

THE UNDERWOOD LECTURE FOR 2004

‘MODERN TECHNOLOGIES’: 75 YEARS OF PROGRESS, BUT WHAT ARE THE FUTURE NEEDS OF OUR INDUSTRY?

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In accepting the honour of presenting this eleventh Underwood Lecture I am conscious not only of the footsteps in which I follow, but also of the session in which the Lecture appears this year. ‘Modern Technologies’ (nowadays) inevitably mean encompassing the power of genomics, proteomics and metabolomics, and no one can dispute the advances that these innovations are starting to facilitate. Yet, animal science is still essentially an integrative discipline, and the need to translate the molecular knowledge generated from such technologies into physiology and husbandry is as important today as it has ever been. Furthermore, whilst we now seem to be in an age of specialisation, the need for multidisciplinary approaches has never been greater in terms of protecting our industry and taking it forward over the first part of the 21st Century. Later I would like to develop the theme of our future needs for integration and communication. To my mind, 1 of the essentials for future progress is an ability to ‘think outside the box’ and to communicate across disciplines, a theme which, of course, is totally in keeping with an address that commemorates the contribution made to animal science by Eric John Underwood.

It is 75 years ago this year since Eric Underwood graduated with an Honours degree in Agriculture from the University of Western Australia (hence the title of this lecture). Over this period, there has been a plethora of important advances in technologies, each of which, in their own way, has allowed us to advance our knowledge to what it is today. There is no doubt that our knowledge of nutrition and genetics now exceeds anything that the young Underwood would have learned in his degree course, yet for all this extra knowledge it has been the ability to translate basic understanding into practical solutions that has taken agriculture forward. This was perhaps 1 of the main strengths that Eric Underwood brought to the area of micronutrient nutrition, and it may be appropriate on this, the 75th anniversary of his graduation, to attempt to develop an historical perspective of how ‘modern technologies’ have been harnessed to develop scientific understanding in physiology and nutrition over the last three quarters of a century.

DEVELOPING TECHNOLOGIES: AN HISTORICAL PERSPECTIVE IN ANIMAL NUTRITION

Underwood’s early chemical and biochemical identification of the importance of cobalt in preventing ‘Denmark wasting disease’ in Western Australian sheep in the 1930s has been well documented (Moir 1984; Lee 1986), as have the challenges in terms of the limitations of the techniques of the day. A spectrophotometer was an expensive rarity, chromatography was in its infancy, and there were no atomic absorption spectrometers, no automated analytical procedures, and no radioisotopes in the early 1930s’ (McDonald 1988). Yet, using the emission spectrography methods of the day, Underwood and Filmer (1935) in Western Australia, and Marston (1935) in Adelaide, were able to identify the essentiality of cobalt in animal nutrition some 13 years before Smith in the UK and Rickes in the States discovered that cobalt was an integral part of vitamin B₁₂ (see Smith 1948). Underwood (1971) was later to point out that, although the net effect of the early spectrographic studies was a giant step forward, often the data were overextended because the methods lacked sensitivity. Despite these limitations, Eric Underwood quickly gained international status for his work in Western Australia, and by 1940, had written a seminal review in which he coined the term ‘trace elements’ for those elements, such as Cu, Mn, Zn, I and Co, that ‘function in quantities so minute that they may appropriately be described as traces’ (Underwood 1939).

The subsequent development of present-day understanding of the importance of trace elements in plant, animal and human nutrition can be attributed, at least in part, to improvements in analytical techniques and their general availability. In 1955, Walsh, an Australian physicist, developed atomic absorption spectroscopy. Although a single-element-at-a-time method, this greatly improved the

sensitivity and precision of detection and so opened up a much wider range of trace elements of nutritional importance. Later, neutron activation analysis and inductively coupled plasma systems provided the advantage of multi-element techniques. Another advance that was important to the elucidation of problems relating to trace element deficiencies was the ability to conduct animal experiments in ultra-clean environments, where the animals were given ultra-pure drinking water. Before the development of present day, mixed-bed, resin deionisation (Veillon and Vallee 1978), workers had to triple-distil drinking water for animals on trace element studies. The early attempts to create ultra-clean environments went to even greater lengths. Schroeder *et al.* (1963) carried out studies on the affects of chromium and cadmium on growth and survival of mice and rats in 'wooden animal quarters at the top of a remote hill 500 m high at the end of a mile long dirt track in order to avoid airborne contamination especially from motor vehicle exhausts'. So, the first prototype all-plastic controlled environment hood system for use with small experimental animals (Smith and Schwarz 1967) represented a major step forward.

By the late 1980s, a wide range of analytical approaches had been devised, including atomic spectroscopy techniques (atomic absorption, atomic emission and atomic fluorescence), nuclear-radioactivity techniques (neutron activation, X-ray fluorescence, substoichiometric extraction), mass spectrometry (isotope dilution) and spectrophotometry of metal complexes (see Wolf 1987), and the importance of trace elements in metalloproteins was becoming evident. As these technique developments improved sensitivity, so the understanding of the involvement of trace elements in mammalian metabolism increased. A typical example of this can be seen in the case of selenium. The consequences of selenium deficiency in terms of myodegenerative problems such as white muscle disease (severe) and impaired response to infections (mild) in farm animals has been well recognised for some time (see Villar *et al.* 2002). Similarly, in clinical medicine, epidemiological studies were showing strong negative correlations between selenium intake and the incidence of cardiovascular disease (Salonen *et al.* 1982) and cancer (Clarke 1985) as long ago as the 1980s. However, with the present power of genomics, it has been possible to identify that the importance of selenium is linked, at least in part, to the presence of 25 genes for different selenoproteins in the human genome. The importance of these selenoproteins in thyroid metabolism and in the regulation of cellular integrity through the antioxidant role of the glutathione peroxidases is now becoming clear and will be referred to later.

Eric Underwood's early career was focussed predominantly on the grazing ruminant and, indeed, the majority of the previous Underwood Lectures have addressed aspects of ruminant nutrition. Despite the lack of sophisticated analytical tools, animal scientists in the 1930s opened up other interesting areas that were later to benefit from technological developments. Rose (1938) in the States identified those amino acids that had to be supplied by diet, and the Barcroft School in Cambridge started to identify the role of the volatile fatty acid (VFA) in ruminant nutrition (e.g. Phillipson and McAnally 1942). With the subsequent development of partition chromatography for the quantitative separation of amino acids (Martin and Synge 1941) and gas/liquid partition chromatography for the separation of VFAs (James and Martin 1952), these subjects became easier to study. In ruminant nutrition, many of the earlier studies centred around rumen fermentation and the measurement of VFA molar proportions in the rumen, their metabolism during epithelial absorption (particularly the conversion of butyrate to β -hydroxy-butyrate) and even their uptake into the venous drainage. However, it was not until the 1960s, with the introduction of dilution kinetics, using radio-labelled VFA tracers, that accurate quantitative appreciation of the interconversion of VFA within the rumen and their rates of production in both housed (Leng and Leonard 1965) and grazing (Leng *et al.* 1968) animals became clearer. Other important technological advances in calorimetry (see Blaxter 1962) led to the elucidation of diet/energy expenditure interacts in forage-fed animals, and an appreciation that the types of VFA produced during rumen fermentation determine the efficiency of utilisation of a forage for growth (Armstrong and Blaxter 1957).

By the 1960s, several centres were developing surgical techniques combined with marker dilution procedures that allowed quantification of digesta flow at different points down the gastro-intestinal tract of ruminants (see Faichney 1975; MacRae 1975). Use of these procedures started to indicate how little glucose was absorbed by ruminants on forages, but more importantly, how the rumen fermentation influenced amino acid absorption from the small intestine, with major losses of nitrogen between the feed and the duodenum in grazing animals (MacRae and Ulyatt 1974). When the

technique was combined with whole-body tracer kinetic procedures, scientists started to get a much better appreciation of the complexity of intermediary and protein metabolism in ruminants. Rates of whole body protein synthesis were found to be 3-4 times higher than rates of amino acid absorption (and many times higher than the rate of protein deposition, as measured by comparative slaughter or N balance). Rates of protein synthesis in individual tissues were shown to vary widely with, for example, the rates in the gastro-intestinal tract and liver being greater than those that comprise skeletal muscle. The tracer approach also indicated the degree of inter-conversion of metabolic carbon between glucose and certain amino acids (not just in terms of gluconeogenesis, but also in terms of glucose carbon use in amino acid synthesis (e.g. Black *et al.* 1955), and started to elucidate those factors that influence nutrient partition and metabolic efficiency in productive animals.

Metabolism is a complex and integrative phenomenon, and over the last 30-40 years, our understanding of nutrient use and metabolic efficiency has been greatly aided by the development of computer-based modelling techniques (see e.g. France *et al.* 1988; Hanigan and Baldwin 1994) and the ability to think across the disciplines of physiology, endocrinology, immunology, genetics and nutrition. Nowadays, as we enter challenging times for the animal industry, we perhaps need to think more widely still, a consideration I will return to later.

DEVELOPMENT OF THE ‘MODERN TECHNOLOGIES’

Nowadays, the drive is to embrace the so-called ‘omics revolution’ in an attempt to understand how genes and gene-products are influencing cellular function and, hence, in animal agriculture terms, productive performance. Over recent years the advances in these areas have been truly staggering and have taken molecular genetics to a new level.

Genomics

Our present day understanding of cellular and molecular biology began with the discovery of the citric acid cycle (Krebs and Johnson 1937) and the double helix (Watson and Crick 1952). In less than a decade after the elucidation of the structure of DNA, Gurdon *et al.* (1958) had cloned frogs from differentiated cells, demonstrating that all the information required to encode for an entire animal was present in the nucleus of a cell. The DNA technology began to accelerate following the isolation of DNA ligase, a key enzyme for cloning technology in 1967. This was closely followed by the isolation of the first restriction enzyme in 1970 and then the creation of the first recombinant DNA molecules by Morrow *et al.* (1972). From that point on, there was an explosion of activity spurred on by the isolation of more restriction enzymes, and the development of bacterial- and virus-based vectors for cloning larger and larger pieces of DNA from a variety of sources. Another significant landmark was the development of hybridisation techniques to identify specific sequences in a complex array of genomic sequence (Southern 1975). This was the basis for the current microarray technology that allows gene expression patterns to be assessed for a whole transcriptome of a cell or tissue. However, the development of polymerase chain reaction (PCR) technology in 1983 was a critical advance, since it enabled the amplification of gene sequences from a single copy of a gene to microgram quantities of sequence. Using homology-based approaches, PCR technology made possible the discovery of new genes previously unidentified due to expression levels and insufficient similarity between sequences for standard cloning techniques of the time.

Most of the initial drive for these advances came from the pharmaceutical industry drug development programs. However, certain sectors of the agricultural industry, particularly the areas of animal genetics, plant science and microbiology, have found much benefit from embracing the techniques to identify which genes are being activated in order to achieve the biological response under consideration.

Proteomics

The fact that a gene is transcribed into mRNA does not necessarily lead to the translation of that gene product into a biologically active protein. Yet it is the protein that is essential in the cellular function. Proteomics has paralleled the development of genomics, providing a snapshot of cellular protein exposure. Modern day proteomics can be traced back to the development of 2-dimensional (2-D) electrophoresis using pH gradients to separate proteins by isoelectrofocussing (IEF) (O’Farrell 1975). The reproducibility of this technique improved considerable with the advent of more stable film-back polyacrylamide-based pH gradients (Gorg *et al.* 1988). Today, in addition to increased stability, the

strips are also made commercially with very narrow pH gradients where 17-24 cm strip lengths can be cast allowing increased protein loading and the visualisation of low copy number proteins without loss of resolving power. The increased reproducibility of gels and the development of sophisticated image analysis software packages has permitted meaningful and statistically significant comparisons of the quantities of individual proteins in 2D gels run under identical conditions, but with samples from control and experimental sources. For example, protein expression from normal and diseased tissue can be studied to highlight differences in what is now called the proteome, i.e. the global expression of all proteins present in a cell or tissue at a moment in time.

The ability to identify individual proteins has also been revolutionised with the advent of mass spectrometry (MS), allied to the accumulation of databases through the genome projects. Using Matrix Assisted Laser Desorption Ionisation time of flight (MALDI-tof) MS, a peptide mass fingerprint can be prepared of all the proteins separated on a gel. This can then be compared with known or theoretical matches in a range of public domain databases. Alternatively, secondary fragmentation can be used to generate sequence ladders (MALDI tof/tof) for unknown proteins. Electrospray mass spectrometry (ES) is now also being applied to provide full sequence analysis of tryptic-derived peptides and further information on post-translational modifications that are becoming recognised as being vitally important in a wide range of control pathways.

Needless to say, the information that can be derived from this battery of sophisticated analytical technologies is only as good as the experiments to which they relate. The equipment is extremely expensive (the Rowett has invested almost £2(A\$5) million in its genomics and proteomics equipment since 2000, and this is run by 6 full-time, highly skilled, technical assistants). Even the cost of the consumables needed to run the sequencing, microarray and proteomics laboratories, plus the bioinformatics infrastructure needed to analyse the outcomes, can be a major drain on resources unless experiments are well planned in order to maximise the chances of achieving meaningful data. In this respect, in biomedical science, the mouse has become the experimental model of choice because of the knowledge already available on its genome, and the ability to development transgenic lines. Two such lines currently in use at the Rowett are the metallothioneine-null mouse, which has been depleted of the gene that transcribes the carrier proteins for zinc, and so can be used to help elucidate how this micronutrient interacts with the immune system (e.g. the inflammatory responses generated in endothelial cells by oxidised low density lipoproteins during the early stages of atherosclerosis; see Beattie and Kwun 2004), and the apolipoprotein E*3-Leiden mouse which has a human-like lipoprotein profile and an impaired ability to clear remnant lipoproteins, and so is a sensitive model to study the early stages of diet-induced hyperlipidaemia and atherosclerosis (De Roos *et al.* 2003).

THE FUTURE NEEDS OF OUR INDUSTRY

Whilst there is little doubt that the 'omics' technologies are already providing information that was not available even 10-15 years ago, there is growing evidence that, for the translation of this knowledge into practical situations (be that agricultural or bio-medical science), another skills set is required (termed post-genomics) to examine the responses in metabolic physiology that result from these changes in gene/protein expression. It would be my contention that these skills are precisely those that were identified in the earlier historical developments that took the area of animal nutrition forward, particularly from the 1960s to the present day. In this respect, it could be argued that the animal industry is well placed to capitalise on present day technologies, but with one proviso; that we have a vision of where we want the industry to be in the next 10-15 years.

It would be inappropriate for a speaker from the other side of the world to try to set out a view of how Australian animal production should go forward over the next 10-20 years. Instead, therefore, I want to focus the rest of this talk on trying to illustrate my own views on how the ruminant sector of UK agriculture should proceed, hoping that there are at least some parallels that can be drawn with the situation here in Australia. In the UK, the present drive is to take ruminant production down a sustainability route, with more dependence on home-grown forages, and a growing awareness of the need to reduce the environmental burden of the production systems. Three issues emerge as major challenges:

1. The need to improve the productive efficiency of forage-fed animals,
2. The need to develop biological ways of controlling endemic infections associated with grazing, and

3. The need to identify ways of sustaining/enhancing the markets for ruminant products. None of these issues are new, but this should not stop scientists thinking ‘outside the box’ in terms of potential solutions. One essential in such an approach is cross-disciplinary communication, and each of these challenges can benefit from the combined expertise that this can generate.

1. *Improving the productive efficiency of ruminants*

Most consumers nowadays are looking to purchase high protein/low fat ruminant products. Yet, it is well recognised that the efficiency with which the animals produce these proteins from forages is notoriously poor (15-25% conversion of dietary protein into animal product in growing animals; 25-30% efficiency into milk in high-yielding dairy cows). One major contributor to this inefficiency is the capture of microbial protein from degraded forage protein. In the eastern parts of the UK, forage maize has been used to advantage, particularly for dairy cows, but in the wetter, western regions, livestock farmers are more dependent upon grazing and conserved grasses and clovers as the basis for ruminant production. Furthermore, on these farms, sustainability practices have encouraged the use of only minimal (strategic) amounts of concentrate feedstuffs. Therefore, the emerging challenge for ruminant scientists is to consider whether there are ways in which the basic forage can be altered in order to increase the efficiency of microbial capture of forage proteins in the rumen.

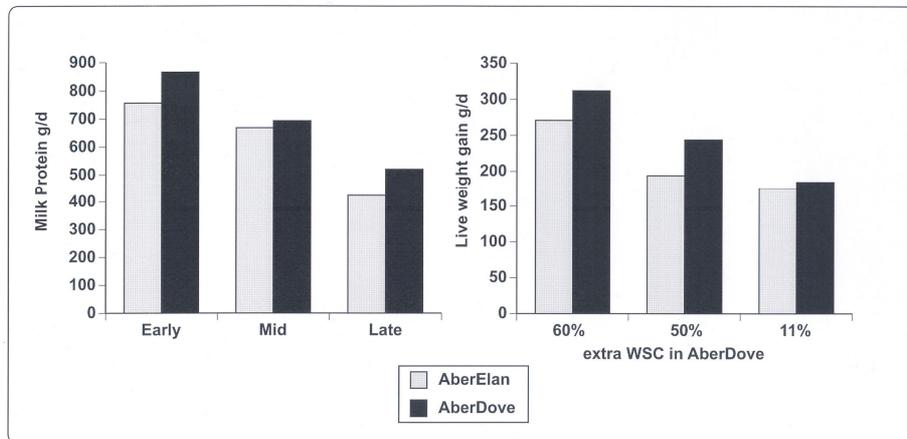


Figure 1. Responses in milk protein output of zero-grazed dairy cows and liveweight gain (lwg) of grazing lambs on higher-water-soluble carbohydrate AberDove perennial ryegrass.

This is where communication between animal and plant scientists has become so important. Forage physiologists and breeders have been quick to embrace the genomics technologies. The QTLs for important traits and marker-assisted selection of genes coding for these attributes are integral parts of most of their breeding programs. However, until fairly recently, their breeding objectives were being directed predominantly at agronomically important traits such as yield per hectare, pest and disease resistance, and extending the growing season. Attributes that might improve the utilisation of the forages by ruminants were not considered because these were not important criteria in terms of seed certification. An example of the progress that can be made by cross-disciplinary communication can be seen in the responses in performance of dairy cows and grazing lambs obtained since the animal and plant scientists at IGER, Aberystwyth, have come together to consider the importance of increasing the water soluble carbohydrates (WSC) content of forages. Figure 1 shows data from zero-grazed dairy cows (Miller *et al.* 2001; Moorby *et al.* 2001) and grazing lambs (Lee *et al.* 2001) that demonstrate substantial increases in production from animals fed the higher-WSC AberDove variety. The increased milk output in the dairy cows was partly due to a repartition of metabolic nitrogen towards milk protein and away from urinary N excretion, but partly because intake increased by up to 25% on the high-WSC forage. Interestingly, in the lamb grazing trial, the extra performance from the AberDove forage was only achieved in periods 1 and 2 of the trial where the differential in WSC was 50-60%. In period 3, where the differential had fallen to 11%, performance was similar for both forages.

The rationale behind selecting for increased WSC content of forages lies in the concept that, during rumen fermentation, the rate at which the microbes degrade the (soluble) forage protein is much

greater than the rate at which energy (ATP) is released from the breakdown of the structural carbohydrates. As a result, substantial amounts of ammonia can be absorbed from the rumen before the microbes can use it in the synthesis of their own protein. The readily available energy released from the WSC can help capture more ammonia into microbial protein, and so increase the efficiency of transfer of dietary protein into digesta protein reaching the small intestine for host-animal digestion and absorption. An alternative strategy for improving microbial efficiency in animals given forages would be to protect proteins in order to reduce the rate at which they become degraded to ammonia. The benefits of this approach have been easy to demonstrate experimentally, but the translation of the principles into commercial practice has so far proved to be more intractable. Thus, the freezing of forage (which reduces plant protein solubility by as much as 50% (MacRae *et al.* 1975)) leads to increased synthesis of microbial protein (25%) and increased absorption of amino acids from the small intestine (15%) (see MacRae 1976). Similar increases in duodenal nitrogen flows per unit nitrogen intake can be seen in animals fed forages that contain different levels of tannin that cross-links with the forage protein, making this less rapidly released during microbial fermentation (Barry and McNabb 1999). This protection can be reduced if polyethylene glycol (PEG) is infused into the rumen, where the PEG competitively binds to the tannin, thus making it less effective in terms of protecting the forage protein. Unfortunately, to date, where breeders have attempted to manipulate the plant genome of the main forages, to introduce polyphenolics into commercial varieties, their efforts have proved to be disappointing in terms of sward hardiness, and so the application of this approach has been restricted to the strategic use of alternative crops, such as lotus spp. and sainfoin that naturally contain tannins (see Barry *et al.* 2001). Currently, there is interest in the possibility that polyphenol oxidase (PPO) in red clover may be acting as a natural protein protector in plants. If so, this could be contributing to the increased performance of animals fed grass/red clover forages. An important strategic use of this protein protection approach in grazing systems that may warrant further consideration is in the biological control of parasitism.

2. Developing biological ways of controlling endemic infections associated with grazing

The risks of developing antibiotic resistance in the area of clinical medicine, through the over-use of antibiotics for performance enhancement, as well as prophylaxis in the livestock industry has been well documented. Yet, there is no doubt that 1 of the major challenges in any grazing system is the control of animal infections. Anthelmintics have been routinely used in most grazing systems to control gut helminths, but there is growing evidence of resistance in some gut parasites to the present generations of anthelmintics used for pasture management (Bartley *et al.* 2001).

Any incidence of sub-clinical infection can greatly influence the productive efficiency of an animal, because both the primary infection and the immune response raised against this infection take preferential use of available nutrients that would otherwise go to productive performance. In this respect, it is important that the young grazing animal has a ready supply of available (metabolic) protein to help off-set the severity of any parasite challenge, and to drive the immune response that it will raise to the infection. Older grazing animals have usually already developed immunity to parasitic challenges. However, during the peri-parturient period of the reproduction cycle (latter stage of pregnancy and early stage of lactation), this immune competence is relaxed and the mother sheds parasite eggs in her faeces. Unfortunately, these eggs can later develop into 3rd stage larvae that migrate up the forage leaves and are, potentially, consumed by the young (naive) offspring, thus perpetuating the infection into the next generation. Scientists in Edinburgh have recently shown that the protein status of the mother during the peri-parturient period can have a major influence on the number of eggs shed in the faeces (Houdijk *et al.* 2001). The protein nutrition of the young can also influence the severity of the worm burden subsequently incurred (Sykes and Coop 2001). These findings would indicate a substantial benefit to supplementing the mothers and off-spring with extra protein, but such a strategy would perhaps not sit comfortably within environmental guidelines for the reduction of nitrogen inputs into grassland management. However, if the protein status of the mother and offspring could be boosted in other ways, (e.g. by improving N-use efficiency in the rumen, thereby altering protein partition) then this might make a further contribution to the concept of sustainability. The presence of tannin has been reported as helping in this respect (Athanasiadou *et al.* 2000) and the strategic use of tannin- or PPO- containing alternative forages may be worthy of future investigation. Equally, if the high WSC-containing grasses can boost the metabolic protein supply, they too might make a meaningful contribution in this area of sustainability.

3. Identifying ways of sustaining/enhancing the markets for ruminant products

One of the overriding requirements of any agricultural enterprise is the economics of the system and the security of markets for the products that leave the farm gate. In this respect, nowadays, the consumer's perception of the quality and health-enhancing benefits of the products may be crucial to longer-term sustainability. Over recent years, in the UK and Europe, the food chain, and particularly its animal produce suppliers, have been frequently challenged by clinicians and public health authorities with health scares, such as salmonella in eggs, *E. coli* in meats, BSE and FMD. These have, in some cases, had dramatic affects on consumer choice, leading to major economic losses in the sectors supplying the produce. Since 1999, the Rowett Institute, which has a long history of nutrition research on farm animals, has re-focused its research into the area of diet and (human) health, with programs that are attempting to elucidate how specific nutrients can help to off-set the incidence of human non-communicable diseases, such as coronary heart disease (CHD), colon cancer and obesity. It would seem appropriate that animal industries around the world, perhaps through the auspices of their national societies of animal science, should be reviewing the information that is beginning to emerge from these biomedical programs in order to help develop specific health benefit messages that can promote animal products in terms of their ability to help prevent some of the non-communicable diseases.

There is a vast literature linking, either through epidemiology, or within more mechanistic studies, the inappropriate consumption of specific nutrients with the incidence of CHD (Krauss *et al.* 1996; Hu *et al.* 2001) and cancer (Wynder *et al.* 1997; Department of Health 1998). Consideration of some of this may provide a basis for future strategies within the animal sector of the feed industry.

Omega-3 polyunsaturated fats. From the human health perspective, there has been a long history of concern about the lipid components of foodstuffs, with animal (especially ruminant saturated) fats being viewed with particular suspicion. Starting back in the 1960/70s, epidemiologists identified strong relationships between the proportion of the dietary calories that were consumed as saturated fat and the incidence of CHD and colon, prostate and breast cancer. The CHD story related to the influence of saturated fat on the development of high levels of cholesterol in the circulating serum lipoproteins, with polyunsaturated fats (PUFA) seemingly modulating these rises in risk factor (Hegsted *et al.* 1965). This led to recommendations for a reduction of saturated fat in the diet. Consumers were not slow to take up this message, as witnessed by a major reduction in full-fat liquid milk sales in the UK between 1984 (90% of total) and 1997 (<25% of total).

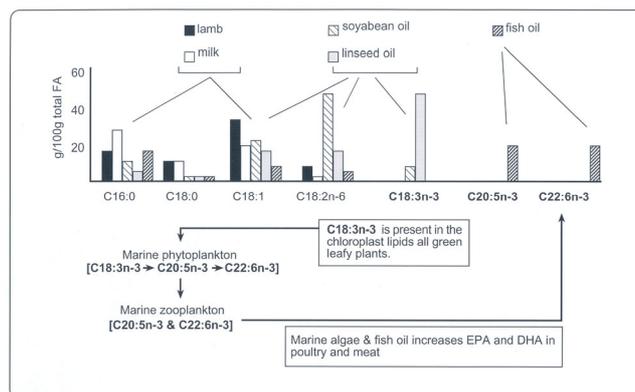


Figure 2. Relative concentrations of the longer-chain fatty acids in meat, milk, plant oils and fish oils, and the basis of the high n-3 fatty acids (FA) content of fish oils.

However, over the last 15 years, research has started to show that the different PUFA are not all equally beneficial in terms of preventing the onset of the non-communicable disease. Inflammatory responses are important components in the development of CHD and cancer. In the early 1990s, immunologists identified that the omega-6 (n-6) PUFA (e.g. linoleic acid, C_{18:2}) are less beneficial than the omega-3 (n-3) PUFA (e.g. linolenic acid, C_{18:3}; eicosapentaenoic acid, C_{20:5} (EPA); docosahexanoic acid, C_{22:6} (DHA)) in terms of reducing this inflammatory response. Pro-inflammatory eicosanoids are generated during the post-absorptive human metabolism of the n-6 fatty acids (FA). The n-3 FA seem to have the ability to modulate this inflammation, by competing with the

n-6 metabolites for incorporation into the membrane phospholipids of immune cells (Gibney and Hunter 1992). Present recommendations are to increase the intake of n-3 FA towards a dietary optimum n-3:n-6 FA ratio of 0.4-0.5. However, most human foodstuffs have a ratio nearer to 0.1-0.2, hence, the health benefits of fish oil products (Leaf *et al.* 2003), that contain high levels of n-3 FA (Figure 2).

The reason why fish oils are an abundant source of the longer-chain n-3 FA relates to the high linolenic content of chloroplast lipids. The presence of chloroplasts in marine phytoplankton is the basic building block of the Marine Food Web. They are also the crucial component of fresh forages and so, not surprisingly, recent research has started to identify substantial increases in the n-3:n-6 PUFA ratio in the intramuscular lipids of animals fed fresh forages rather than concentrates (Figure 3; Dewhurst *et al.* 2003). Advocates of the current drive for sustainability may see this as an important longer-term positive (and specific) health message to help promote the sales of pasture-reared beef and sheep, and the milk from forage-fed dairy cows.

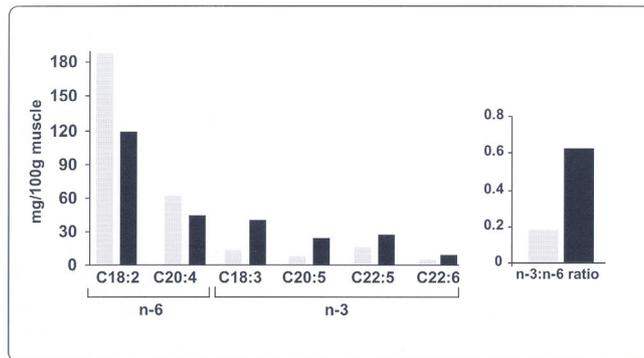


Figure 3. The n-3 and n-6 polyunsaturated fatty acids content of intramuscular lipid from lambs fed either a concentrate or a pasture diet.

Conjugated linoleic acids. Over the last 5 years, a plethora of laboratory animal studies has reported apparent health benefits from the consumption of conjugated linoleic acids (CLA). These have included reducing the severity of cholesterol-induced aortic lesions in rabbits (Lee *et al.* 1994), reducing the incidence of carcinogen-induced mammary tumours in mice (Ip *et al.* 2001) and even altering the composition of body weight gain (higher protein/lower fat) in mice (Park *et al.* 1997). Recent studies at the Rowett would suggest that, at least in the CHD area, 1 of the main mechanistic attributes of the CLA is their ability to modulate the inflammatory mechanisms at the level of adhesion molecule transcript in endothelial cells. The expression of these adhesion molecules, that lead on to plaque formation, is thought to be stimulated by cytokines generated from the oxidation of low-density lipoproteins (LDL) in the tissue that underlies the endothelial cells. The CLA seem to modulate these cytokine signals, thereby reducing the inflammatory response (Cook *et al.* 1999).

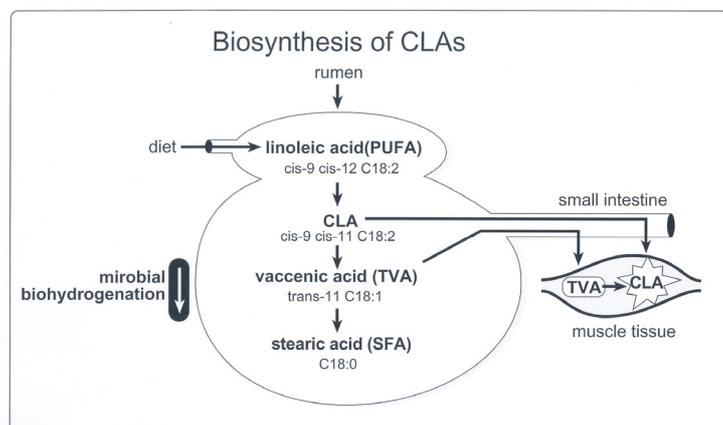


Figure 4. Biosynthesis of conjugated linoleic acids (CLA) in ruminants during microbial hydrogenation of polyunsaturated fatty acids (PUFA).

The CLA are very much an attribute of ruminant products, being formed as an intermediary metabolite in the biohydrogenation of C_{18:2} (unsaturated) linoleic acid to C_{18:0} (saturated) stearic acid during rumen fermentation (Figure 4). Hence, levels of CLA in milk, cheese, butter, lamb and beef (4-7 mg/g total FA) are considerably higher than in non-ruminant products (chicken, pork, fish, olives; <1 mg/g). Unfortunately, these levels could never provide sufficient CLA intake per day to make any meaningful contribution in terms of off-setting inflammation in humans. At present, a person would need to consume over 3.5 kg of cheese per day to take in a meaningful therapeutic dose of CLA. Currently, attention is being focussed on the regulation of biohydrogenation in rumen microbes, either by dietary manipulations (Loch and Bauman 2003), or by examination of those microbes that regulate the latter steps in this process, and whether these could be manipulated to boost the CLA content of muscle and milk products (J. Wallace, *pers. comm.*). If this could be achieved, then it may represent another specific health benefit with which to make ruminant products more attractive to the consumer.

Selenium. One of the underlying mechanisms associated with the onset of CHD and cancer is disruption of the normal cellular processes. In this respect, lipid oxidation is a major stressor, because the cytotoxic hydroperoxides formed can cause membrane damage. Intra-cellular free radical generation, accelerated in exercise, infection and even the stress of high performance, is another stressor. To modulate these processes, cells depend on antioxidants such as vitamin E and a number of glutathione peroxidases (GPX). The GPX are selenoproteins and, to date, 25 have been identified in the human genome. It is not surprising, therefore, that early epidemiology studies indicated clear links between the availability of selenium (Se) in the human diet (Se levels in blood) and the incidence of CHD (Salonen *et al.* 1982) and cancer (Clark 1985). Some of the selenoproteins are important also in thyroid metabolism and in the redox control of cells, and so inadequate selenium intake has been linked with impaired thyroid metabolism, reduced response to viral infection, infertility and, in more serious situations, cardio- and skeletal-myopathies.

One concern for clinicians in the UK and other parts of Europe over the last 10-15 years has been the substantial reduction in daily Se intake that has occurred as a result of the switch from selenium-rich high-protein North American wheat to lower-Se UK and European wheat for flour making in the mid 1980s. The volcanic and sandy soils across major sectors of the UK have had a lot of their Se washed out, leading to low levels of Se in cereals, fruit and vegetables, and as a result, the daily intake of Se in the UK population (approx. 35 µg/d) is less than half of that in the USA (80-100 µg/d). The question of whether the UK should follow the example of Finland and New Zealand and add sodium selenate to fertilisers, or Se to bread flour, was raised in the late 1990s (Rayman 1997), but as yet nothing has been done.

Selenium deficiency in farm animals has been well recognised for many years. In cattle, severe deficiency will result in myodegenerative problems, such as white muscle disease, whilst marginal deficiencies have been linked to elevated levels of mastitis, scours, cystic ovaries and retained placenta (Villar *et al.* 2002). As a result, Se has been included in most mineral supplements for farm livestock since 1978. Therefore, the Se content of animal products (e.g. meat and poultry are 100 and 160 µg/kg, respectively; FSA 2002), and particularly liver and kidney (>400 µg/kg), are considerably higher than in the plant components of the UK diet (fruit and vegetables <10 µg/kg; cereals 20-25 and bread made from UK and European wheats, 50-55 µg/kg; see FSA 2002). It has been estimated that animal products presently provide over 60% (18 µg per day) of this intake. The present reluctance of governments to implement supplementation policies lies in the potential toxicity problems associated with over-consumption of selenium (>900 µg per day). However, there seems no danger of reaching these dangerous levels by advocating the promotion of health-enhancing animal products, (alongside the odd Brazil nut!) to help replete the marginal selenium intakes that have developed over the last 20 years.

Value-added aspects of animal proteins. The year before Eric Underwood graduated from Western Australia, John Boyd Orr, the first director of the Rowett Institute, was demonstrating how milk caseins can deliver calcium and phosphorus to help prevent rickets and improve growth in Scottish children (Orr 1928). That research was the basis on which, subsequently, he persuaded the UK government to provide free school milk for all primary school children in the UK from the 1930s until Mrs Thatcher (the UK's Prime Minister of the time) phased it out in the 1980s. Interestingly, some

Scottish local authorities are now beginning to reintroduce it into their primary schools. In just the same way that milk protein acts as a delivery system for calcium and phosphorus, meat protein can help deliver a number of trace nutrients, including iron. Considerable clinical attention has been focussed recently on the problems associated with low iron intake in pregnancy, where as many as 1 in 5 women are clinically iron deficient, and many more suffer from marginal iron deficiency. If this anaemia is not corrected, there is an increased risk of poor pregnancy outcome, premature delivery and/or low birth weight. In addition, the child has a higher risk of developing cardiovascular disease or non-insulin dependent diabetes later in life. Consequently, pregnant women with anaemia are always prescribed iron supplements. However, these can cause unpleasant side effects (e.g. gastric upset, nausea and constipation) and so compliance rates are low.

The basic problem with inorganic iron supplements is 1 of bioavailability. Less than 10% of ingested inorganic iron, or indeed, the non-haem iron of most plant foodstuffs, is absorbed from the gastrointestinal tract. In comparison, haem iron, (i.e. the iron bound up in the porphyrin ring structures of haemoglobin and myoglobin (Figure 5), present in meat and fish, has a much higher bioavailability (20-30%; Figure 5; Roughead and Hunt 2000). Given the clinical problems referred to above, and the experimental information from rodent models, linking iron deficiency in pregnancy and the subsequent development of hypertension problems in the growing off-spring (Gambling *et al.* 2003), there would appear to be considerable potential for promoting meat as a means of alleviating anaemia, not just during pregnancy, but through other periods of high iron requirement such as adolescence and menstruation. This could be helped by the fact that the iron-carrying capacity of different qualities of meat seems to be linked to eating quality as perceived by taste panel assessment, a relationship built on the fact that differentials in meat tenderness can be correlated with differences in the area and frequency of the slow twitch oxidative fibre in the muscle (Maltin *et al.* 2001) with higher myoglobin content.

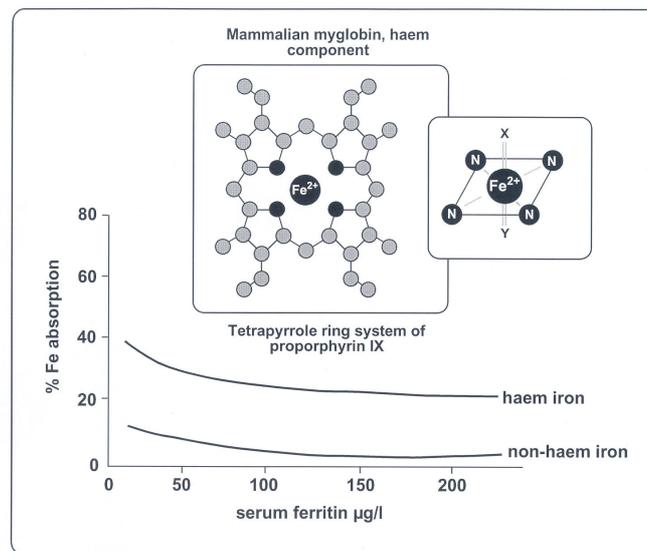


Figure 5. Bioavailability of haem and non-haem iron.

Meat is also a major dietary source of other micronutrients such as zinc, selenium and copper, and the vitamins, folic acid and B₁₂, indeed B₁₂ is only found in animal and fish products. Each of these nutrients has been linked to the prevention of non-communicable diseases and to the maintenance of a healthy immune system, and so it is perhaps time for the animal industry to start to consider the products that it delivers to market, not just in terms of consumer (organoleptic) preference, but also in terms of specific health benefits. Promotion of such messages may need a refocus of research effort, however. Over the last 5 years, there have been more than 1000 research papers from studies that focussed on the tenderness, texture, juiciness and flavour of meat products. Perhaps, as we go through the next 5 years, more thought should be given to the nutritive value of the products placed on the market. Indeed, increasingly we could be faced with the realisation that healthy products are the essence of an (economically) healthy industry.

CONCLUSIONS

The new technologies of today are starting to open up new horizons in terms of molecular mechanisms and the interactions between nutrients, the environment and the genetics of plants and animals (including humans). If animal agriculture is to survive and thrive within the present challenging economic climate, it will need a strengthening of our abilities to 'think outside the box' and communicate (and collaborate) across disciplines. This paper has attempted to illustrate just 2 such interactions that would seem to offer longer-term objectives for UK animal science. Whilst the details of these may not have total relevance to the Australian scene, the need to improve nutrient use efficiency in ruminants, through working with forage breeders and physiologists, and the need to draw on biomedical information that might be used by the animal industries in the promotion of specific health messages to support niche markets, are surely universal. Eric Underwood's contribution to the basic understanding of trace element nutrition may never be surpassed. Certainly, his ability to translate basic science into practical solutions to agriculturally important problems was an inspiration to generations of younger scientists, not just in Australia, but world-wide. I would suggest that if he were still with us today, he would be at the forefront of advocating these approaches to the solution of our present-day problems.

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