

Host associated factors that influence rumen microbiome composition

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The gastrointestinal tract is host to a diverse microbial ecosystem that can vary depending on both host genetic and environmental factors. Studies have shown that even minor shifts in these populations can have significant impacts on livestock nutrition and productivity. Recent work has reported that the microbiome structures that are naturally occurring in the rumen are highly correlated with, and predictive of the feed efficiency phenotype of an animal (Ben Shabat *et al.*, 2016). The potential for a host-specific microbiological uniqueness of the ruminant was first noted in the protozoal community (Eadie, 1962), and much later in the fibrolytic bacterial community (Weimer *et al.*, 1999), prior to the development of advanced molecular tools to characterize the gut community. The concept of host microbiome individuality has now achieved substantial attention, primarily as a result of recent studies of the human gut microbiome. Such studies have revealed that the human gut contains a “core microbiome” (i.e., a set of taxa present in all subjects in the study), but also a large number of taxa whose presence or abundance varies among hosts.

If the microbial community within each rumen is unique to its host, then two main questions arise:

- i) What are the host-associated factors that drive the uniqueness of such microbial ecosystem?
- ii) At what stage of life is this community assembled to the point where it can be regarded as compositionally and functionally unique?

This review will present the main findings with regards to the above questions:

- i) A recent work conducted as part of the Rumen Global Census project (Henderson *et al.*, 2015), determined the foregut microbial community composition in ruminants (742 samples from 32 animal species and 35 countries). Although the diet was the main driver, some patterns were associated to the animal species. For example, *Fibrobacter* abundances presented significantly higher levels in bovines compared to deer, sheep, or camelids. Under recent FP7-RuminOmics project an experiment involving the exchange of ruminal digesta from cows into reindeer was performed. Comparison of the microbial communities in the rumen of reindeer and cows before digesta exchange revealed that both species share a core microbiome. About 80% of identified bacterial taxa did not differ between species, with 10% being more closely associated with either the reindeer or cow host. Following the transfer of digesta from the cow into reindeer, a gradual time dependent increase in bacterial communities specific to this species was detected. Such findings indicate a clear host effect influencing bacterial communities specific to reindeer. In another experiment (a twin cow-cow rumen exchange) no clear evidence was found confirming that the rumen microbiome is consistently more similar in genetically related animals than between unrelated cows. It appears that while there are some indications of host effects on the composition of certain taxonomic groups, there is a stochastic component that can lead to even bigger differences than originally introduced by the exchange of rumen contents.

The importance of different host-specific factors (saliva production, VFA absorption, digesta passage rate, rumen contractions and volume, immune system) will be presented and discussed at the meeting.

- ii) The rumen is quickly colonized by all type of microorganisms straight after birth and the colonization pattern may be influenced by several factors such as presence/absence of adult animals, the first solid diet provided, and the inclusion of compounds that prevent/facilitate the establishment of some microorganisms or the direct inoculation of specific strains. Recent studies (Yáñez-Ruiz *et al.*, 2015) suggested that it is possible to promote different microbial populations establishing in the rumen of the young animal by manipulating the feeding management early in life that persisted in later life. This would create differences in adaptive capacity due to different early life experiences, leading to the idea of ‘microbial programming’. However, despite significant research effort, there is still a lack of understanding of the mechanisms governing microbial/host cell interactions, the immune factors involved, the development of the rumen and its microbial community, and the implications for the host when microbial colonization patterns are altered, especially the long-term effects.

The latest results from the FACCE-JPI RumenStability project, which aims at addressing some of these issues, will be presented at the meeting.

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References

- Ben Shabat S K, Gore S, Doron-Faigenboim A, Durman T, Yaacomy S, *et al* 2016. *The ISME Journal* 10, 2958–2972.
- Eadie J M 1962. *Journal General Microbiology* 29, 579–588
- Henderson G, Cox F, Jonker A, Global Rumen Census Collaborators and Jansen P 2015. *Scientific Reports* 5, 14567.
- Weimer P J, Waghorn, G C, Odt C L and Mertens, D. R. 1999. *Journal of Dairy Science* 82, 122–134.
- Yáñez-Ruiz D R, Abecia L and Newbold J 2015. *Frontiers in Microbiology* 6, 1133.

Diet effects on the rumen microbiome

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Application Studies of the microbiome in a variety of ecological environments is one of the most rapidly evolving areas of science, with the rumen being recognized as one of the most complex microbial ecosystems known.

Introduction Ruminants are unique in their ability to convert lignocellulosic biomass (i.e., grasses and crop residues) into high quality meat and milk protein for humans. Ruminants do not produce cellulolytic or hemi-cellulolytic enzymes to degrade ingested plant material; instead they rely on symbiotic associations with bacteria, fungi, methanogens and protozoa that reside within their gut. The rumen microbiome is highly complex and is comprised of bacteria (up to 10^{11} cells/mL), protozoa (10^4 - 10^6 cells/mL), fungi (10^3 - 10^6 zoospore/mL), methanogens (10^6 cells/mL) and bacteriophages (10^7 - 10^{10} particles/mL). It has been estimated that at least 85% of the species inhabiting the rumen remain uncultured. Recent projects like the Hungate 1000 (<http://genome.jgi.doe.gov/TheHunmicrobiome/TheHunmicrobiome.info.html>) and the global rumen census have been undertaken to increase our understanding of the genomics of this unique ecosystem. The Global Rumen Census project further emphasized the impact of diet on the composition of the gastrointestinal microbiome in a variety of herbivores (Henerson *et al.* 2015). Central to ruminant productivity is the ability of symbiotic microbes to express a vast array of carbohydrate active enzymes (CAZymes) that digest fibre and enable the host to derive energy from end products of fermented sugars.

Discussion The composition of the rumen microbial community has an impact on the health and productivity of ruminants. This community is highly dynamic and changes rapidly in response to changes in diet. Generally, the major components of the diet of ruminant livestock are forages, grains and by-product concentrates with ratios of these ingredients varying substantially among diets. The physiochemical nature of feed and the metabolic end products that are formed during digestion select for the growth of certain microbes while inhibiting the growth of others. The composition of a ruminants' diet has been identified as the most important determinant of the composition of the rumen microbiome. *Streptococcus bovis*, *Ruminobacter amylophilus*, *Prevotella ruminicola*, *Butyrivibrio fibrisolvens*, *Succinimonas amylolytica* and *Selenomonas ruminantium* have traditionally been seen as the principle starch digesters in the rumen. Recent culture independent studies utilizing metagenomics and fluorescence *in situ* hybridization to study the microbial basis of grain digestion have revealed that up to 80% of the bacteria that form the biofilm on the surface of barley and corn are Ruminococcaceae, most of whom are not the amlyolytic species described above. Furthermore, there were distinct differences in the community in different individual animals and with different grain types (i.e., barley vs corn). These findings indicate that there is still a great deal about the microbial basis of starch utilization in the rumen that is unknown. In high forage diets, the rumen microbiome consists of a diverse array of cellulolytic bacteria, protozoa and fungi. Culture based studies attributed the majority of the fibre digesting activity in the rumen to four bacteria, *Fibrobacter succinogenes*, *Ruminococcus albus*, *Ruminococcus flavefaciens* and *Butyrivibrio fibrisolvens*, and to a lesser extent to rumen fungi. More recently, metagenomics and metatranscriptomics have described a more diverse, largely uncultured and uncharacterized microbial community that plays a central role in plant cell wall digestion. Examining the expression of CAZymes in the rumen of cattle fed high fibre diets has revealed the absence of some carbohydrase families, possibly shedding insight into the development of next-generation enzyme based technologies that could further improve fibre utilization in ruminants.

Conclusion Understanding how the rumen microbiome adapts in response to diet could lead to improved ruminant productivity and health and more informed approaches to manipulating the rumen microbial community. This review will focus on recent insight into how the composition of the rumen microbiota is impacted by alteration in feed and how these changes can positively or negatively affect the health and performance of ruminant livestock.

Acknowledgements The authors gratefully acknowledge the financial support of the Agriculture and Agri-Food Canada Agri-Innovation Program and the Alberta Crop Industry Development Fund.

References

Henderson *et al.* 2015. Rumen microbial community composition varies with diet and host, but a core microbiome is found across a wide geographical range. *Scientific Reports* 5:14567 | DOI: 10.1038/srep14567

New Antibiotics, New Alternatives: Can we win the AMR arms race?

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Antimicrobial resistance threatens to render ineffective our current arsenal of clinically effective antibiotics and bring us towards a post-antibiotic era, where infections caused by microorganisms resistant to conventional antibiotics exert a heavy toll both on patients and on the economy. Although antibiotic resistance may be considered an ancient phenomenon, the often inappropriate use of antibiotics in clinical medicine and across a wide range of sectors including Agri-Food, has led to the emergence of resistance to all classes of antibiotics in clinical use. This situation is mirrored by a steady decline in new antibiotics in late-stage development and the exit of nearly all the major pharmaceutical companies from the antibiotic R&D space. The majority of clinically useful antibiotics were discovered during the 'Golden Age' of antibiotic discovery, from the 1940s to the early 1970s. Since then, the discovery and approval of new antibiotic entities has not kept pace with the emergence of resistance and loss of efficacy of conventional antibiotics. Therefore, there exists a critical, unmet need for the discovery of new antibiotics, particularly new classes of antibiotics, since no new class of antibiotic has made it to the market in the last 30 years. In this presentation the current approaches to antibiotic discovery will be analyzed and the question of whether we can recover the lost art of antibiotic discovery addressed.

Furthermore, are new antibiotics all that is required or should we adopt a broader approach to treating infection and combatting AMR? The Wellcome Trust and Department of Health recently established an 'Alternatives to Antibiotics' working party to examine the potential of non-compound approaches as alternatives to conventional antibiotics. The most advanced of these alternatives are in Phase II and Phase III trials but are likely to be adjuvant therapies (rather than true alternatives), indicating conventional antibiotics are still needed for the coming decades. This presentation will focus on the potential approaches to combat AMR through the reinvigoration of the antibiotic discovery pipeline, the quest to bring antibiotic alternatives from the laboratory to clinical reality, the barriers to antibiotic discovery and lack of a business incentive, and to reflect on the key findings of the O'Neill Review.

References

O'Neill, J. (2014-2016) The review on antimicrobial resistance: Tackling drug-resistant infections globally.

(<https://amr-review.org>)

Czaplewski et al., (2016) Alternatives to antibiotics – a pipeline portfolio review. *Lancet Infect Dis* 16:239-51.

Antimicrobial resistance and its possible impact on sustainable cattle production systems

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Antimicrobial resistance (AMR) is a growing concern world-wide both in medicine and veterinary medicine. Individuals, scientific bodies and lobby groups may argue over the importance of antimicrobial use in livestock production, but the fact remains that human health is at risk due to AMR globally. While as scientists we must argue for evidence based policy making, there is already enough evidence to support the need to reduce antimicrobial usage in all livestock sectors. The scientific arguments have now in part been overtaken by a political drive for change at the very highest levels. In 2016 AMR was discussed by leaders of the G7, G20 and United Nations and it is likely that all three bodies will continue to call for substantial changes on a global scale as outlined by Lord O'Neill (2016).

The responsibility for antimicrobial stewardship on farms rests ultimately with the veterinary profession as it is only veterinary surgeons that have the right to prescribe such medications; however livestock farmers, their other advisors and those controlling every step in the food supply chain also have a responsibility, as do those responsible for the production, promotion and supply of veterinary medicines. Milk buyers, processors and retailers of all animal products are increasingly monitoring, benchmarking and seeking to influence medicine usage on farm.

After giving an insight into medicine use on farms, this presentation will outline some of the research undertaken by members of the AMR Force research group at the University of Bristol (@AMRForce). This group is seeking to answer fundamental questions about AMR in livestock, the environment and in human patients, and has shown that it is possible to keep healthy, productive, commercially viable dairy cattle without the need for critically important antimicrobials such as fluoroquinolones and 3rd and 4th generation cephalosporins. In fact studies by the Bristol group have shown that with appropriate management; health, fertility and production can all be improved while at the same time phasing out the use of these critically important antimicrobials for human health.

The author will also discuss briefly the future implications of AMR on farming practises and production systems, and their possible impact on sustainable livestock production.

Acknowledgements

This paper draws upon the work of many members of the University of Bristol, AMR Force research group (@AMRForce). The author fully acknowledges the efforts of all members of this group.

References

O'Neill J, 2016. amr-review.org/sites/default/files/160525_Final%20paper_with%20cover.pdf

Alternative treatment strategies to address the challenge of antimicrobial resistance

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Application The issue of antibiotic resistant microbes remains a global public health problem and it is the subject of much media coverage. Inappropriate use of antibiotics, in both human and animal medicine, is contributing to the problem and the agriculture sector cannot afford to be complacent.

Introduction Policy makers in many jurisdictions often introduce policies in proportion to the media coverage of the issue rather than the extent of the problem. Sensational headlines like superbugs and killer microbes and stories of factory farming generating multidrug resistance microbes reflect badly on the sector. There is a disconnect between consumers and modern agricultural practices which leaves fertile ground for sensational stories to gain traction and for inappropriate policy responses.

The Challenge The more you use antibiotics the quicker you lose them. Selective pressure whether it is in a human hospital or in a farm environment results in the generation of resistant microbes by a variety of mechanisms. The microbes are quite promiscuous with their genetic material and this resistance capability can be transferred to other microbes who can act as a reservoir for resistance genes in both commensals and environmental organisms. Antibiotics are necessary for the treatment of sick animals but are not a substitute for good husbandry practices. Much progress is being made in modern animal and poultry production in the areas of genetics and nutrition. Genetics creates the potential for superior performance and nutrition delivers on this potential, however suboptimal animal health status undermines the gains from the former two. Antimicrobial resistance is a global problem and big pharma has not got a pipeline of new antibiotics so they have to be regarded as a precious resource. The Agri sector has to play, and be seen to play, its part in tackling the problem.

Antibiotic use has to be reduced to the absolute minimum and alternative strategies to prevent infectious diseases from spreading have to be exhausted. Biosecurity, quarantine, immunisation and dietary interventions to boost immunity must be part of the strategy to maintain animals healthy. In animals and poultry breeding performance and production traits has been paramount but increasingly disease resistance is being seen as a highly desirable trait. Slower maturing, more robust poultry and animals may come more desirable.

The epidemiology of microbial resistance is complex and even with a reduction of antibiotic use microbes may not immediately revert to being sensitive. Heavy metal induced antibiotic resistance is a complicating factor that receives limited airtime.

The Solution An emphasis on prevention of disease, both performance only related and zoonotic, has to be the way forward. The best way to reduce the use of antibiotics is to reduce the need for antibiotics so having healthier stock has to be the objective. AMR is not a poultry problem and it is not a pig problem, none of these demand an antibiotic. It is a people problem and we need to see a behavioural change by both farmers and veterinarians if alternative strategies are to become the norm.

Gene sequencing is enabling the molecular scientists to track resistant genes and study resistance acquisition mechanisms. Perhaps compounds can be found to block these mechanisms and when added to antibiotics could render current resistance microbes susceptible again and hence rejuvenate and extend the life of the current portfolio of antibiotics.

Control of AMR should not be considered in isolation to controlling all infectious agents. This should be a component of all herd, flock and shoal health programs - not a stand-alone entity. You will never see the epitaph "*Here lies the body of my beloved Granny: - thank God it was a sensitive bug that killed her!*".

Development and application of a net feed efficiency estimated breeding value to a commercial population of Stabiliser cattle in the United Kingdom and Ireland

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Application The Stabiliser Cattle Company (SCC) have recognised the need for a highly efficient suckler cow and use a multi-trait selection approach for traits of economic interest. Net feed efficiency (NFE) is the latest trait to be added to the programme. It is important because approximately 70% of the feed energy in a suckler system is used for maintenance.

Introduction Suckler beef systems work because they utilise low quality forages and/ or make use of marginal land. Suckled calves are then finished effectively on forage based rations supplemented with cereals to give a high energy density feed. The SCC manages the breeding programme of 10,000 suckler cows in 95 herds. All animals are performance recorded for 20 economically important traits and selection of animals as parent stock is based on an economically weighted selection index that is designed to optimise profit margin. The index is a balance between the maternal traits, to ensure low production costs, and the growth and carcass traits required to meet market specification. The inclusion of NFE in the selection index will strengthen the profitability of the users of Stabiliser genetics. Identifying individual animals in the breeding programme that are more feed efficient than average will have two beneficial effects, firstly daughters that become cows will have a lower lifetime maintenance costs and secondly finishing animals will require less feed to meet the required market specifications.

Material and methods Previous research has established the techniques for measuring NFE in a breeding population. In our programme 160 young breeding bulls (10 months old) per year are tested. They have a 4 week acclimatisation period followed by a 56-day test period. Dry matter intake is measured through a Growsafe system and live weight is measured every 7 days. All bulls are ultrasound scanned for back fat depth at the end of test. NFE is calculated correcting for growth rate, live weight and fatness. Over 1000 animals have been tested and careful selection of animals to test means the important families in the breeding programme are tested for NFE. Sons and grandsons of influential sires that have been tested in the first phase of the work are now being tested themselves.

Results The key measure of profitability to which the selection index works is weight of beef produced from the feed resources available. In a suckler system there will be contributions to this figure from: number of calves produced (number of cows kept, fertility, mortality), feed used (for maintenance and growth) and carcass traits (age, weight, fatness, yield, marbling). An important consideration for the SCC is to maintain a balanced approach to selection on all traits of economic importance to optimise profit margin.

NFE is a component of the overall selection process but has to be balanced against the financial contribution made by increased growth rate, reduced cow size, higher fertility, lower birthweights, etc. The heritability of NFE in the Stabiliser population tested has been calculated to be 0.37 which is similar to estimates from other work around the world and means good rates of genetic progress can be made by breeding from the highest merit animals. The monetary value of the trait in the UK Stabiliser population has been calculated to be worth around £100 per cow per year when comparing the average of the top third animals to the average of the bottom third animal tested. To illustrate the point in a practical sense this means that for the same growth rate the top third animals on test would have a dry matter intake of 9.5kg compared to the bottom third consuming 11.5kg. The NFE EBV has been available as a stand-alone EBV since January 2017 and will be integrated into the new selection index (£Profit) from September 2018 so progress for this trait in the breed in the UK cannot yet be quantified but the development phase indicates that selection pressure for improved NFE will be achievable. Evidence from similar work in the US on Stabilisers, which has been in place since 2003, is showing excellent progress in the population with continued improvements in growth rate, a capping of mature size and lower DMI intakes.

For beef producers in the UK using NFE is a vital step to improving profitability. Selection for growth rate alone has led to ever larger cattle that cannot be fattened within the required age and weight specifications required by processors and their retailer customers. Furthermore these high growth genetics used in a suckler cows increase maintenance costs and reduce output per hectare. The thorough recording and disciplined selection used by SCC, and the future use of the £Profit selection index with NFE included, will produce increasingly profitable suckler cows by reducing input costs whilst meeting retailer specifications.

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Greener Pigs: Self-sustaining livestock building for commercial production

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This 36 month project aimed to develop a working prototype for sustainable high welfare intensive pig houses integrated with renewable energy generation, introducing innovative civil, chemical and mechanical engineering solutions to livestock housing to create self-sustaining commercial pig housing.

Project background, context and need

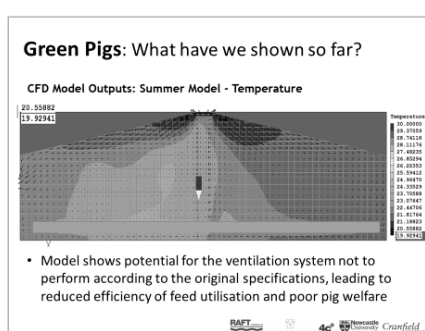
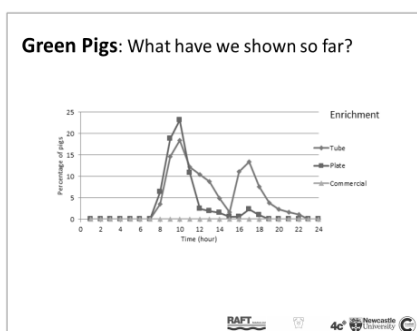
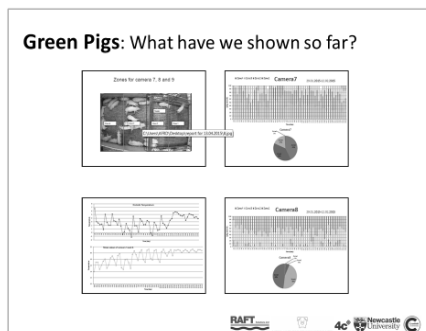
Self sufficiency in UK pig production has fallen by 30% since 1998 as UK producers struggle in the face of disease challenge, volatile grain prices and animal welfare reforms (CRESC report, Bowman *et al.*, 2012). 2013 statistics from BPEX reveal UK producers are still only able to satisfy 52% of the country's demand for pork. Making the UK more competitive and therefore able to deliver a higher percentage of UK pork demand is key to the industry's growth. 60% of UK total slaughter (5.4M pigs) originates from straw based or part slatted buildings where food conversion efficiency is 10% worse (£7/pig produced extra cost) than from fully slatted intensive units. While these systems satisfy the EC Directive (2001/93/EC) which states that "pigs must have permanent access to sufficient quantity of material to enable proper investigation and manipulation activities..." and attract some retailer premium (6p/kg) due to the provision of manipulable materials (e.g. straw, compost, leaf litter), the higher cost of production means UK producers still cannot compete on price as well as welfare – and managing production costs is key.

Project goals

- to develop engineered systems to introduce straw etc. for first time into intensive slatted floor systems to promote animal health & welfare whilst maintaining low production costs.
- to minimise energy costs and polluting emissions by using bedding material as part of a whole farm energy system, using waste disposal systems to transport slurry and de-odourise air, capture remaining energy through bio-digesters and CHP for on-farm energy generation
- to develop a working prototype for sustainable high welfare intensive pig houses integrated with renewable energy generation, considering NEW BUILD and RETRO-FIT situations.

The project has brought together teams from RAFT Solutions: veterinary support; project management; PROJEN plc; civil, mechanical and industrial engineering, strategic engineering project management; Newcastle University; pig welfare behaviourists; engineering department (modelling and energy design); Cranfield University: UK's leading GHG and life cycle assessment modelling scientists; Midland Pig Producers: one of the UK's largest pig producers; provide test centre, pigs, on farm labour and engineers; 4c Design: product design and modelling.

Work packages in manipulable materials exploration and delivery; flushing systems; waste management and life cycle analysis have been progressed.



Results are being processed but have concluded:

- the optimum manipulable material for use in slatted floor systems with flushing capabilities (maize silage) and the optimum delivery method (plate delivery)
- how pig behaviour is influenced by ventilation and temperature patterns in slatted floor systems
- the shape and design of a pig building for commercial production, given pig health and welfare needs and to minimise energy (and cost) inputs.

Animal personality: what does it mean for our understanding of animal welfare?

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Application Personality is likely to confound our assessments of welfare, leading to large amounts of unexplained variation unless we account for it.

Introduction Animal welfare is at heart a science of the individual. The propensity of individuals to suffer is central to our understanding and quantification of welfare issues. Animal personalities, also known as ‘behavioural syndromes’ (Sih *et al.*, 2004) or ‘coping styles’ (Koolhaas *et al.*, 1999) are defined as consistent individual differences in behaviour across situations and time (Sih *et al.*, 2004) and they may have consequences for fitness (Smith and Blumstein, 2007; Biro and Stamps, 2008). The way in which individuals react to environmental stressors has been studied from the perspective of coping styles and these are characterized by consistent individual behavioural and physiological traits termed ‘reactive’ and ‘proactive’ coping styles’ (Koolhaas *et al.*, 1999). Cognitive bias has become a popular way to access non-human animal mood, though inconsistent results have been found. In humans, mood and personality interact to determine cognitive bias, but to date, this has not been investigated in non-human animals.

Material and methods Weaned at four weeks, 36 pigs (commercial crossbreed PIC337 (large white × landrace), n = 24 males, n = 12 females) were assigned (pseudo-randomly controlling for sex, weight and dam) to either a high- or low-level enriched environment in two groups of 18, replicated three times. Six pigs from each environment and replicate were selected for training. Both environments had solid floors, a slatted area and wooden blocks on chains as enrichment. More enriched environments had deep straw and a larger space allowance (more enriched: 0.62 m² pig⁻¹; less enriched: 0.41 m² pig⁻¹). Personality testing occurred at six and eight weeks of age; cognitive bias training and testing was completed by 7–10 weeks of age. A linear mixed-effects model with restricted maximum-likelihood was used to analyse log ‘time to run’ as the outcome variable (using lme in nlme package (Pinheiro, 2005)). Individual differences were accounted for as models were weighted by speed of approach to location P, pig and pen identity were included as random effects, P–R scores were covariate and the fixed effects were: treatment (environment), sex and probe location.

Results In humans, information processing biases are dependent on both current mood state and personality (Pinheiro, 2005; Marshall *et al.*, 1992). We found an analogous effect on cognitive bias in pigs. The speed of approach to the probe locations was significantly affected by an interaction between the location of the probe, personality and housing environment (which is likely to have affected mood; $p = 0.005$). Separate analyses on the interaction term revealed that there was no difference between the environments in pigs’ speed of approach to the ‘near positive’ probe ($p = 0.183$), or effect of personality ($p = 0.345$). To the ‘near negative’ and ‘middle’ probes, there was an interactive effect of environment and personality (near negative: $p = 0.028$; middle: $p = 0.025$); pigs in the more enriched environment were more optimistic if they were more reactive. However, pigs in the less enriched environment became more pessimistic to the near negative probe if they had a more reactive personality.

Conclusion We are only just starting to uncover the myriad ways in which animal personality alters our understanding of how animals perceive the world around them. The individual differences highlighted by results so far suggests a level of internal complexity previously unconsidered. Measuring welfare at this level is a clear reminder that individuals are indeed individual, and assumptions of uniformity are unfounded.

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References

- Biro, P.A., Stamps, J.A. 2008. Trends in Ecology & Evolution 23, 361–368.
- Koolhaas J, Korte S, De Boer S, Van Der Vegt B, Van Reenen C, Hopster H, De Jong I, Ruis MA, Blokhuis H. 1999. Neuroscience & Biobehavioral Reviews 23, 925–93.
- Marshall GN, Wortman CB, Kusulas JW, Hervig LK, Vickers RR Jr.. 1992. Journal of Personality and Social Psychology 62, 1067–1074.
- Pinheiro J. 2005 In Encyclopedia of Biostatistics. Wiley Online Library.
- Sih A, Bell AM, Johnson JC, Ziemba RE. 2004 Quarterly Review of Biology 79, 241–277.
- Smith BR, Blumstein DT. 2007. Behavioral Ecology 19, 448–455.

What has animal science ever done for us, and what does it need to do now?

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Increasingly, public funders of research ask for predictions and evidence of economic, societal or other impacts from the research they fund. Introducing the evaluation of impact to the UK Research Excellence Framework (REF2014) is a case in point. So, what has animal science achieved, and what does it need to do now to help meet future societal challenges?

Some of the early advances in animal science were made by Sir John Hammond and his colleagues in the early-mid 1900s, in animal physiology, growth and development. During Hammond's career and the following few decades, great advances were made in understanding the nutritional requirements of farm animals – and in evaluating feeds, formulating diets and developing feeding systems to meet them, with benefits for animals, farmers and consumers. While this area appeared to mature by the end of the last century, it has been reinvigorated by the need to reduce the environmental impact of livestock, to understand the wider role of the gut microbiome in animal health and production, and by opportunities to better tailor animal nutrition to suit the animal's and consumers' needs ('designer nutrition'). Hammond's work on growth and development was an important foundation for developing more efficient livestock production systems, and later responding to consumer demand for leaner meat, and improved meat eating quality. Since the mid-1900s, there have been many advances in animal breeding and genetics, including in the design of breeding programmes, development of selection criteria and measurement techniques, prediction of genetic merit, understanding the consequences of selection, and addressing unintended consequences. Scientific advances have stimulated development of major global breeding companies in the poultry, pig and dairy cattle sectors, with increasing vertical integration in pigs and poultry. Global industry breeding programmes in all three sectors typically achieve cumulative genetic gains of 1-3% per annum, which has had a major impact on the cost and environmental footprint of livestock products in industrialized countries. Multi £ billion benefits are estimated in the UK alone. From the mid-1980s, molecular genetics was expected to deliver a new paradigm for animal breeding, based on molecular tests for traits of interest. While there was some success in traits influenced by one or a few genes (e.g. scrapie resistance), it is the relatively recent advent of genomic selection – in which prediction of genetic merit from pedigree and performance data is augmented by information from genetic markers throughout the genome – that has begun to revolutionize breeding for more complex traits. New genetic and genomic technologies are delivering already, or promise to deliver, even faster change – one of the key achievements of animal science in the last few decades. Artificial insemination, championed by Hammond and colleagues, has been pivotal in delivering and disseminating genetic improvement, in cattle especially. Other reproductive (and GM) technologies have yet to have such wide impact in livestock production, though many have laid the foundations for developments in human fertility and medicine. The first cloning of a mammal from an adult somatic cell (Dolly the sheep) was one of the major scientific advances of the last few decades. This too, has provided a platform for advances in other branches of biology and medicine. Animal welfare is another key area of achievement in animal science – improving our understanding of welfare from the animal's perspective, delivering tools for welfare assessment, and solutions to some key welfare challenges in management, housing and production systems, transport and slaughter, often implemented through government policy or industry assurance schemes. Interactions with veterinary science in areas such as management, nutritional and genetic influences on livestock diseases have produced economic, welfare and 'one health' benefits. Animal welfare, economic and environmental impacts were the most commonly cited primary impacts (43, 31 and 14% of impact case studies, respectively) of animal science in REF2014.

Society is facing major challenges related to global population growth, food security, environmental change, human diet and health. The challenges are urgent, massive, complex and interconnected. Solutions will need inter/trans-disciplinary and systems thinking. Livestock production is often part of these challenges, and animal science and innovation need to be part of the solution. Developments in molecular biology, genetics and genomics, informatics, data science, remote sensing, precision farming, and many other areas, offer a plethora of new opportunities – in established and 'newer' farmed species e.g. in aquaculture. As well as being innovative and dynamic, future livestock systems need to be socially acceptable, environmentally sensitive and economically viable (three pillars of sustainability). This is easy to say, much harder to measure and achieve. As well as scientific excellence, animal science needs practitioners who can engage with the interests of society at large, work across disciplines, develop more coherent frameworks to allow the costs and benefits (in the widest sense) of livestock systems to be objectively evaluated, and managed at different scales. Animal scientists also need to communicate the challenges and potential solutions effectively with a wide range of audiences (including the public and policy makers), and to help target more basic research for maximum impact. BSAS has an important role in developing the next generation of animal scientists to rise to these challenges, as Hammond and his colleagues did last century.

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Use of transcriptomics to identify mechanisms of improved growth and feed efficiency

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Feed efficiency is normally determined either as feed-to-gain ratio (i.e. kg feed per kg weight gain) or gain-to-feed ratio (i.e. kg weight gain per kg feed). More recently the concept of residual feed intake (RFI) has been developed (Gilbert *et al*, 2017), which is how much more (high RFI) or less (low RFI) food an animal eats compared to the group average, for the same rate of growth. Hence low RFI animals are more efficient, since they consume less food for the same rate of growth; whereas high RFI animals are less efficient, since they consume more food for the same rate of growth. In order to investigate the mechanisms for differences in feed efficiency, a number of groups have used transcriptomic methods to identify which tissues are important and which genes are differentially expressed within those tissues and therefore might be responsible for the different efficiencies. Transcriptomics relates to the simultaneous measurement of expression of thousands of genes (i.e. mRNA levels) within a tissue, with the most common method utilising microarrays. For example, a pig microarray currently available is able to measure the levels of around 40,000 genes simultaneously. After introducing the basic principles of microarrays, the main talk will focus on 2 areas of research using microarray approaches to identify the mechanisms for improved feed efficiency.

Firstly, some of our work at Nottingham (Brown *et al*, 2016) comparing the transcriptomic effects of known anabolic agents, growth hormone and beta-agonists, administered to both sheep and pigs, with particular focus on their effects on skeletal muscle. This work has identified a potential novel mechanism for increased muscle growth, whereby metabolic pathways (particularly glycolysis and the tricarboxylic acid (Krebs) cycle) appear to be induced to synthesise biomolecules (e.g. serine, glycine, choline, phospholipids) for increased growth and protein synthesis, rather than their normal role in the generation of energy (i.e. ATP).

Secondly, transcriptomic studies in tissues from animals (particularly pigs) genetically selected for low and high RFI will be presented. A number of groups around the world have independently developed genetic selection lines for low and high RFI (Gilbert *et al*, 2017; Grubbs *et al*, 2014; Jing *et al*, 2015; Vincent *et al*, 2015) and there appear to be subtle differences in the mechanisms involved, as indicated by the tissue transcriptomic analyses. Skeletal muscle appears to be an important tissue contributor to the improved efficiency seen in low RFI animals. There are also some similarities in the gene expression profiles both across the different genetic selection lines and in comparison to the effects of anabolic agents, including a common reduction in expression of mitochondrial genes.

These studies are beginning to identify novel mechanisms for feed efficiency, including specific genes that might be used as markers in future breeding programmes. Those genes may also be alternative drug targets that might avoid some of the ethical and safety issues relating to the use of some anabolic agents, such as beta-agonists.

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References

- Brown DM, *et al*. 2016. Scientific Reports 6, 28693. doi: 10.1038/srep28693.
- Gilbert, H, *et al*. 2017. Animal, 1–13. doi: 10.1017/S175173111600286X.
- Grubbs, JK, *et al*. 2014. Journal of Animal Science 92, 1995-2007.
- Jing, L, *et al*. 2015. Scientific Reports 5, 11953. Doi: 10.1038/srep11953.
- Vincent A, *et al*. 2015. Journal of Animal Science 93, 2745-58.

Is precision control of the rumen microbiome possible?

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Introduction The rumen plays a central role in the ability of ruminants to produce human edible food from resources that are otherwise not available for consumption by mankind. Fermentation in the rumen also has the potential to influence the health and wellbeing of both the host and man through the nutritional quality and safety of meat and milk and through potential deleterious environmental consequences due to emission of greenhouse gases and excessive N excretion in faeces and urine. Given the importance of the rumen fermentation, it is perhaps not surprising that a great deal of effort has been devoted to investigating methods for manipulating this complex ecosystem and the possibility of precision control of the rumen microbiome is a highly attractive target. Here I will attempt to address two of the major limitations that will need to be overcome to achieve this aim.

Describing the rumen microbiome Traditional studies on rumen microbiology have relied on our ability to culture and characterise microorganisms from the rumen. Whilst significant progress has been made using these techniques over the years, it is recognised that only a relatively small proportion of the microbes within the rumen are recovered leaving us ignorant about the roles and activities of the vast majority of the rumen microbial ecosystem. Molecular techniques are allowing both quantitative and qualitative studies on microbial populations in the rumen to be carried out. Ribosomal genes have been used both to quantify different how specific microbial groups respond to quantitatively and to more qualitatively describe the rumen microbial population and changes induced by manipulation through the characterization of 18/16S rRNA gene pools through massively parallel amplicon sequencing. However, increasingly studies are expanding to not only to consider which microbes are present in the rumen but also the functional genes present, their expression in the rumen and how this might ultimately allow an increased understanding of the role of the rumen in the health and wellbeing of man and animals. Progress in this area has been rapid but is now limited by our ability to culture and characterize major components of the rumen microbiome. Only a limited subset of the rumen bacteria, archaea and fungi are available in culture collection, whilst to date there has been no successful axenic culture of the rumen protozoa and available cultures are maintained in co-culture with rumen bacteria. Significant efforts, including the Hungate 1000, are underway to improve our ability to culture rumen microbes including the use of metagenomic information to identify nutrient requirements of specific organisms.

Factors that influence the rumen microbiome Diet and dietary additives are the most obvious factor influencing the rumen microbiome and using the techniques noted above we are rapidly developing an understanding of not only the overall effect of diet on the composition of the rumen microbiome but also its function. The development of network based models is allowing us to explore both the temporal and spatial development of microbial populations within the rumen, in particular in regards to the colonization and degradation of dietary fibre entering the rumen. Evidence is mounting that the host itself might have an effect on the rumen microbial population. Indeed, it is now apparent that within a flock sheep of the same breed on the same diet some animals will segregate into 'low' or 'high' methane producers and that to an extent this is heritable. The mechanisms by which the host might control the rumen microbial population remain unknown but factors such as modifying the gene expression of the rumen epithelium and possible variation in rumen outflow or volume have been suggested. In addition to heritable host factors we have also recently investigated the possible role of early life nutrition on microbial population structure and function in adult ruminants. During rumen development, in young ruminants ingested microbes colonise and establish in a defined and progressive sequence. The coexistence of the host and microbial gut communities is clearly immunologically driven, and we are only beginning to understand the complex ways in which they adapt to each other. We have reported that a simple nutritional regime (forage vs. concentrate) applied early in life modified in lambs the bacterial population colonizing the rumen and that the effect persists over 4 months and have shown that treating lambs with chloroform (a potent inhibitor of methanogenesis) from birth up until weaning had significant effects on methane production and rumen function 4 months after the chloroform treatment stopped and there were still indications of altered rumen function 12 months after the treatment ceased. Clearly there is a need for more research in this area but if the concept that additives used in early life can affect rumen function in adult life can be confirmed then it will fundamentally change our approach to rumen manipulation.

Conclusion As yet the concept of precision control of the rumen microbiome is not possible, however as we continue to develop our ability to both describe and understand the factors that control the rumen microbiome it is perhaps an achievable dream.

The use of insects in the animal production sector with an insight into consumer perception of insects as food or feed and potential to replace antibiotics

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It is widely accepted that by 2050 the world will host 9 billion people. To accommodate this number, current food production will need to almost double. The livestock feed market is large and growing; global demand for animal feed is estimated to be worth £236 billion. There is a growing interest in alternatives to traditional livestock feed - soya bean, fish meal and other processed animal protein (PAP) – which bring significant environmental and, as a result, financial costs. Projections of global meat demand suggest massive increases so there is a concern to find new ways to address this. Alternatives have to have high protein content with the right amino acids and be digestible and palatable to the livestock.

The UN's Food and Agriculture Organisation has identified that insects could have a valuable role to play in this both as a component of human diets and as a source of feed for livestock. As result, there is a growing body of research into the many different aspects of this – usefully reviewed recently by Dossey *et al* (2016). There is a wide spectrum of research in the UK and Europe looking into sustainable protein, including calls through the Sustainable Agriculture and Food Innovation Platform, Innovate UK and Horizon 2020.

Studies have established that insects will not be able to match the nutritional characteristics of fish meal, however insects could become a major animal feed source. House fly and black soldier fly are rich in protein and have clear potential as a protein source in animal nutrition. Additional nutritional components that add value to insect products include fats/oils and vitamins & minerals. As a result, insect meals could partially replace fish meal for some livestock and may even be able to completely replace some vegetable or soy meals for monogastric livestock (pigs, poultry). The PROteINSECT programme conducted poultry feeding trials and the results indicated that no significant differences could be observed in animal performance. Insects are also highly efficient in the rapid conversion of a range of “waste” substrates into biomass and they require much less land than equivalent quantities of feed alternatives. Insect-based feed products could have a similar market to fishmeal and soy, which are presently the major components used in feed formulae for aquaculture and livestock.

The use of insects as feed is a relatively new practice on a commercial scale, and many questions remain to be tackled, particularly regarding safety concerns. However, the European Food Safety Authority (EFSA) scientific assessment of the possible use of insects in feed believe the evidence suggests that when currently allowed feed materials are used to feed insects, the possible occurrence of any microbiological hazards should not pose any additional risk compared to other feeds. In the European Union, the use of insects as a source of protein for animal feed for animals raised for human consumption is currently not possible due to requirements under Regulation EC 999/2001. Under EC regulation 1069/2009, insects reared for the production of Processed Animal Proteins (PAP) would currently be considered ‘farmed animals’ and are therefore prohibited from being fed on manure or catering waste. The Commission has indicated, however, that they will allow insect protein to be fed to fish by summer 2017. We expect this to be extended to other livestock shortly. The cost of farming insects is the second major factor making the insect protein a high cost alternative to traditional feeds and research is underway to develop technologies to make insect farming less labour intensive and more cost effective.

PROteINSECT carried out consumer perception research and found a high level of support for insects as a protein source in animal feed. They also found many who would like more information on this topic. On behalf of the UK Government Office for Science, Which also found similar views in the context of a discussion about options for addressing the major challenges facing food production – issues were raised about what the insects might be fed on.

One recent development that could significantly increase the value to the farmer (and consumer) of insects as feed is a technology, Immunity Generation, that can stimulate insects to create the antimicrobial peptides (AMPs) which enable them and creatures that eat them to resist diseases. This has been proven at scale by studies carried out by the School of Veterinary Science, University of Bristol. The studies focused on establishing resistance in poultry to *Campylobacter* and members of the family of bacteria that includes *Salmonella* and *Escherichia coli*. When stimulated, insects are fed to livestock, the AMPs are accepted by the poultry in their guts and confer immunity to a range of poultry associated diseases. This approach can be varied to stimulate the production of different AMPs, with the expectation that this would mean they could be targeted at different livestock and different pathogens. It could tested to see if it would work with other animals. Trials are also needed to see if it could be used to protect livestock from viruses such as avian flu.

Dossey, A. T., Morales-Ramos, J. A., Guadalupe Rojas, M. 2016. *Insects as Sustainable Food Ingredients - Production, Processing and Food Applications*. Elsevier.

Structure-activity relationships of condensed tannins and their effects on gastro-intestinal nematodes in livestock

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Application Several tannin-containing forages are nutraceuticals, i.e. possess nutritional and anti-parasitic properties and have potential for combatting the effects of gastro-intestinal nematodes (anthelmintic effects) in livestock (Hoste *et al* 2016). This review summarises the anthelmintic effects of different tannin types and links these to current evidence from animal feeding trials.

Introduction Plants produce an enormous diversity of different tannin compounds. Therefore, it is important to establish, which feeds and types of tannins are best able to interfere with the life cycle of gastro-intestinal parasites. With the aid of tests that can mimic several stages of the parasitic life cycle in the laboratory, we have identified the key structural features in tannins that are most inhibitory.

Materials and methods Recent research established new methods for purification of condensed tannins in mg to g quantities. This allowed *in vitro* testing of different tannin types against parasitic nematodes from ruminants (cattle, sheep, goats) and non-ruminants (pigs) with nematode species that reside in different parts of the digestive tract (*Ascaris suum*, *Cooperia oncophora*, *Haemonchus contortus*, *Oesophagostomum dentatum*, *Ostertagia ostertagi*, *Trichostrongylus colubriformis*) and also at different life stages and biological processes (e.g. egg hatching, exsheathment of infective larvae, motility of larvae and adults, and feeding of newly hatched larvae).

Results It generally holds true that high proportions of prodelphinidins and galloylation within tannins tend to lead to good anthelmintic effects (Ramsay *et al* 2016; Desrues *et al* 2016; Quijada *et al* 2015). However, longer tannin polymers also proved to be more potent against some gastro-intestinal nematode species than shorter polymers (Quijada *et al* 2015; Desrues *et al* 2016). It is thought that prodelphinidin-type tannins are more effective than procyanidin-types, as they possess more phenolic groups that can bind to parasite proteins, and longer tannins contribute most to protein aggregation and precipitation (Ropiak *et al* 2017). Taken together, this could explain why prodelphinidins, which generally (but not always) occur as larger polymers, tend to have better anthelmintic properties (Hoste *et al* 2016). Many tannins caused physical deformations on parasite surfaces and plaque formations around orifices (Desrues *et al* 2016; Kommuru *et al* 2015; Quijada *et al* 2015) and led to external and internal damage in nematodes (Ropiak *et al* 2016; Williams *et al* 2015). Interestingly, tannins in combinations with a few flavonoids and cinnamaldehyde acted synergistically against nematodes (Klongsiriwet *et al* 2015; Ropiak *et al* 2016), which opens new opportunities for enhancing the anthelmintic effects of tannins by selecting plant sources containing multiple bioactive plant compounds.

Conclusion We conclude that interdisciplinary research is needed as very few studies have linked plant composition and tannin structures with anti-parasitic effects in feeding trials. We also need to establish what the nutritional implications are of feeding anti-parasitic plants. Results by Cherry *et al* (2014) and Terrill *et al* (2012) suggest that some tannin-containing forages can be used to generate both nutritional and anti-parasitic benefits. It is hoped that such studies will contribute guidelines for bioactive feed formulations or for breeding of new bioactive plant varieties.

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References

- Cherry N M, Bullinger M, Lambert B D, Muir J P, Whitney T W, Miller J E and Sawyer J T 2014. *Journal of Applied Animal Nutrition* 2:e15.
- Desrues O, Fryganas C, Ropiak H M, Mueller-Harvey I, Enemark H L and Thamsborg S M 2016. *Parasitology* 143, 444–454.
- Hoste H, Torres-Acosta J F J, Quijada J, Chan-Perez I, Dakheel M M, *et al* 2016. *Adv Parasitol* 93, 239–351.
- Klongsiriwet C, Quijada J, Williams A R, *et al* 2015. *Internat J Parasitol: Drugs Drug Resist* 5, 127–134.
- Kommuru D S, Whitley N C, Miller J E, Mosjidis J A, Burke J M, Gujja S, *et al* 2015. *Vet Parasitol* 207, 170–175.
- Martínez-Ortíz-de-Montellano C, Arroyo-López C, Fourquaux I *et al* 2013. *Exp Parasitol* 133: 281–286.
- Quijada J, Fryganas C, Ropiak H M, Ramsay A, Mueller-Harvey I and Hoste H 2015. *J Agric Food Chem* 63, 6346–6354.
- Ramsay A, Williams A R, Thamsborg S M and Mueller-Harvey I 2016. *Phytochemistry* 122, 146–153.
- Ropiak H M, Desrues O, Williams A R, Ramsay A, *et al* 2016. *J Agric Food Chem* 64, 8795–8805.
- Ropiak H M, Lachmann P, Ramsay A, Green R J and Mueller-Harvey I 2017. *PLoS ONE* (accepted 11 Jan 2017).
- Terrill T H, Miller J E, Burke J M, Mosjidis J A and Kaplan R M 2012. *Vet. Parasitol.* 186, 28–37.
- Williams A R, Ramsay A, Hansen T V A, Ropiak H M, Mejer H, Nejsun P *et al* 2015. *Nature Sci. Rep.* 5, 14791.

Tannins and animal health: modulation of immunity and inflammation by dietary tannins during gastrointestinal parasite infections

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Application Tannins are a class of plant compounds with documented effects on animal health such as reducing gastrointestinal parasite infection. Here, we explore the effects of dietary tannins on host immunological responses during parasite infection and show that tannins may reduce inflammation and promote balanced immune responses. This may have broad relevance for animal health and production in an era of reduced synthetic drug usage.

Introduction Heavy reliance on the use of synthetic drugs to control bacteria and parasites is an increasingly important issue in livestock production, due to increasing reports of drug-resistance of zoonotic relevance as well as consumer demand for animal products produced with a minimum of synthetic chemical inputs. Bioactive diets, including forages or plant-based nutraceutical supplements containing bioactive secondary compounds, may play a role in reducing drug use by regulating pathogen burdens and promoting balanced immunological responses in the host (Xu *et al.*, 2012). Tannins are a widespread class of plant secondary compounds that are well-known for their antioxidant, anti-bacterial and anthelmintic effects. It is now well-established that tannins can exert anthelmintic effects through direct binding to and neutralization of parasites, and that this bioactivity is determined by a complex set of biochemical characteristics of the tannin molecule (Hoste *et al.*, 2016). We hypothesised that, in addition to direct anti-parasitic effects, low levels of dietary tannins may modulate the immunological and inflammatory response of the host to parasitic infection. Therefore, we have conducted a series of *in vivo* studies in pigs as well as *in vitro* experiments using primary cell cultures to test the effects of tannins on the initial host immune response to gastrointestinal helminth infection.

Material and methods For *in vivo* experiments, growing pigs (20-40 kg) were fed either a basal diet or a diet containing 5% tannin-containing grape pomace, resulting in a daily intake of around 4-6 g of condensed tannins, and then infected with the porcine roundworm *Ascaris suum* or left as uninfected controls. After 14 days infection, pigs were killed and tissue samples taken for histology, gene expression and metabolomic and microbiota analyses. For *in vitro* experiments, fresh human or porcine blood was used to generate primary macrophage or dendritic cell cultures which were then stimulated with purified tannins and/or parasite antigens. Cellular responses were determined by measurement of cytokine production, microarray analysis and fluorescence microscopy.

Results Dietary tannins modulated the host response to *A. suum* infection. Infected pigs fed tannins had higher levels of granulocytes and parasite-specific antibodies, with a decreased expression of inflammatory-related genes, indicating a shift from a pro-inflammatory environment to a more Th2-type environment which is important for protective immunity to parasite infection as well as wound-healing and mucosal repair. Tannins also modulated *A. suum*-induced alterations in the host metabolome and gut microbiome. To explore the mechanistic basis for these effects, cells were exposed *in vitro* to tannins which resulted in a suppression of multiple inflammatory pathways, and a shift towards regulatory/Th2-type cytokine production. These cellular responses synergized with the Th2-inducing activity of parasite antigens *in vitro*.

Conclusion Our results indicate a profound effect of dietary phytonutrients on immune function, and suggest that tannins (which may be found in many inexpensive dietary supplements) could play a role in reducing inflammation and promoting animal health and performance during gastrointestinal parasite infection.

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References

- Hoste H, *et al.* 2016. *Advances in Parasitology* 93, 239-351.
Xu Z, Du P, Mesier P, and Jacob C 2012. *Natural Product Communications* 7, 381-388.

Effects of tannins on N utilisation and energy partitioning in ruminants

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Application Dairy cows receiving sainfoin (*Onobrychis viciifolia*) based total mixed rations show evidence that nutrient utilisation is changed by redirecting their energy metabolism towards body protein accretion at the expense of body fat.

Introduction It is well established that condensed tannin containing forage crops like sainfoin can have beneficial effects on animal health, nutrition, and on lowering the environmental impact. This can be attributed to the condensed tannins present in the forage. Tannins have been reported to prevent bloat, exhibit anthelmintic properties, reduce enteric methane emissions and nitrogen (N) losses, improve fatty acid profile in milk and meat, and seems to alter energy partitioning (Purchas and Keogh, 1984; Mueller-Harvey, 2006; Girard *et al.*, 2016; Huyen *et al.*, 2016). Changes in fat deposition in lamb receiving lotus (*Lotus pedunculatus*; Purchas and Keogh, 1984) and changes in energy partitioning in sainfoin fed dairy cows (Huyen *et al.*, 2016) suggest condensed tannins may have a role in re-directing metabolic pathways in ruminants. Literature reports that tannins can be digested and absorbed, and after phase II metabolism can provide biologically active metabolites. This contribution briefly illustrates the fate of tannins following digestion, absorption and subsequent metabolism, and the possible mode of action how fatty acid and glucose metabolism is affected. This will be supported with data from an *in vivo* study with dairy cows in which we investigated the effects of replacing grass silage by sainfoin (*Onobrychis viciifolia*) silage in a TMR based diet on nitrogen utilization and energy balance in dairy cows.

Materials and methods The experiment followed a crossover design with two dietary treatments and six rumen cannulated lactating multiparous dairy cows with a metabolic body weight of $132.5 \pm 3.6 \text{ kg}^{0.75}$ (mean \pm SD), 214 ± 72 days in milk and an average milk production of $23.1 \pm 2.8 \text{ kg/d}$ at the start of the experiment. Cows were first allowed to adapt to the control diet (CON) for 7 days prior to the start of the experiment. The CON diet was composed of grass silage, maize silage, concentrates and linseed prepared as a TMR. Subsequently, cows were paired based on parity and milk production and among pairs randomly assigned to receive either a CON or a sainfoin based (SAIN) diet over two experimental periods of 25 days each. In the SAIN diet half of the grass silage was replaced by sainfoin silage. The cows were housed in tie-stalls for a 21-day period to adapt to restriction in movement and to the experimental diets. After the adaptation period, the cows were housed in climate controlled respiration chambers for 4 days to determine feed intake, apparent digestibility, metabolisability, milk production, nitrogen utilization and energy balance. Data were analysed using the mixed procedure of SAS with Diet and Period as fixed factors and Animal considered as random factor.

Results Total daily DMI was similar between the CON diet (17.8 kg/cow/d) and the SAIN diet (18.6 kg/cow/d). Milk yield averaged was 2.0 kg greater higher ($P = 0.042$) for the SAIN diet (24.1 kg/d) than for those on the CON diet (22.0 kg/d). Nitrogen intake, faecal nitrogen and nitrogen retention expressed in ($\text{g/kg BW}^{0.75}/\text{d}$) were higher ($P \leq 0.038$) in the SAIN diet (3.97, 1.37 and 0.27, respectively) than in the CON diet (3.53, 1.19 and 0.11, respectively). Nitrogen retention as a percentage of N intake tended ($P = 0.083$) to be higher in the SAIN diet (6.88%), compared to in the CON diet (3.21%). The GEI was similar among the two diets. However, the energy retention (ER, $\text{kJ/kg BW}^{0.75}/\text{d}$) as body mass was nearly 2 times higher ($P = 0.025$) in the CON diet cows (199.6) than in the SAIN diet cows (108.3). Interestingly, in term of the energy retained, the ER as body protein was higher ($P = 0.038$) in the SAIN diet (40.62) compared to the CON diet (16.3). In contrast, the ER energy retained as body fat was lower ($P = 0.007$) in the SAIN diet (67.7) than in the CON diet (183.3).

Conclusion This study suggested that sainfoin silage can be used in TMR diets for dairy cows in to improve nitrogen utilization and milk production. Moreover, sainfoin silage seems to redirect metabolism in late lactation dairy cows towards body protein accretion at the expense of body fat.

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References

- Girard, M., Dohme-Meier, F., Silacci, P., Ampuero Kragten, S., Kreuzer, M., and Bee, G. 2016. Journal of the Science of Food and Agriculture 96, 1923-1933.
- Huyen, N.T., Desrues, O., Alferink, S.J.J., Zandstra, T., Verstegen, M.W.A., Hendriks, W.H., and Pellikaan, W.F. 2016. Journal of Dairy Science 99, 3566-3577.
- Mueller-Harvey, I., 2006. Journal of the Science of Food and Agriculture. 86, 2010-2037.
- Purchas, R.W. and Keogh, R.G. 1984. Proceedings of the New Zealand Society of Animal Production 44, 219-221.