Forty-five years in the rumen; aroma, discovery, and fun

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Abstract

I was the youngest student in our B. Agr. Sc. Class of 1967 at Lincoln College, and only went to university because my paraplegic father had come to realize the value of an education. I preferred physical work to lectures and often avoided the latter, but worried that my time spent welding and building was not relevant to science. How absolutely wrong; I can talk to engineers about science, and make things. Only at the age of 27, I realized what I wanted to do: understand biology from a molecular standpoint; energetics, ATP, enthalpy, molecules, biochemistry, and the like. A very good scholarship enabled study toward a PhD (modelling analyses on bovine mammary and liver metabolism) with Lee Baldwin (University of California, Davis).

My first paid research was a radiology study of rumen motility and digesta mixing in sheep, as well as bloat research (a physiologist’s graveyard). Following my return to Department of Scientific and Industrial Research (DSIR), Palmerston North from California, I became involved in a series of digestion studies, and serendipitously moved into condensed tannin research, which I still do ‘voluntarily’. More recently there was the Feed Conversion Efficiency program at DairyNZ with LIC, and genetic markers have been developed to identify dairy cows requiring less (or more) feed for milk production. There have been many other fun aspects to my work: surgery (sheep and cows), stomach tubing to sample rumen digesta, determining the relationship between nutrition and ovulation in sheep, methane measurements, forage endophytes, parasitology, configuration of feed troughs and rail spacing for live sheep export, diets for breeding kakapo, inflating cows and some fairly intensive sheep and cow feeding trials where ‘everything’ is measured. My successes have depended on great support, but especially students that I have tried to advise, avoidance of things I don’t enjoy (‘management’), sharing ideas, and an overriding philosophy that research must be fun, and boundaries must be challenged.

Introduction

I grew up in an era of full employment in a fairly socialist system, where governments provided money (in reality, an annual donation) for good science, guided by senior staff, with few restrictions and no managerial milestones. There was a shortage of scientists, and many were imported from the United Kingdom. The University paid us to attend and this arrangement led to a fairly good time. In research, there was freedom to try things, providing your boss agreed – and this contributed to innovation. I was employed at the Applied Biochemistry Division of DSIR as an assistant to Cam Reid, and later reported to Marc Ulyatt; both were leaders in digestive physiology research. I remember being interviewed one day, and offered a job the next; these people could make decisions, and I liked that! Neither the university, nor research environments were perfect, but the freedom to test ideas contributed to success, until government questioned the wisdom of their funding model.

When funders require justification for research, based on the ‘return on investment’, some fields do well. For example, scientists researching disease, product development (animal products or forage germplasm), fertility or biosecurity etc. easily justify their existence, but not in ruminant digestive physiology. That is why we are few in number, and this is a field best avoided if you have a mortgage! Funding uncertainty has also contributed to my diverse career; when you have no funds – you pick up what you can, and in my case this led to a lot of interaction with plant breeders, and later, microbiologists and atmospheric researchers.

My life in science

I have listed (Table 1) some areas of my research that have benefited either humans, animals, or the planet. Some projects are ‘complete’, others ongoing, and we have a lot more learning to do. Some passions are not included in this list, for example my frustration at the continued application of excess nitrogen, especially on dairy pastures to make grass grow when it doesn’t want to; bad for the animal, the pasture, and our world. I am concerned about excess potassium application, but taking water from aquifers with so little consideration of consequences is offensive. Perhaps worst of all, animals need and deserve shade; we remove it for a whole range of reasons, none of which are justifiable. I am less concerned about temperature than radiant energy, and the pain associated with burning. Don’t just think about these issues – do something, or our customers or animal welfare organisations will!

This summary provides an overview of some research that has ‘made a difference’, or I hope ‘will make a difference’, and some brief results. All has been collaborative, but details of some have been left out, especially my involvement with ruminant microbiologists at AgResearch. This is simply because my enthusiasm greatly exceeds my understanding, and my explanations will not do my colleagues justice. However, I believe microbiology will be the next step in improving animal health and productivity - once animals have shade, and nitrogen intakes of dairy cows are reduced.

Condensed tannins

Tannins are polyphenolic compounds that bind to protein, and are used to tan skins to create leather. They
account for up to 8% of dry matter (DM) in commonly grazed forages, and much more in some browse. The early impetus for research concerning condensed tannins (CT) was driven by a serious disorder of cattle; bloat. Bloat killed over 1% of cattle each year during the 1960s, 70s and into the 80s, but forages containing CT in their foliage (birdsfoot trefoil (lotus corniculatus), broad-leaved dock (Rumex obutisfolius), sainfoin (Onobrychis vicifolia), sulla (Hedysarum coronarium)) prevented deaths, whereas clovers (Trifolium spp.) and lucerne promoted bloat, especially in cattle. Tom Barry (Massey University) had been investigating the effect of CT on digestion in sheep fed Lotus pedunculatus, and had shown negative impacts on apparent digestion of crude protein (Barry 2011) and other constituents. I took advantage of some high- and low-CT Lotus corniculatus at DSIR in Palmerston North and measured similar parameters, but included absorption of amino acids from sheep fed the two feeds. The CT was associated with a higher outflow of nitrogen from the rumen (because less was degraded) and more essential amino acids were absorbed (Waghorn et al. 1987).

This discovery set in process a large number of research projects, involving students and colleagues, and in fact became too popular. I knew that most CT were anti-nutritional, and the benefits associated with L. corniculatus were atypical and required diets dominated by this forage, which was less productive than most legumes and grasses and difficult to maintain as a pure sward. Nevertheless, benefits of CT in L corniculatus were evident in sheep, and resulted in improved daily gains, higher lambing percentage, higher wool production and less dags, as well as lower methane emission from cattle (relative to ryegrass; Woodward et al. 2004) and more milk from sheep and cattle (Waghorn 2008). It was important to separate the ‘CT’ responses from ‘legume effects’ in some trials, but better was to come.

A bit of banter about whether or not dock was a weed with Ian Popay (a weed scientist at AgResearch), resulted in a single-page experimental proposal in which I only sprayed the dock out of half the lucerne paddock. The ensuing cow trial confirmed the views of some farmers that dock prevented bloat, my view that it was a plant rather than a weed, and most important - that the CT in dock bound with the protein in lucerne (Waghorn & Jones 1989).

Then a comment by Yeap Foo, a tannin chemist at Industrial Research in Lower Hutt, about raspberry canes and soil nematodes led to some real discovery. Working with John Niezen, Abdul Molan (AgResearch) and Tony Charleston (Massey University) we spent several years demonstrating the capability of CT to prevent nematode egg hatching and larval development; the CT in sulla, but not L pedunculatus, also killed adult nematodes in sheep (Niezen et al. 1995; 1998). Jessie Chan undertook a project on CT extracted from pine bark, and this did nothing to the nematodes, but I knew that Sericea Lespedeza (Lespedeza cuneata) contained tannins and killed nematodes. Were the types of CT different? Yes; virtually opposites, but details might not be in the public domain. There is money to be made controlling intestinal nematodes.

Other work had convinced me that the concentration of CT was probably less important than its structure, clearly demonstrated by differences in the effectiveness of CT from different plants for reducing egg hatching and larval motility of nematodes, and reducing adult worm numbers in sheep. Similar conclusions were reached in relation to digestibility when lotuses were fed with pasture to sheep. A meeting with Irene, a tannin chemist, in a pub in Reading (UK), about 25 years ago opened the doors to understanding how the differences in structure affect CT efficacy for reducing methane (Huyen et al. 2016), altering products of digestion (Hatew et al. 2016) and killing intestinal nematodes (Hoste et al. 2015). These are three of many papers originating from programs that were supported through the LegumePlus (http://legumeplus.eu) research grant, led by Irene Mueller-Harvey (University of Reading, UK). I was their international science advisor, from 2012-2015.

**Table 1**

<table>
<thead>
<tr>
<th>What</th>
<th>How</th>
<th>Outcome</th>
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<tr>
<td>Condensed tannins (CT)</td>
<td>Fed high and low CT lotus to sheep</td>
<td>Found CT increased absorption of EAA</td>
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<tr>
<td>Nutrition</td>
<td>Fed a range of CT forages to sheep and cows</td>
<td>Realised CT structure was very important</td>
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<tr>
<td>Nematodes</td>
<td>Evaluated effects of several CT on parasitized sheep</td>
<td>Identified types of CT that kill nematodes</td>
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<tr>
<td>Interdisciplinary collaboration</td>
<td>Finding &amp; introducing scientists with open minds</td>
<td>A spectacular advance in knowledge</td>
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<td>Feed conversion efficiency</td>
<td>Building a facility to measure intakes and live-weight gain of 1050 weaners for 7-8 weeks</td>
<td>Genetic marker to identify H/F cows that use feed more or less efficiently than average</td>
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<td>Forage mixed rations</td>
<td>Comparing forage digestion for sheep and cows</td>
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<td>Physical aspects of digestion</td>
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<td>Help Kakapo lay eggs</td>
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<td>Inflating cows</td>
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<td>Improving live sheep export</td>
<td>Defined rail spacing &amp; feed trough height for sheep</td>
<td>Standards for live sheep shipment</td>
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EAA, essential amino acids; BCAA, branched chain amino acids; H/F Holstein/Friesian

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### References

Irene Mueller-Harvey (University of Reading, UK). I was their international science advisor, from 2012-2015.
As with most programs, funders tire of ‘more of the same’ and look for something new; John Niezen and Abdul Molan departed AgResearch, and in the past decade most progress has been made outside of New Zealand. This research will help overcome effects of gastro-intestinal parasitism (especially where there is resistance to proprietary anthelmintics), improve efficiency of feed utilisation, and I expect will enable partial replacement of antibiotics used in intensive animal production systems. And legume bloat will be prevented! The work is ongoing, much of which is carried out in Europe, and in North America.

I have presented seminars on CT in five continents, and in 2015 I visited Yap Foo (he has long retired) and told him what has evolved from his comment about raspberries and soil nematodes; he had no idea that about 50 people are working on CT and parasitism now!

My research with CT and efforts to encourage scientists from different disciplines to collaborate will become my main contribution to science. The involvement of chemistry has been fundamental to success, and I suspect the real impact will be in the anti-microbial and anthelmintic arena (rather than nutrition), and will become evident in 10-15 years.

**Feed conversion efficiency**

In 2007 I submitted an application for funding, in collaboration with LIC, AgResearch and Australian researchers, to identify dairy cows that differed in the efficiency of energy utilisation for milk production, and to identify genetic markers for efficiency. My reasoning was that we could measure intake and daily gain in calves aged 6-8 months and that divergence in feed requirements would have a molecular basis, and therefore, be applicable to lactating cows. The application anticipated a 1-3% improvement in efficiency, had industry support and utilised new electronic and gene technologies.

We were successful, but the challenges were considerable; a new facility had to be built (in Hawera), contactors organised and Gallagher accepted the challenge of developing a system to weigh the 28 bins (50 times/s) and identify the animals eating. The data had to be provided in a manageable format, bins designed and rotomoulded, and hundreds of tonnes of cubes imported; initially lucerne from Canada, then pasture-based cubes from Victoria, Australia. This sounds simple, until you try and import something that MPI is not familiar with, and the suppliers need to provide certification that they haven’t done before. Even unloading 40 foot containers with 26 t cubes in each was a challenge, and had to be done quickly as the cost of container rental increased dramatically four days after arrival in Auckland.

Kevin Macdonald (DairyNZ) managed the programme, whilst I worried about bits of science. We measured intakes of 1050 weaners, the Australians did the same with an additional 900, and LIC were responsible for undertaking the gene analysis and relating genes to efficiency. The trial was remarkably successful; the most and least efficient 10% of the calves grew at the same rates, but one group needed 21% less feed than the other (Waghorn et al. 2012). When they became adults, the cows that were efficient as weaners used 3% less feed than their inefficient counterparts for lactation (Macdonald et al. 2014). The marker was used for screening 3300 cows from commercial herds, the extremes evaluated in the facility in Hawera, and the efficient cows used 2.7% less feed than did the inefficient ones (Davis et al. 2014). So, the marker worked, and this efficiency trait is now part of the LIC herd-improvement scheme.

I believe we were successful in this project because it had a sound (biochemical) basis, it used good technology and didn’t promise too much. However, in New Zealand, a 1-3% increase in efficiency would enable an additional 60-180,000 cows on our farms, with no reduction in individual milk production. The most important ingredient was enthusiastic and dedicated people, especially the ‘level-headed’ scientist with decades of experience in managing and watching cattle. We were a good team and won the Kudos award (2013) for our efforts. Thanks Kevin!

**Forage mixed rations**

Perennial ryegrass is the main forage sown in New Zealand; ruminants eat it and normally are reasonably productive. But, this animal thought, ‘one feed (with a few weeds) - how boring’; how about a mixture? Then Sharon Woodward (DairyNZ) asked if I would be interested in collaboration to create a ‘forage mixed ration’. Outcomes included in vitro and in sacco evaluations of about 20 forages, sheep feeding trials (Burke et al. 2000), followed by detailed evaluations of ryegrass harvested at different stages of regrowth (Chaves et al. 2006). My plan was to incorporate in vitro volatile fatty acid and ammonia production, and in sacco degradation rates, into a mechanistic model to simulate optimal rations to meet ruminant requirements in a range of physiological states. The objective was never quite achieved, and although recent trials have involved feeding brassicas and fodder beet to dairy cows, and measurement of N-fertiliser regimens on forage degradation (Elena Minneé, DairyNZ), the focus is on environmental pollution and nitrogen excretion rather than ruminant nutrition and welfare. Looking after our livestock should be our priority. Animals need to be fed properly every day and this is challenging when a single feed dominates their diet, whether it be ryegrass, fodder beet or anything else.

Making best use of information generated from large and complicated trials is challenging, and often the results from good research are under-utilised. I believe all large programmes should be based around (existing) mechanistic simulation models that use current knowledge to predict outcomes, indicate deficiencies in our understanding, and direct research to meet these needs. New findings should be incorporated in the model, and tested to ensure simulations are improved, perhaps also improving return on investment!
Physical aspects of digestion

Many measurements concerning nutrition are made on dissolved substances; VFA, ammonia, glucose, urea etc., and feed evaluations used to be undertaken with dried and ground dietary components. However, in New Zealand, ruminants eat fresh forage, which is chewed, mixed in the rumen and chewed again to make the solids smaller, enabling rapid microbial digestion and passage from the rumen, so there is room for more feed. My first paid work included radiological study of digesta mixing in the sheep rumen (Waghorn & Reid 1977), and more than 40 years later I play a small part in the supervision of Stephen Waite who is modelling rumen contractions to gain a better understanding of how the solids are processed, and gain a PhD in the process.

I have tried to understand how fresh forages are processed, from ingestion to excretion, and improve methodology for evaluating diets. This has been challenging because there isn’t a clear revenue stream associated with understanding digestion of solids! However, funds were obtained by John Caradus (AgResearch, Palmerston North) to evaluate 100 accessions of white clover, by mincing and incubating fresh minced material (Waghorn & Caradus 1994). Later, Warren McNabb (AgResearch, Palmerston North) and I incubated fresh minced and freeze-dried and ground L. pedunculatus, and showed that the fresh preparation was more suitable for studies of Rubisco degradation. It took several years to refine the mincing method and achieve repeatable results with fresh forages; nothing is straightforward in method development.

More recently Elena Minneé and I have developed a method for measuring the energy required to mince fresh forages, and in conjunction with particle size separation (wet sieving) we can define the consequences of chewing to better understand the physical makeup of ingested feed and rumen digesta. For example, the proportion of cells ruptured by chewing during eating can be higher for tough, than for soft and flexible forages; the amount of fibre (NDF) affects toughness, but plant anatomy and the strength of the glue holding cells together (lignin) are most important. Cell rupture affects the rate of crude protein release during digestion and circadian patterns of urinary nitrogen deposition on pastures, which some scientists apparently find exciting!

The New Zealand ‘rumen’ processes about 1 million tonnes of forage/day (150,000 t DM; MFE 2003) and understanding its operation is fundamental to efficient nutrient capture from feed. Microbiologists can measure the microflora easily, and microbial function should be incorporated into a mechanistic simulation of rumen digestion. Integration of chemical, physical and microbial processes could provide plant breeders with criteria to assist in cultivar selection, and optimise diets for production. Of course, diets should not contain harmful endophytes or other toxins, but may include secondary compounds, such as condensed tannins, in some instances. This is the type of information that I believe is essential for efficient and sustainable production from ruminants grazing pastures.

Cool stuff

These are a few trials that were fun (in no particular order):

1. Keith Lassey (NIWA) and I undertook the first methane measurements from sheep and cattle in New Zealand using the SF6 marker-dilution technique (yokes to sample respired breath). Then 20 years later, Erin Garnett and I used the first commercially available GreenFeed machine (www.c-lockinc.com), which enabled emissions to be measured from many grazing cattle, without the need for a laboratory. Later we built respiration chambers with clear plastic sides, for sheep and cattle; one benefit was the ‘immediate’ adaptation to the chamber, because subjects can see animals around them.

2. John Smith (MAF, Ruakura) had established the relationship between dietary protein, DM intake, and ovulation in ewes, and asked what was the nutrition link with ‘flushing’? With a few colleagues, we fed high- and low-CP diets at high and low intakes to wether sheep (notoriously devoid of ovaries) and measured ‘everything’: digesta flow, amino acids, hormones etc. Our conclusion was that an increase in flux of branched chain amino acids (valine, leucine and isoleucine) would be responsible for sheep making two eggs, rather than one (Smith et al. 1990). Later trials with ewes, showed an infusion of BCAA (but not other amino acids) during the luteal phase did increase ovulation rate (Smith & Stewart 1990).

3. Kerry James (DSIR) and I were asked if we could help make Kakapo lay eggs. Someone had collected faeces from the 26 remaining birds in ‘breeding’ and ‘non-breeding’ years (they bred one year in four). We gave this a bit of thought and decided their diet was mostly deficient in protein (and lipid), so suggested provision of almonds and brazil nuts (I like these). Some peanuts were added, probably to save on nut costs. I thought, maybe add apple and kumara too – a more-balanced diet. Reports suggested the kakapo were eating everything offered, and this was too much; I was worried we would end up with fat birds. However, night-vision cameras showed they threw the apple and kumara off the feeding stations and the rats took them away. Anyway, supplementation worked and 33 chicks fledged in 2016; not bad for a couple of days work (James et al. 1991)!

4. We wanted to know about the aetiology of bloat, and one question was how much gas was involved. So we first tried to inflate a cow with CO2, via the fistula, and with a device held in the cardia to prevent eructation. My assistant (John Kook) suffered a bit from the effort of holding everything in place, the cow entered a trance, and we then changed to weather balloons. We needed 70 L to ‘bloat’ a small cow; those that had never bloated kicked up a fuss, but ‘bloaty’ cows just chilled out. One was going ‘click, click, click’; I asked Dave Shelton, “what’s that noise Dave?” “She
can’t breathe” – i.e., death imminent! We unplugged the hose pretty quick!

5. Neville Grace (AgResearch) wanted to measure cow responses to iodine; my interest was in providing a source for humans, because New Zealand has an endemic iodine deficiency, and we were being advised to reduce our (iodised) salt intake. We undertook a trial with about 300 cows, and established the response in milk iodine to intramuscular injections every 100 days (Grace & Waghorn, 2005). Now there is a route for “natural” supplementation, should we need additional iodine in our diet.

6. I was approached by the live-sheep exporting industry to look at ways to reduce the fluidity of excreta in pens by feeding bentonite clay, and to establish criteria for trough height above the ground and rail spacing to prevent animals getting out of their pens (1% escaping each day = 800 sheep on big ships). Some of the work was done at 30°C and high humidity (throw water on the floor), and we videoed the pen trial with hungry sheep trying to get through a 25 cm gap; some could; novel in 1993.

And the list goes on; stomach tubing alpacas (they have a good memory and chew your thumb), rumen contents from water buffalo, infusions of B carotene (intravenous), intra-ruminal oxygen (11 L/day into a sheep had no effects from water buffalo, infusions of B carotene (intravenous), a good memory and chew your thumb), rumen contents etc. etc.

Conclusion

I need to acknowledge some of people who contributed so much to the fun I have had; Yuxi, Warren, Jennifer, Alex, Simone, Emma, Vicki, Kirsty, Elena, Stephen, Talia, Jessie, Erin, as well as honours students, interns from overseas and a lot of techs. And to a young woman that I married so she could come with me to live in California; Alison endured 40 years of rumen smells (some very advanced – when I gave up using gloves). I actually promised not to open any sheep cannula on the morning of our wedding (I was measuring rumen motility).

Freedom to experiment is a scientist’s dream. But too often the “carrot of power” is placed in front of good scientists and most grasp it. They become managers; good scientists are rarely good managers. I avoided this temptation; my philosophy was to enjoy every day at work, and my passion was science. I do understand the need to regulate expenditure, but excessive regulation stifles innovation. If we were regulated by milestones and meticulously ticking boxes in 1990, I doubt there would be many kakapo.

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