:0 fatty acids in milk from elephant and white rhinoceros. The milk composition of most Bovidae, that is ruminants such as cow, sheep, goat, and antelope, do not differ extensively. However, high amounts of medium to medium long chain fatty acids, 10:0 and 14:0, with a consequently lower long chain fatty acid content, are found in the Elaphinae sub-family, that is the blesbok, black and blue wildebeest. The reason for these differences lies in the synthesis of the fatty acids. Please remember this; I'll refer to it later.

44) In all cells fatty acids are synthesized by the enzymes of the fatty acid synthase complex to a final length as required, or the capabilities of the enzyme. In the mammary gland the length of the fatty acids is determined by a second enzyme, thioesterase, which terminates the elongation between 8 and 14 carbon length. It is probably the difference in activity of this enzyme that is responsible for the high amounts of short to medium long fatty acids found in the milk fat of rhinoceros, elephant and wildebeest.

45) To illustrate the phylogenetic effect of the fat synthesizing enzymes, primate milk can also be used as example. It is specifically fatty acids of 8, 10 and 14 carbon length which are affected. The phylogenetic effect on oligosaccharides is also included in this diagram.

What is the benefit of this knowledge to technology?

46) There are people who are born with a metabolic defect which renders them unable to digest certain fatty acids. One of the fatty acid digesting enzymes, acylCoA dehydrogenase, is defective. While some of these people are not able to digest long chain fatty acids, they can still digest short chains. The remedy lies in providing fat that is digestible to them. Mother's milk and cow's milk contains the wrong fatty acids for these patients. For at least two of the cases, defective long chain acylCoA synthase and Adrenoleukodystrophy, milk from elephant, rhinoceros or wildebeest might be a remmedy. Of course this is not possible because I do not know of a dairy farmer who is milking a herd of elephant rhinoceros or wildebeest! However, these species provide evidence that specific fatty acid synthase and thioesterase enzymes may be genetically controlled. Since the wildebeest is a ruminant of the bovidae family, and therefore closer related to the cow, the question is whether this property is found in the cow?

47) A comparative study of the milk from indigenous African cattle races show that some breeds are able to produce milk fat with a higher content of the medium to medium-long fatty acids (8:0 -14:0) and consequently less long chain fatty acids. It is to note that the nutrition of all these animals was the same, so that the nutritional effect is ruled out. These data would indicate that it might be possible to alter milk fatty acid composition of cow's milk by breeding or even genetic manipulation to produce custom made milk for the mentioned patients.

48) High amounts of medium chain fatty acids in milk fat also have an effect on the technological possibilities. Ordinary butter is known to be hard and difficult to spread, especially on cold winter mornings. That is due to the molecules with long chain fatty acids melting only at high temperature. At a pleasant environmental temperature of 22°C, butter contains at least 25% solid fat, while 75% is liquid, which renders the butter as hard. In the case of milk fat from the blue wildebeest, the solid fat content at the 22°C is only around 7%, which would make it easily spreadable.

49) The digestive problems of people with the metabolic disorder of the defective acylCoA dehydrogenase and the effect of fatty acid length on the hardness of butter, directed me to a topic for future research. It is known that the fatty acid length of milk fat is ascribed to the fatty acid synthase complex and thioesterase. Small structural changes in these enzymes in different species may be responsible for the different fatty acid compositions in milk. It is specifically the structure of these proteins from various mammalian species that should be studied.

50) Having mentioned proteins in connection with the saccharides and lipids, it is now appropriate that the last macronutrient should be described: the proteins.

<u>51) PROTEINS</u>

Of all the milk nutrients, the proteins are the least studied in species other than the human, cow and a few domestic animals. All milks consist of the caseins and whey proteins. Whey protein is a collective description for a wide variety of proteins and includes enzymes, immunoglobulins, as well as major proteins β-lactoglobulin and α-lactalbumin. They are the major proteins and have been studied in detail. These proteins may exist in different forms due to post-translational modification. This means that they are modified by the addition of phosphate, sugars and lipids, or they may be truncated by proteolysis. Multiple forms are also possible due to genetic differences amongst cattle breeds. The minor proteins mainly consist of enzymes. It is not clear whether they have a function in milk or whether they are only present because they have been carried over from the mammary cells.

52) By electrophoresis, proteins are separated in an electric current according to size and surface charge. With this technique differences in amino acid composition as well as structural differences may be detected to some extent. In this photograph it can be seen that the migration distances of the β-caseins of the different milks do not differ much, which might indicate that the proteins are very similar, not only when the ruminants are compared, but also non-ruminants. Comparison of the α-lactalbumins between species shows that greater differences may be expected. While the migration distances of this protein is very close between ruminant proteins, great differences are obvious for other species.
53) 2-D electrophoresis is a more powerful technique in which the electrophoretic migration is exerted under two different conditions. With this technique it is possible to study the extent of multiple protein forms and post translational modification of the proteins, while completely different proteins are easily identified.

54) Reported studies of milk proteomics are relatively limited, the area is growing at a rapid rate, with virtually all published studies in the area having appeared over the past few years. This is also the field in which the progress of my own research is the least, however, this is where the future research lies, with the aims of investigating the enzyme systems that are responsible for the synthesis of the saccharides and oligosaccharides, as well as the enzymes involved in the synthesis of the medium chain fatty acids.

55) The data of this research should explain the biochemical mechanism why milk of some mammals contains high amounts of ologisaccharides, as well as the biochemical reason of the synthesis of different fatty acid lengths in the mammary glands of some mammals.

56) In the distant future I foresee that the information that has been obtained from the comparative studies with non-dairy species could be implemented in a commercial milk producing animal. I specifically point to genetic manipulation or genetic modification of the cow. This should not be too difficult, as examples of

the target milk compositions are found in other closer related ruminants within the Bovidae family.

I think that you will now agree why I included a ? behind the word “well-known” in the title of my talk; there is still much to be explored about milk. Furthermore I hope to have convinced you that there is much to be learnt about the biodiversity of our planet.

57) Thank you for your attention.