

Integrating QTL mapping, gene-expression and next-generation sequencing to unravel a QTL affecting meat quality in chicken

D J de Koning^{1,4}, J Nadaf^{3,4}, X Liu^{1,2}, X Li¹, E Le Bihan-Duval³, I Dunn⁴

¹Swedish University of Agricultural Sciences, Uppsala, Sweden, ²Uppsala University, Uppsala, Sweden, ³INRA, UR83, Recherches Avicoles, Nouzilly, France, ⁴The Roslin Institute, Edinburgh University, Edinburgh, UK

Email: dj.de-koning@shu.se

Introduction Genetical genomics has been shown to be a promising approach for dissecting complex traits. However high cost and therefore low sample size has been a problem. One strategy to overcome this problem is to focus on only one or a few identified QTL and select homozygote genotypes in the segregating population for the follow up gene expression study (targeted genetical genomic). In this study, we focussed on a QTL affecting the initial pH of meat on chicken chromosome1. Meat pH is one of the most important indicators of chicken meat technological quality, and can be indicative of changes in meat colour and meat drip loss.

Material and methods From 698 F2 chickens used for QTL mapping, gene expression profiles of 24 birds (12 from each homozygote genotype at the QTL) were investigated. RNA samples from breast muscle were assayed with Agilent 44K chicken microarrays using a dye-balanced design. Statistical analyses were performed in R using the limma routines in the Bioconductor library. The refined QTL region was subsequently analysed for putative causative SNPs (quantitative trait nucleotide or QTN) using next generation sequencing (NGS). Ten birds that were homozygous for either the high (QQ) or low (qq) QTL allele were selected for targeted sequencing. DNA was enriched for 1 Mb around the QTL using Agilent SureSelect Target Enrichment. These libraries were then sequenced using the Illumina Genome Analyzer. The gene expression analyses and NGS procedures were carried out by Ark Genomics (<http://www.ark-genomics.org/>).

Results We identified up to 16 differentially expressed genes in the QTL area (potential cis-eQTL). These were much more significant than any genes outside the QTL region (Figure 1). These genes span an area less than 1 Mb, reducing the QTL interval considerably. Several of these local signals corresponded to unknown genomic elements, while others corresponded to known genes, which could contribute to variation of the trait due to the QTL. Combining these results with analysis of downstream effects of the QTL (using gene network analysis) suggests that the QTL is involved in the pH variation by controlling oxidative stress. Selected genes have been confirmed using qRT-PCR. Using paired-end libraries with an average insert size of 300 kb, we obtained about 200X coverage for each of the 10 homozygote genotypes. The candidate SNPs were identified from key regions, such as the 5 base pairs flanking a splicing site, CpG islands, promoter sequences, transcription binding sites, utr regions, and exons.

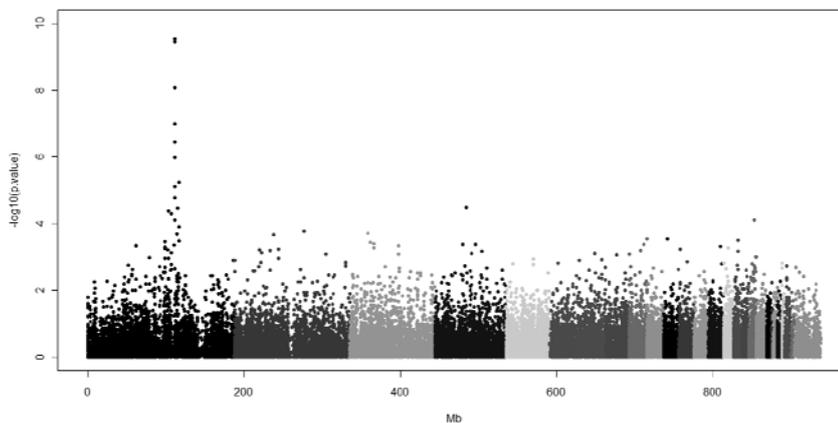


Figure 1 Gene expression effects of a QTL affecting meat pH on GGA1. Each dot represents a transcript from the microarray experiment. The X-axis indicates the location of the transcript on the chicken genome. The Y-axis shows the transformed P value of the t-test for differential expression.

Progress towards improving neonatal lamb survival and reproductive efficiency in Australia

F Brien¹, G Hinch², C Shands³, A Thompson⁴

¹South Australian Research and Development Institute, Roseworthy, South Australia, Australia, ²University of New England, Armidale, New South Wales, Australia, ³New South Wales Department of Primary Industries, Glen Innes, New South Wales, Australia, ⁴Department of Agriculture and Food WA, South Perth, Western Australia, Australia

Email: forbes.brien@sa.gov.au

Introduction Poor lamb survival is a major cause of reproductive inefficiency in sheep flocks in many countries. Since 2007, the Australian Cooperative Research Centre for Sheep Industry Innovation (the Sheep CRC) has had a major project on reproduction efficiency to help industry wean an extra 1 million lambs per year by 2014 from the national flock, the key target being improved neonatal lamb survival. In addition, work is also directed at improving post-weaning survival. This paper provides an overview of the project, its key findings and achievements to date, focussing on neonatal lamb survival.

Outline of Sheep CRC Reproduction Efficiency project The project has research and technology transfer activities. Research is focussed on improving lamb survival by genetic means, largely using data generated from the Sheep CRC's Information Nucleus (Banks *et al.* 2006). A number of PhD projects have also been conducted, researching the physiology and genetic control of thermogenesis and vigour of new-born lambs. Technology transfer consists largely of producer training courses on optimal ewe and lamb nutrition (based on the findings of the Lifetime wool project, Trompf *et al.* 2011) and workshops conducted in conjunction with providers of ultrasound scanning services for pregnancy and litter size diagnosis in sheep flocks (scanning contractors).

Findings and Achievements to Date *Research.* From analyses of Information Nucleus data, the direct heritability of lamb survival to weaning (LSW) was estimated at only 0.01, however LSW had moderate genetic correlations with crown to rump length, rectal temperature and time taken for the new-born lamb to bleat after release, with these traits having heritabilities of 0.30, 0.10 and 0.11, respectively, much higher than LSW itself (Brien *et al.* 2010). These parameter estimates have been confirmed in subsequent analyses with more data and improved pedigree information that have allowed the partitioning of maternal variance components, with maternal heritability of LSW estimated to be 0.04. This suggests there is additional scope for genetic gain through improved maternal ability. Indirect selection as a supplement to direct selection is predicted to substantially improve genetic gain compared to direct selection alone (Brien *et al.* 2010). Further, progeny-testing of ram lambs for LSW could be a useful way of increasing genetic gain for lamb survival and especially the number of lambs weaned per ewe joined – net reproduction rate (Brien *et al.* 2011). Further work is underway to investigate the impact of causes of lamb death and environmental conditions at lambing on the interpretation of results.

Technology transfer. By the end of 2010, 221 producers had completed Lifetime Ewe Management (LTEM) courses, which incorporate the research findings of the Lifetime wool project, economic modelling and producer guidelines. The 182 participants surveyed had increased their whole-farm stocking rates by 14%, increased lamb marking percentages by 11–13% depending on enterprise type, and decreased ewe mortality rates by 43% (Trompf *et al.* 2011). Two-thirds of the increase in lambing marking percentages was attributable to improvements in lamb survival (Trompf pers. comm.). A further 402 producers have completed or are currently completing LTEM courses as of January 2012, taking the total number to over 600 producers undertaking LTEM courses since they were first offered (D. Hallam, pers.com). As of the end of 2011, 55 workshops half-day workshops focussing on improving reproductive efficiency in sheep flocks had been conducted around Australia, attended by 1,132 producer clients of scanning contractors. Collectively, the producers attending workshops own 2.18 million sheep, approximately 3% of the national flock. Based on survey responses from 43 workshop participants, 51% stated that attending workshops had a moderate to major impact on the reproduction rate of their sheep flocks. A database of the results generated by scanning contractors is being developed for national use. This database will be able to be used for evaluation purposes, with the ability to monitor the achievement of regional and breed-specific targets (S. Hatcher, pers. com.). Producers can also undertake training through the Making More From Sheep program (<http://www.mla.com.au/research-and-development/extension-and-training/making-more-from-sheep>, accessed 23 January, 2012), which has a specific module “Wean More Lambs” that provides information on reproductive efficiency. Producers completing this module are encouraged to undertake the more comprehensive LTEM course.

Conclusions Results suggest that modest genetic gain in lamb survival is possible, but selection accuracy needs to be improved by information from correlated traits and relatives. Progeny-testing of ram lambs for lamb survival is a possible way of improving net reproduction rate. Finally, steady progress is being made in increasing the adoption rate of sheep nutrition targets through Sheep CRC training courses and this is leading to improvements in lamb survival, net reproduction rate and farm profitability.

References

- Banks, R.G, van der Werf, J.H.J. and Gibson, J.P. 2006. Proceedings of the World Congress on Genetics Applied to Livestock Production. Communication 30-12.
- Brien, F.D, Hebart, M.L, Smith, D.H, Hocking-Edwards, J.E, Greeff, J.C, Hart, K.W, Refshauge, G, Bird-Gardiner, T.L, Gaunt, G, Behrendt, R, Robertson, M.W, Hinch, G.N, Geenty, K.G. and van der Werf, J.H.J. (2010). *Animal Production Science* 50, 1017-1025.
- Brien, F.D, Hinch, G.N, van der Werf, J.H.J, Brown, D.J. and Swan, A.A. (2011). Proceedings of the Association of Animal Breeding and Genetics 19, 151-158.
- Trompf, J.P, Gordon, D.J, Behrendt, R, Curnow, M, Kildey, L.C. and Thompson, A.N. (2011). *Animal Production Science* 51, 866-872.

Using systems biology to understand feather patterns in poultryD Headon¹, K Wells¹, K Painter², A Cahaner³, Y Hadad³, D Ben-Avraham³, J Hillel³¹The Roslin Institute, Edinburgh, UK, ²Heriot-Watt University, Edinburgh, UK, ³The Hebrew University of Jerusalem, Jerusalem, Israel *Email: denis.headon@roslin.ed.ac.uk*

Introduction The extent of feathering on broiler chickens is associated with production in hot conditions and several natural mutations exist that reduce feathering, providing heat tolerance benefits. Feather density is established prior to hatching, with theoretical work going back several decades highlighting the likely role of signalling molecules with mutually antagonistic functions in defining feather size and location. We have been using genetic approaches to identify the molecular nature of mutations that influence feather coverage in the chicken, followed by experiments aimed at understanding the mechanisms that control feather density and extent. By taking a systems approach we are getting a picture of how feather density and size are set down in the embryo.

Methods We use genetic mapping and sequencing to identify mutations that cause reduced feather coverage. We then explore the roles of the molecules thus identified and their integration into signalling networks by experimental manipulation of cultured embryonic chicken skin. Treatment of skin with different pharmacological agents or proteins alters feather patterning, which we visualise by *in situ* hybridisation. Computer simulations are then used to interpret data and design further experiments.

Results I will summarise our identification of mutations that influence feather coverage in the chicken, such as Naked neck (Mou *et al.*, 2011), and illustrate how we explore the roles of the molecules altered by mutation in skin development. This approach has allowed us to define small networks of interacting signals with the potential to produce a wide variety of feather (and other) patterns. Such networks require relatively small tweaks to produce a range of feather sizes and densities, and searching for these kinds of changes underlying different feather characteristics on different body sites of an individual bird, or defining different feather patterns between individuals, is now our major area of interest.

Acknowledgements This programme of work is supported by the BBSRC

Reference

Mou, C., Pitel, F., Gourichon, D., Vignoles, F., Tzika, A., Tato, P., Yu, L., Burt, D.W., Bed'hom, B., Tixier-Boichard, M., Painter, K.J., and Headon, D.J. 2011. PLoS Biology 9, e1001028.

One Health – One Welfare: Can we sustain welfare standards in a food hungry world

N Waran

Jeanne Marchig Professor of Animal Welfare. Director (Jeanne Marchig International Centre for Animal Welfare Education). Royal (Dick) School of Veterinary Studies, University of Edinburgh, Midlothian, Scotland, UK

Email: natalie.waran@ed.ac.uk

By 2050, the world population is predicted to rise from 7 billion to 9.1 billion including 7 billion people living in the developing world, where 1 billion of those people are unable to satisfy their basic daily energy requirements. It is estimated that food production must double if we are to feed this growing population. In addition, alongside this population growth is a shift in consumer expectations, with greater urbanisation and changing food preferences which have led to an increasing demand for high quality and affordable animal derived food products. Although food security is a major global concern, food safety issues cannot be ignored if we are to ensure the health and welfare of this expanding human population. There is increasing recognition that emerging health issues are linked to increasing contact between humans and wildlife, intensification and integration of food production and the increasing numbers of companion (pet) or stray animals living in close proximity with humans. It is claimed that many zoonotic threats can be traced to the globalized, industrialized production of meat and animal products.

There is a growing body of scientific evidence to show that animal welfare is intrinsically linked to human welfare. As with human health and welfare, animal welfare involves safeguarding the physical and psychological health of an animal, with good animal welfare practises designed to minimise the suffering of animals over which we have a duty of care. It is important to recognize that promoting best practice in welfare goes beyond an ethical obligation since it is also essential for improving public and environmental health. At the level of the individual animal, it has been shown that animals in a poor state of welfare do not perform to the best of their ability, with poor health and lowered production related to poor animal management, handling, transport and housing conditions. There is a growing appreciation that higher animal welfare standards have both a direct and indirect impact on improvement in food safety, food quality and public health issues.

Is there a conflict between increasing food production in order to feed a growing world whilst at the same time safeguarding animal welfare standards or should it be our aspiration to achieve both? To address this challenge, possible solutions include; increasing productivity of available land, improved use of technology, greater intensification of production methods, improved breeding and feeding, improved management of herd and individual health and welfare, enhanced awareness and control of food waste and changing attitudes towards diet preferences. In exploring these possibilities I will highlight opportunities and limitations and discuss the concept of ‘One Health- One Welfare’ as the underpinning philosophy which needs to be adopted if we are to protect and enhance human and animal welfare in a food hungry world.

A multivalent vaccine and single platform diagnostic for bacterial respiratory disease in pigs: progress so far

A Tucker¹, P Langford², A Rycroft³, B Wren⁴, D Maskell¹

¹University of Cambridge, Cambridge, UK, ²Imperial College, London, UK, ³Royal Veterinary College, London, UK, ⁴London School of Hygiene and Tropical Medicine, London, UK Email: awt1000@cam.ac.uk

Background Respiratory infections constitute one of the most important endemic diseases of growing pigs at a global level. Endemic diseases of pigs in general tend to be accommodated by producers and herd veterinarians and yet they impact significantly on pig welfare, public health and production efficiency. Poor health contributes to lower welfare through general debility and reduced competitiveness. Examples of specific welfare effects of low health include pain linked to the pathology of streptococcal arthritis or pleurisy, reduced feed and water intake consequent to systemic illness, and ultimately increased mortality consequent to disease.

Poor pig health impacts on human health through increased shedding of both food-borne and directly zoonotic pathogens and through increased use of antimicrobials. While the important bacterial food-borne infections can be shed without clinical disease, five separate studies have shown that a history of recent antimicrobial treatment for endemic disease control was linked to increased shedding of *Salmonella* in fattening pigs at slaughter, and seven studies found that intercurrent disease (such as enteric or respiratory infections) had a similar effect (Fosse *et al.*, 2008). The relationship between low health and increased antimicrobials use is perhaps obvious but not well documented. Farms with long-term moderate respiratory disease (>10% prevalence of pleurisy in slaughter pigs) were more likely to have 2 or ≥ 3 ongoing group level medications compared to control farms (odds ratio 3.6 and 9.6 respectively) (Jäger *et al.*, 2012).

The impact of pig health on production efficiency cannot be over-emphasised. These losses can arise through diverse routes such as reproductive loss, mortality, and also reduced feed conversion efficiency or reduced feed intake leading to longer finishing periods. As examples, Jäger *et al* reported a 0.26 day extension to slaughter weight for every 1% increase in prevalence of pleurisy in UK slaughter pig batches (Jäger *et al.*, 2010). Baekbo *et al* (2002) found that each 1% of lung volume obliterated by enzootic-pneumonia like lesions contributed a 7.1g/day reduction in daily gain between 20 and 24 weeks of age (Baekbo *et al.*, 2002).

While there has been good penetration of the pig industry by *Mycoplasma* vaccines, application of vaccines to control other respiratory bacterial infections has been much less impressive – primarily due to lack of cross-protective and robust field efficacy for those products that have reached the market. Emerging genomics-based technologies provide an excellent opportunity to develop a new generation of multivalent vaccines with optimised local immune responses, and a complementary set of molecular-based diagnostic and epidemiological tools.

This paper gives a progress report on aspects of a collaborative project aimed at the development of better tools for the diagnosis and vaccine-based control of pathogenic respiratory infections of pigs. The paper focuses on *Streptococcus suis* and *Haemophilus parasuis*, but this is a multicentre project also including work on *Actinobacillus pleuropneumoniae* and *Mycoplasma hyopneumoniae* through partner institutions at Imperial College, the Royal Veterinary College and the London School of Hygiene and Tropical Medicine.

Methods Complementary strategies of population biology and functional genomics are being applied to identify diagnostic targets and candidates for recombinant subunit and/or live attenuated vaccine approaches for *S. suis* and *H. parasuis*. Population biology approaches included the generation of large archives (>200 isolates) of each organism with detailed clinical and antimicrobial sensitivity history. Next generation, high throughput sequencing is being applied to produce sequence databases for analysis of phylogeny, recombination characteristics and clinical associations. Functional genomics approaches are based on the use of transposon directed insertion site sequencing (TraDIS). This requires the construction of large pooled (10^4 - 10^5) libraries of unique mutants, generated using transposons – small mobile genetic elements that insert and disrupt single genes at random. Next generation sequencing technology is then applied to these libraries both before and after exposure to an ex vivo model of colonisation. By reading the DNA sequences from the transposon out into the disrupted gene for each mutant, and then quantifying the relative frequency of each mutant in the pool before and after exposure to the biological system, TraDIS can identify and prioritise genes essential for survival or colonization (Langridge *et al* 2010). Vaccines targets will progress to evaluation in live animal protection studies, while diagnostic and epidemiological markers capable of discriminating disease- from non-disease related strains will be evaluated in field trials.

Results to date Genomic sequencing of more than 200 isolates each of *S. suis* and *H. parasuis* has been completed and sequence databases have been built to permit ongoing in depth analysis. TraDIS libraries have been generated from pools of *S. suis* mutant libraries harvested before and after exposure to ex vivo nasal and tracheal pig organ culture systems. Available results will be presented and discussed at the meeting.

Acknowledgements This project is funded by the BBSRC (Longer and Larger Initiative), with industrial support from Pfizer Animal Health.

References Baekbo P, Andreason M, Wachmann H. (2002) Proceedings of the 17th Congress of the International Pig Veterinary Society Ames, Iowa. pp. 103.

Fosse J, Seegers H, Magras C (2008) Vet Research 39, 1.

Jäger HJ, McKinley T, Wood JLN, Pearce GP, Williamson SM, Strugnell BJ, Done S, Habernoll H, Tucker AW (2010) Proceedings of the 21st Congress of the International Pig Veterinary Society, Vancouver. pp. 191.

Jäger HJ, McKinley T, Wood JLN, Pearce GP, Williamson SM, Strugnell BJ, Done S, Habernoll H, Tucker AW (2012) PLoS One accepted for publication.

Langridge GC, Phan MD, Turner DJ, Perkins TT, Parts L, Haase, J., Charles, I., Maskell, D. J., Peters, S. E., Dougan, G., Wain, J., Parkhill, J. and Turner, A. K. (2009) Genome Research 19, 2308-2316.

Role of animal-derived foods in an obesogenic environment

M Gibney

UCD Institute of Food & Health, University College Dublin, Ireland *Email: mike.gibney@ucd.ie*

Before one can begin to define a putative role for any food category in the current epidemic of obesity, a number of key issues need to be addressed in the context of any long term approach to this public health nutrition. Firstly, the rate of increase in the levels of obesity is often portrayed as being of recent origin and also linear in form. This will be challenged with data that show a wave-like growth in the levels of obesity with periods of strong growth followed by periods of stability. A second area that must be addressed is the genetic component of obesity. Very strong data exists to show that obesity and over-weight are strongly heritable. Indeed, in many countries for which data are available, there is evidence that the rate of obesity has levelled off indicating that the obesogenic environment has met its full genetic potential. The third area that must be considered is that of physical activity and its ability at moderate levels to abate most if not all of the risk factors arising from obesity such as diabetes and hypertension. Finally, we must face the fact that food intake data suffers significantly from energy under-reporting which is not confined to any specific food group but covers all categories of foods ingested. Thus we cannot know the extent to which food intake is distorted by under-reporting since under-reporting can involve the denial of ever consuming a given food, misreporting frequency of consumption and also misreporting portion size. It is impossible to see how these discrepancies can be corrected by any statistical modeling.

Against that background, it is unlikely that any one food group can play a unique role in the development of obesity. It is possible to explore the role of specific nutrients that are mainly derived from animal foods in aspects of obesity. For example, the role of vitamin D in the development of Type 2 diabetes is highly controversial and has been played down by the US Institute of Medicine. Equally, calcium intakes have been linked both to the development of obesity and to the issue of weight loss. Some interesting work with fatty acids such as palmitoleic acid and conjugated linoleic acid will be discussed.

Can milk and dairy products be an aid to body mass index control?

A Dougkas

Lund University, Lund, Sweden Email: anestis.douglas@appliednutrition.lth.se

Introduction As the incidence of obesity is reaching ‘epidemic’ proportions there is currently widespread interest in the impact of dietary components on body weight and food intake. The majority of data available from both epidemiological and intervention studies provide evidence of a negative but modest relationship between milk product consumption and body mass index (BMI) or other measures of adiposity (Dougkas *et al.*, 2011). Furthermore, accumulating evidence indicates an impact of dairy constituents, in particular whey protein derivatives, on food intake regulation and satiety. However, there is a paucity of evidence that considers the effect of different types of dairy products on adiposity, appetite and overall energy intake. The aim was to provide an insight into the likely contribution of dairy foods to strategies aimed at body weight and appetite regulation.

Material and methods Two research approaches including an epidemiological analysis and an acute human appetite study were used. The epidemiological cross-sectional analysis examined the relationship between milk, cheese, cream and butter consumption and BMI. It was based on data from the Caerphilly study, a cohort of 2512 men, aged 45–59 years at recruitment (phase I), which was followed for 10 years with 5-year intervals (phases II and III) (Elwood *et al.*, 2009). In the acute cross-over study, forty overweight men [age: 32 (SD 9) years; BMI (kg/m²): 27 (SD 2)] attended four sessions one week apart and received three isoenergetic (830 kJ) and isovolumetric (410 ml) servings of dairy snacks or water (control) 120 minutes after breakfast. Subjective appetite ratings (hunger, desire to eat, fullness and prospective consumption) were determined using visual analogue scales (VAS) throughout the morning, and *ad libitum* energy intake as a lunch-time meal was assessed 90 minutes after the intake of snacks.

Results Dairy consumption was inversely associated with BMI and examination of the individual dairy products showed that most of the positive effect is due to milk, with the BMI of high milk consumers (>568ml/d) 1.2 to 1.4kg/m² less compared with non-milk consumers (Table 1). Furthermore, results from the acute appetite study showed that among the milk products, yoghurt had the greatest impact on suppressing appetite. Hunger rating was 8%, 10% and 24% ($P<0.001$) lower after intake of yoghurt compared with intake of cheese, milk and water respectively, although there were no differences in energy intake (Figure 1).

Table 1 BMI According to milk intake and phase I

	None	≤284ml/d	>284- ≤568ml/d	>568ml/d	P
Milk intake (ml/d)	0	284	426	625	
Phase I					
BMI (kg/m ²)	26.9±0.8	26.3±0.7	26.0±0.8	25.6±0.8	0.001
Phase II					
BMI (kg/m ²)	27.0±0.9	26.5±0.9	26.1±1.0	25.6±1.2	0.014
Phase III					
BMI (kg/m ²)	27.4±1.0	26.8±1.1	26.5±1.1	26.2±1.3	0.070

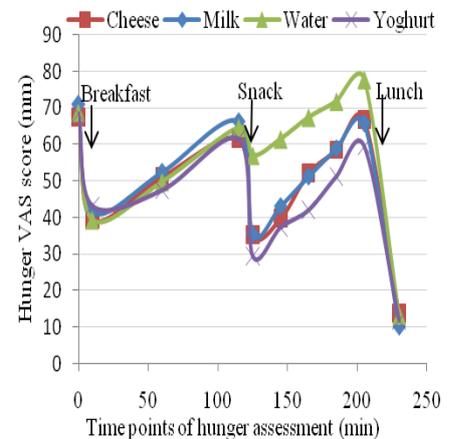


Figure 1 Hunger ratings over the morning

Conclusions These findings are in agreement with the suggested favourable effects of dairy consumption on body fatness but provide new evidence that most of the effect is associated with drinking milk. Results also showed that yoghurt had the greatest effect on suppressing subjective appetite ratings, but did not affect subsequent food intake compared to milk or cheese. Future human studies are required to verify the potential benefits of specific dairy products on weight or appetite regulation and weight loss and provide an insight into the underlying physiological mechanisms.

Acknowledgements The present research was funded jointly by the Barham Benevolent Trust Studentship, DairyCo UK and The Dairy Council UK

References

Dougkas, A., Reynolds, C.K., Givens, D.I., Elwood, P.C. and Minihane, A.M. 2011. *Nutrition Research Reviews*. 15, 1-24.
Elwood, P.C., Pickering, J.E. and Fehily, A.M. 2007. *Journal of Epidemiology and Community Health*. 61, 695–698.

Dairy products and vascular disease prevention: Evidence from prospective studies

P C Elwood

Institute of Primary Care & Public Health, Cardiff University School of Medicine, Cardiff, UK

Email: pelwood@doctors.org.uk

Introduction The belief is widespread that the consumption of milk and dairy foods increases the risk of a heart attack. This perception seems to be based largely on the fact that milk drinking raises cholesterol level.

The consumption of dairy foods does raise cholesterol level, but this observation comes from short term studies, and in most of these the rise in cholesterol is actually relatively small. Furthermore, a fall in blood pressure which has been shown in many similar studies, is usually ignored.

Both these outcomes – cholesterol level and blood pressure – are ‘intermediate’ variables, or ‘surrogate’ measure of health, and no such measure can be a valid basis for balanced and overall conclusions about the health consequences of the consumption of any food item. Nor should any such evidence be a basis for food policy.

Dependable judgements about diet and health can only come from large, long-term studies of large samples of subjects, for whom dietary intake and other life-style factors are recorded, and who are then followed forwards in time so that disease events can be identified and deaths recorded and related to former milk or dairy consumption. Such studies are known as ‘cohort’, or ‘prospective’ studies. Although even more dependable evidence would be obtained from a randomised controlled trial, with death and disease incidence as outcomes, any such a study would have to be very large and long-term, well beyond the resources of any research group. The very best evidence therefore come from an ‘overview’ or ‘meta-analysis’ in which an attempt has been made to identify every cohort study ever reported, and a combing or pooling of the results in all of these, to give an overview of relationships.

Material and methods Medline, an electronic record of medical literature was searched, using a variety of key words (‘milk’ ‘dairy’ etc. as predictors; and ‘heart disease’, ‘stroke’, ‘diabetes’ etc as outcomes). Reports which related to both these groups of key words were inspected and the references in the selected reports were also inspected for further relevant reports. In the end, 24 reports were selected for inclusion in ‘overviews’ relating to the various disease outcomes. The overview of these 24 cohort studies¹ are summarised below. It is the largest yet reported, and the results are in general agreement with the reports of earlier, more limited overviews.²⁻⁵

Results The numbers of clinical events upon which conclusions are based are: 16,212 heart disease events; 9,725 strokes; 7,121 new cases of diabetes and 5,902 deaths. Compared with the fifth (20%) of subjects who consumed little or no milk/dairy, the fifth of subjects (20%) with the highest dietary intakes of milk/dairy show reductions of....

about 8% in heart disease risk (but possible only about 1%)

about 20% in the risk of a stroke (but possibly only about 10%)

about 15% in the risk of diabetes (but possibly only about 4%)

Conclusions Proof is of course elusive, but the benefit indicated above appears to go beyond all reasonable doubt. Thus there is no valid evidence of any harm to health from milk and dairy consumption in terms of the risks of disease, indeed there is evidence consistent with reductions in vascular disease, diabetes and all-cause deaths in subjects who drink the most milk.

References

- Elwood PC, Pickering JE, Givens DI, Gallacher JE. The consumption of milk and dairy foods and the incidence of vascular disease, diabetes: an overview of the evidence. *Lipids* 2010; 45,925-33.
- Gibson, R. A., Makrides M, Smithers LG., Voevodin, M., Sinclair, A. The effect of dairy foods on CHD: a systematic review of prospective cohort studies. *British Journal of Nutrition* 2009; 96, 1-9
- Mente A., de Koning L., Shannon HS., Anand SS. A systematic review of the evidence supporting a causal link between dietary factors and coronary heart disease. *Archives of Internal Medicine* 2009; 169,659-69
- Pittas AG., Lau J., Hu FB., Dawson-Hughes B. The role of vitamin D and calcium in Type 2 diabetes. A systematic review and meta-analysis. *Journal of Clinical Endocrinology & Metabolism* 2007; 92, 2017-29.
- Soedamah-Muthu SS, Ding EL, Al-Delaimy WK, Hu FB, Engberink MF, Willett WC, Geleijnse JM. Milk and dairy consumption and incidence of cardiovascular diseases and all-cause mortality: dose response meta-analysis of prospective cohort studies. *American Journal of Clinical Nutrition* 2011; 93,158-71

The benefits and limitations of fish in the diet

C Ruxton

Nutrition Communications, Cupar, UK *Email: carrie@nutrition-communications.com*

In 2004, UK Government advice stated that consumers should increase fish consumption to two portions a week, one of which should be oily fish. However, data from the National Diet and Nutrition Survey suggest that this advice has not been acted upon by most consumers. Average fish intakes amongst adults are equivalent to 1.85 portions a week, but oily fish contributes only a third towards this figure in regular fish consumers, rather than the 50% recommended for the whole population. Fish intakes amongst children, particularly teenagers, are much lower.

The 'two-a-week' message is underpinned by strong scientific evidence in relation to heart health, where fish nutrients, such as long-chain *n*3 polyunsaturated fatty acids (LC*n*3PUFA), appear to reduce inflammation, reduce serum triglycerides levels, and support normal blood pressure and insulin sensitivity. Weaker evidence links fish consumption and key fish nutrients with a lower risk of immune dysfunction, normal brain development in infants, and maintenance of cognitive function in later life. Certain nutrients found in fish, such as LC*n*3PUFA, protein, selenium, iodine, zinc, potassium, vitamin D and B vitamins have been the subject of approved European Article 13.1 health claims.

Limitations of fish consumption have also been identified, including the risk of contaminants in certain species, such as swordfish, marlin and tuna, and issues around sustainability. Risks were considered in 2004 by the UK Committee on Toxicology resulting in warnings to pregnant women to avoid or limit certain species of fish. The population risks were taken into account for the two-a-week message. The Food Standards Agency consulted on potential tensions between sustainability and the two-a-week message in 2009 but made no change to the recommendation, although consumers were encouraged to seek sustainable options when purchasing fish.

Clearly, further promotion of the two-a-week message is needed to ensure that greater numbers of people respond. Further work is also needed to address barriers to fish consumption, particularly amongst those with the lowest intakes, such as teenagers, young adults and people on low incomes.

Red meat in the diet: is it as bad as we have been told?

L Wyness

British Nutrition Foundation, London, UK Email: l.wyness@nutrition.org.uk

Consumers have been faced with many conflicting messages from the media about the health effects of eating red meat. Views often held by the general public are that meat can cause cancer, meat is high in fat and we should avoid eating meat to benefit the environment. Consumers are increasingly conscious of the relationship between food and their health. Therefore it is important that information and messages to consumer and to the media are clearly and effectively communicated.

From a nutritional perspective, red meat contains high biological value protein and important vitamins and minerals, all of which are essential for good health throughout life. According to European Union health claims legislation, beef, lamb and pork can be classified as a *source* or a *rich source* of several nutrients. For example, lean beef is a *source* of vitamin B₂ (riboflavin), iron, potassium and phosphorus and a *rich source* of vitamin B₃ (niacin), vitamin B₁₂ and the zinc. Some of these nutrients, e.g. iron and zinc, are more bioavailable in meat than alternative food sources. These, and some other nutrients, have been identified as being in short supply in the diets of some sectors of the UK population, such as infants and women of childbearing age; therefore red meat can make an important contribution to intakes.

There have been notable changes recently to the fat content and composition of red meat. For example, the fat content of beef has decreased by around 15% over the past few decades, and considerable progress has been made to enhance the fatty acid profile of beef, for example by decreasing the saturated fatty acid (SFA) content and increasing the polyunsaturated fatty acid (PUFA) content. A high intake of SFA by humans is associated with raised cholesterol levels, which can increase the risk of heart disease. The PUFAs in beef are predominantly linoleic acid (*n*-6) and alpha-linolenic acid (*n*-3) which are considered to be essential. Beef also provides very small amounts of the long-chain *n*-3 PUFAs eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), for which there are very few rich dietary sources apart from oily fish. These long-chain PUFAs have been shown to have favourable effects on heart health.

There is often confusion among consumers regarding the effects of red meat in relation to cancer. In response to the Scientific Advisory Committee for Nutrition (SACN) report (2010), the Department of Health issued new advice for consumers about the amount of red and processed meat that can be consumed as part of a healthy, varied diet. The advice is that people who eat more than 90g (cooked weight) of red and processed meat a day should cut down to 70g/day. This advice is based on evidence that suggests a probable link between eating red and processed meat and a risk of colorectal cancer. However, the advice stresses that red meat, such as beef, lamb and pork, can form part of a healthy diet and that it is a good source of protein, vitamins and minerals.

With increasing demand for food, increasing pressure on natural resources and concern about the associated impact on the environment, the question of whether meat can be part of a sustainable diet is currently being debated. There are many issues to consider when defining the attributes of a sustainable and healthy diet that has a low impact on the environment. Socio-economic and environmental issues need to be considered along with the nutritional contribution that foods such as lean meat can make to a healthy diet.

Key issues regarding neonatal mortality in farmed livestock

S A Edwards

Newcastle University, Newcastle upon Tyne, UK *Email: sandra.edwards@ncl.ac.uk*

Introduction Neonatal mortality in farmed mammalian livestock (pigs, cattle, sheep, goats, rabbits), resulting from death during parturition, occurring shortly after birth or predisposed by fetal and early life events, typically results in a 10–20% loss rate of offspring. It is thus a major source of economic loss to the agricultural sector worldwide, and a subject for animal welfare concern. Recent trends have increased the importance of this problem. Economic pressures have resulted in larger herd sizes, with reduced staff available to provide individual animal attention. Breeding of animals for improved production performance in terms of prolificacy, growth rate, carcass meat yield or milk production has resulted in an increased incidence of placental dysfunction, neonates which are less mature at birth and mothers which show increased parturition difficulty and poorer maternal behaviour. Research into the causes and minimisation of neonatal mortality in farm livestock has been carried out for many years, but has failed to make a global impact on this intractable problem. This is partly because of the increasing challenges outlined above, but also because the research has often addressed only a limited aspect of what is a very complex issue, involving interacting factors associated with the neonate, the mother, the birth environment and the nature of human interventions. At a more fundamental level, the issues encompass many questions with generic relevance across different scientific fields, including the role of embryonic programming, the nature of neonatal vitality and viability, and the regulation of maternal behaviour and lactation.

Reasons for mortality These vary in importance between species, but share common factors which are significant for welfare. In all species, deaths as a direct or indirect result of asphyxia during the birth process contribute significantly to losses. Such deaths are often associated with a difficult or prolonged parturition, and therefore also compromise the welfare and performance of the mother. Subsequent neonatal mortality arises most commonly from the interactive causes of hypothermia, starvation, infection and physical trauma. These may be predisposed by a lack of physiological maturity and vigour of the neonate at the time of birth, and exacerbated by inadequate maternal care from the dam.

Animal characteristics Birthweight is a major determinant of neonatal viability in most species, with excessively small or large offspring both experiencing increased risk of mortality. However, vitality is also apparent as a trait independent of birthweight, and linked to vigour and thermoregulatory ability. More recent research has highlighted the importance of proportionality (shape) as a predictor of survival, emphasising the role of placental function and nutritional adequacy during fetal developmental. In most farm species, early colostrum consumption by neonates is essential for achievement of both energy balance and passive immunity. This depends not only on the neonate's ability to suckle and utilise colostrum, but also on the physiology and behaviour of the dam. Recent work has increased knowledge on the genetic component of a range of key survival traits in both dam and offspring.

Environmental challenges In extensive systems, neonates face challenges from their environment in respect of climatic extremes and predation. In the absence of significant human intervention, survival can only be improved by modification of the intrinsic biological characteristics of the animals themselves. The consequences of imbalances between increased genetic production potential and unimproved low-cost environments may be severe. Thus, understanding the genetic and developmental influences on the relevant characteristics of the animals, and the ways in which they interact, is key to progress through approaches such as modification of genotypes, maternal nutritional inputs or social experiences. In systems where livestock are farmed more intensively, with generally the more prolific species and less developed offspring, external interventions to improve survival are possible. Modification of the birth environment to incorporate artificial aids to survival such as shelter, supplementary heat or protection from crushing have resulted in significant improvements, but also raised welfare issues related to the degree of restriction of behavioural freedom with which such environmental controls are often associated. Understanding the biological mechanisms which underlie these conflicts, and developing innovative practical approaches in building design and engineering to reduce them, is a major scientific challenge.

Human interventions The extent and nature of possible human intervention in the periparturition period is often dictated by the nature of the birth environment because of considerations of practicality, economics and safety. It is known that human-animal interactions before, during and immediately after parturition can be influential. These influences can be both positive, by aiding the parturition process and caring for neonates of marginal viability, but also negative as a result of maternal disturbance or inappropriately timed intervention in the course of delivery. Beneficial interventions require increased labour inputs from skilled staff, but the financial cost-benefit of such activities in different circumstances is a subject requiring better evaluation.

Conclusions Solutions to the intractable problem of neonatal mortality require multidisciplinary approaches, recognising that basic mechanistic understanding must be combined with applied research and economic cost-benefit analyses to synthesise the most appropriate solutions for different species and farm circumstances.

Maternal diet during pregnancy and neonatal survival – new insights into brown adipose tissue (BAT) regulation and function

M Symonds, H Budge

The University of Nottingham, Nottingham, UK *Email: michael.symonds@nottingham.ac.uk*

Since the rediscovery of BAT in adult humans in 2007 there has been a dramatic resurgence in research interest on its role in heat production and thus energy balance. This has coincided with a reassessment of the origins of BAT and the suggestion that brown preadipocytes could share a common lineage with skeletal myoblasts. In newborn sheep, the onset of non-shivering thermogenesis through activation of the BAT specific uncoupling protein (UCP)1 is essential for effective adaptation to cold exposure of the extra-uterine environment. There are thus likely to be novel interventions aimed at promoting BAT growth and development as a result of separate studies in humans and rodents that could be readily translated into sheep production.

We have now shown that in young sheep there are at least three different types of fat located within the body. These distinct depots are either primarily white or brown, or are a mix of brown and white, at least in the fetus and then “become white” during postnatal development. The different anatomical location of each of these depots could be directly related to its early life origin. To date, the most widely studied depot in the sheep is the perirenal-abdominal region in which UCP1 is rapidly activated around the time of birth and then gradually lost as the depot becomes increasingly white. It may, however, retain some BAT-like characteristics and thus be equivalent to “BRown adipose In WhitE (BRITE)” adipose tissue, as identified from rodent studies. This depot, together with the pericardial, is very responsive to changes in the maternal nutritional environment that can have a range of different short and long-term outcomes. These are dependent on the stage of gestation in which the maternal metabolic environment is challenged, together with maternal age and parity, fetal number and the type of postnatal manipulation. There is now a new opportunity to manipulate BAT development in early life in order to not only promote survival of the newborn but also to prevent excess adiposity in later life. This type of intervention could therefore have important economic consequences for meat production but also in the prevention of obesity in the human population.

Key References

Symonds ME, Budge H, Perkins AC & Lomax MA 2011 Adipose tissue development - Impact of the early life environment. *Progress in Biophysics & Molecular Biology*. 106, 300-306.

Symonds ME, Pope M, Sharkey D & Budge H 2012 Adipose tissue and fetal programming. *Diabetologia* (In press).

Mother-offspring behaviour and neonatal survival: From neuroendocrinology to practical applications

C Dwyer

SAC, Edinburgh, UK Email: cathy.dwyer@sac.ac.uk

Introduction All mammalian offspring depend on the appropriate expression of parental care for their neonatal survival. For 97% of mammals, and all domesticated species, this is exclusively maternal care. Mothers play a fundamental role in ensuring the nutritional and thermoregulatory functions of their offspring, and provide protection and opportunities for social learning. The quantity and quality of maternal care expressed by mothers varies with the stage of offspring development at birth: for example with altricial offspring (e.g. rats, mice) which are largely helpless at birth, mothers are solely responsible for cleaning, feeding, protecting and regulating the body temperature of their young, whereas precocious offspring (e.g. sheep, cattle) are mobile soon after birth and their own behaviour can contribute to, for example, accessing the udder and feeding. For these species the coordinated expression of appropriate behavioural responses from both mother and young are important for offspring survival. Studies of causes of neonatal mortality in farmed species have implicated failures of both mother and offspring to display appropriate behaviours, and thus an understanding of the causes of individual variation in these behaviours can contribute to strategies to improve neonatal survival.

Neuroendocrinology of mother-offspring behaviour

Currently much of our understanding of the neuroendocrine basis of maternal behaviour comes from studies in rats and sheep. Although these species show marked differences in the types of behaviours involved in their expression of maternal care, the underlying neurophysiological mechanisms are broadly similar, although some species variation exists. In both species the rapid onset of maternal behaviour with the birth of young is reliant on temporal changes in circulating steroid hormones, oestradiol and progesterone. These act as 'primers' for maternal care by increasing the expression of oxytocin receptors, or binding sites, in key tissues associated with maternal functions (mammary glands, uterus), including specific brain regions associated with maternal behaviour. However, oestradiol and progesterone are not sufficient to elicit maternal behaviour alone. The stretching of the vagina and cervix as the foetus is expelled (called the 'Ferguson Reflex') causes the release of the protein hormone, oxytocin, from the paraventricular nucleus of the hypothalamus, and stimulates maternal behaviour in ewes sensitised to respond through the action of oestradiol and progesterone in late gestation. The central release of oxytocin influences neurotransmitter release in specific brain regions associated with maternal care, and elicits species-typical behavioural responses (licking and grooming, maternal vocalisations, suckling offspring etc.). This mechanism therefore ensures that maternal behaviour is expressed temporally when the offspring are delivered.

Individual variation in maternal care

Expression of maternal behaviour is influenced by maternal experience, by breed or strain, by nutrition or stress in pregnancy, and to some extent by the behaviour of her offspring (Dwyer, 2008). First parity mothers generally show specific deficits in maternal behaviour, particularly an increase in agitation, aggression and rejection, and have high offspring mortality than more experienced mothers. In sheep, although inexperienced ewes have similar concentrations of circulating oestradiol and progesterone as experienced ewes, they have fewer oestradiol- α receptors, and are thus less sensitive to the neuroendocrine signals leading to maternal responses. Breed or strain variation in maternal behaviour has been reported in many species, and can also affect the mortality rate of offspring. In the sheep, breed differences in maternal care is associated with variation in circulating oestradiol in late gestation, although whether this is causal of the behavioural differences seen has yet to be established. Undernutrition, or psychological stress, in late gestation is also associated with alterations in maternal care which, in the case of nutrition, may also be related to impacts on circulating steroid hormones in late gestation, and suggest that, at least in the sheep, the expression of maternal behaviour is sensitive to external events in late pregnancy.

Offspring behaviour

In precocious species, the offspring play a crucial role in ensuring their own survival – neonates need to express coordinated responses to stand and move towards the udder. In many species, offspring also need to learn to discriminate their mother from other females and to express following behaviour. The ability of the offspring to perform these behaviours has been shown to be associated with survival in pigs and sheep. Appropriate expression of offspring behaviour is affected by birth weight, litter size, gender and by breed or strain. However, much less is known about the control of neonatal behaviour than maternal care, thus some of the underlying reasons behind this variation is unknown. Our recent work, however, suggests that in sheep the origins of variation in behavioural responses may occur early in prenatal development.

Practical applications

Improvements in offspring survival can be achieved through careful management during pregnancy and at birth, and via selection for appropriate characteristics in mother and/or young if sufficient genetic variation exists and the traits can be measured. For example, we have recently demonstrated that lamb behaviour can be recorded simply on farm, and this is a trait with moderate heritability, suggesting that improvements in survival can be made via this route. In addition, understanding the biological basis for individual variations in behaviour can help to identify vulnerable or sensitive periods when management can have an impact on behavioural expression, with consequences for neonate mortality.

References

Dwyer, C. M. (2008) Individual variation in the expression of maternal behaviour: a review of the neuroendocrine mechanisms in the sheep (*Ovis aries*). *Journal of Neuroendocrinology* 20, 526-535.

Prenatal stress, immunity and neonatal health in farm animalsE MerlotINRA, Saint-Gilles, France *Email: elodie.merlot@rennes.inra.fr*

In farm animal species, management practices dedicated to improve neonatal survival and health are mainly focusing on the young animal itself. Although this approach seems reasonable, it does not take into account that events occurring before birth can play a significant role. Until now, the prenatal treatments that have mainly been tested concern nutritional supplementation of the mothers with various feed additives. The aim of this presentation is to provide data supporting the idea that management practices impacting maternal welfare could also influence the health of the young after birth. First, maternal stress or well-being can directly affect maternal immunity and sanitary status, and thus the immune and microbial inheritance that the mother transmits to its progeny before or immediately after birth. Secondly, maternal stress can impact the developing foetus. Indeed, a comprehensive number of epidemiological and animal studies suggest that prenatal events are important determinants for disorders later in life. Among them, prenatal stress (i.e. stress experienced by the mother with impact on foetal ontogeny) influences the developing immune system with consequences on the passive, innate and acquired immunity of the offspring. According to rodent and primate studies, the passive placental transfer of maternal immunoglobulins to the foetus is relatively resilient to maternal stress. However, cell-mediated immunity of the offspring after birth is decreased by prenatal stress, an effect which resembles the consequences of chronic stress in adults. The effects of prenatal stress on humoral immunity or inflammatory responsiveness are more inconsistent. Some of these immune alterations are transient and disappear soon after birth whereas others are long lasting, reflecting a programming effect of prenatal stress. These effects have been shown to be partly mediated by the foetal exposure to stress-induced high levels of glucocorticoids. A recent literature has emerged demonstrating comparable data in farm animals, mainly focusing on pig species. It has been shown that maternal welfare during gestation might affect the transfer of colostral immunity of the piglets. Blood leukocyte counts can be transiently decreased after birth, whereas lymphocyte functions such as proliferation or inflammatory responses to immune challenges were shown to be altered by prenatal stress until weaning age or later. The nature and persistence of prenatal stress effects probably depend on the time of application of maternal stress relatively to the fetal stage of development. Because ungulates and rodents have dissimilar prenatal timing of maturation (rodents are less mature at birth), specific studies investigated the existence of critical developmental windows of vulnerability of the immune system to environmental programming in pigs. However, more studies are still needed to determine whether prenatal stress predisposes to increased morbidity and mortality, and data concerning other farm species are still lacking.

The evolving face of the animal feed industry: Impact of the consumerH D RaineAB Agri Ltd, Lynch Wood, Peterborough, UK *Email: helen.raine@abagri.com*

This paper will consider a number of changes that have occurred across the feed industry since the 1980s and the emergence of BSE and CJD in particular. Prior to this point, compounders were focussed on supplying feeds to support the required output of milk, meat and/or eggs as cost-effectively as possible. Safety concerns tended to be focussed on the animals *per se* rather than on the products produced.

There has been a raft of EU legislation aimed at ensuring both feed and food safety since that time and the industry has taken a pro-active stance since the mid-90s which will be described. Increased monitoring and reporting means that issues still arise across the EU (often due to imports) but the industry is quick to respond to these and works closely with the Food Standards Agency and / or DEFRA towards resolution.

The use of GM technology and concerns about the development of antimicrobial resistance are other threats which have impacted the industry. However, feed and food safety is not the only consequence of consumer awareness. The recognition of global warming has led to numerous initiatives aimed at reducing GHG emissions on farm and energy usage across the sector and has given nutritionists incentive to reduce further methane output and improve nutrient utilisation. Finally, the worldwide drive towards sustainable food and livestock production has and is providing a challenge to the industry which, as always, means opportunity for those who react proactively.

Reproductive diseases in the equine and how they can be minimised with good 'stud practice'

J R Newcombe

Equine Fertility Clinic, Warren House Farm, Brownhills, UK Email: john@newcombeandeast.co.uk

This presentation will discuss all the various infectious and non-infectious diseases which have an adverse effect on the ability of the mare to reproduce. A whole range of anatomical malformations of the genital system occur together with a range of physiological malfunctionings.

Infectious diseases significantly affect reproductive capacity. Of paramount importance are those bacterial infections of the uterus causing endometritis, reduced conception rates and increased embryo loss rates. The mare appears to be more susceptible to uterine infection than other species. The cervix is a muscular organ which dilates during oestrus so that ejaculation is intrauterine. This results in bacterial contamination which must be eliminated before the embryo enters the uterus about 6 days after ovulation. The ability of the mare to mount the normal massive inflammatory reaction to the presence of sperm, bacteria and debris is vital to the process of elimination. The ability of the cervix to dilate and remain dilated, and the ability of the uterus to contract and void all unwanted material is paramount. Any reduction or impairment of the efficiency of this process may allow bacteria and inflammatory fluid to persist. Once the cervix closes and blood progesterone levels becomes elevated, the efficiency of voiding fluid and the ability of phagocytes to eliminate bacteria become progressively impaired. Any bacteria surviving beyond 3-4 days postovulation will be able to multiply rapidly. The inflammation resulting from this causes premature release of endometrial prostaglandin so that the corpus luteum is lysed, progesterone levels fall to basal and the mare returns to oestrus.

The source of these organisms is the external genitalia of the stallion but mainly that of the mare herself. Her vulval region supports a wide variety of saprophytic bacteria two of which, *Streptococcus zooepidemicus* and *E. coli* are also opportunist pathogens. The penis of the stallion does not normally harbour these organisms but can either temporarily or even permanently harbour, without any clinical disease, three organisms which if inoculated into the uterus during copulation, will cause endometritis. These organisms, *Klebsiella aeruginosa*, *Pseudomonas* spp. and *Taylorella equigenitalis* are known as venereal pathogens. A thorough but voluntary screening program instituted many years ago has kept the UK and most of the world free of equine venereal disease with only occasional limited outbreaks.

The opportunist pathogens cannot be eliminated since they are present in all mares. Control is by measures which limit their introduction to the uterus but primarily by aiding the natural defence mechanisms with their removal. Some, usually older mares, seem particularly prone to infection and various drugs and procedures are used to treat these 'susceptible' mares. However even young and apparently normal mares can still become infected and therefore it is common practice in stud medicine to treat preventatively all mares after mating or artificial insemination. Regimes include large volume saline flushes soon after mating followed at intervals by instillation of broad spectrum antibiotics and finally oxytocin to induce uterine contraction. The intensity of this treatment will vary with the known degree of susceptibility of the mare.

Improving sperm quality for equine artificial insemination

J Morrell

Swedish University of Agricultural Sciences, Uppsala, Sweden Email: jane.morrell@shu.se

The use of artificial insemination (AI), particularly with cooled semen, is steadily increasing in Sport horses, apart from Thoroughbreds. In several European countries in recent years, AI has been used in more than 90% of warmblood mares bred. The advantages of AI, among others, are in preventing disease transmission, overcoming geographical and chronological barriers, allowing flexibility in the choice of stallions. However, some problems are particularly pertinent to equine breeding, including sperm quality in semen doses, preservation of semen (cooled, frozen), trying to match supply and demand for semen particularly when horses are competing away from the stud, lack of feedback from recipient studs, and disease transmission.

Researchers have been endeavouring to improve semen processing methods to overcome some of these problems. Single Layer Centrifugation (SLC) through a species-specific colloid is a modification of the density gradient centrifugation (DGC) method used to separate different populations of cells. Whole stallion ejaculates can be processed easily with SLC, whereas DGC was too impractical for use with stallion semen. SLC-selected sperm samples show improved motility, normal morphology, membrane integrity and chromatin integrity than their uncentrifuged counterparts. Since some of these parameters of sperm quality have been related to fertility, it is anticipated that the fertility of SLC-samples may be better than for uncentrifuged semen doses and AI trials are currently underway to explore this possibility. Spermatozoa from “problem” ejaculates certainly show improved fertility after SLC (1) but SLC-selected sperm samples from stallions of normal fertility have also given better pregnancy rates in AI. In a different AI trial, SLC-selected sperm doses stored for 48-96h after semen collection resulted in pregnancies after insemination (2). In other studies, SLC-selected spermatozoa were shown to survive freezing better than spermatozoa prepared by “cushion” centrifugation (3), enabling more batches to have an acceptable level of sperm quality on thawing. Furthermore, it was possible to separate spermatozoa from viruses and bacteria in boar semen using a similar technique, although recent studies with equine arteritis virus and *Taylorella equigenitalis* in stallion semen conducted at the Gluck Equine Research Center, Lexington, have shown that the level of contamination is reduced but not completely removed. It remains to be seen whether the reduction is sufficient to prevent infection via semen doses in AI.

In conclusion, SLC appears to offer a solution to at least some of the challenges facing the equine breeding industry. Research is continuing to optimise the conditions for using this technique.

Funded by The Swedish Foundation for Equine Research, Stockholm, Sweden.

References

Morrell JM, Mari G, Kutvölgyi G, Meurling S, Iacono E, Mislei B, Rodriguez-Martinez H. Spermatozoa from stallion ejaculates processed by Single Layer Centrifugation with Androcoll™-E are capable of fertilization after artificial insemination. *Reproduction in Domestic Animals* 46, 642-645.

Lindahl J, Dalin A-M, Morrell, JM (2011) Pregnancies in mares inseminated with spermatozoa selected by Single Layer Centrifugation and stored for 48h or 72h. *Reproduction in Domestic Animals* 46, Supplement. 3, P158.

Hoogewijs M, Morrell JM, Van Soom A, Govaere J, Johannisson A, Piepers P, De Schauwer C, de Kruif A, De Vliegher S. (2011) Sperm selection using single layer centrifugation prior to cryopreservation can increase post thaw sperm quality in stallions. *Equine Veterinary Journal*, 43 (Supplement 40) 35-41

What makes them move: an overview of anatomical imaging in animals

R Weller

Royal Veterinary College, North Mymms, Hatfield, Herts, UK *Email: rweiler@rvc.ac.uk*

There are six different imaging modalities available to assess the musculoskeletal system of animals: radiography, ultrasonography, computed tomography (CT), magnetic resonance imaging (MRI) and fluoroscopy. Each of those has its advantages and disadvantages and which one is best depends on the type of question that needs answering and the nature of the tissue in question.

Radiography is the oldest and most commonly used imaging modality. It is relatively cheap, portable and easy to perform in live animals as well as cadavers. It does however involve the use of ionising radiation and only produces planar images of static objects. Radiographs are commonly used to show bones and provide information about the general density, shape, size, outline and position of these. It can also provide some limited information on soft tissue, such as general outline, but does not allow the differentiation of soft tissues, e.g. muscle, tendon, subcutaneous tissue. **Fluoroscopy** is a special form of radiography that allows the radiographic assessment of moving structures. These systems are however expensive and involve high rates of radiation.

Ultrasonography is also relatively cheap and portable. Its use is usually a bit more challenging and requires a very thorough understanding of the anatomy. It is an excellent method to assess superficial soft tissues, but does not go through bone or air. Doppler Ultrasonography employs the Doppler Effect to assess the direction and velocity of structures (usually blood) in relation to the transducer and can be used to assess blood flow.

CT and **MRI** are both tomographic imaging modalities which represent a three-dimensional object as a series of two-dimensional slices and hence avoid superimposition of structures. Both modalities allow for viewing the structure in different planes after acquisition and its reconstruction in three dimensions. CT uses x-rays and allows the accurate measurement of the radiodensity of tissues, which can then be used to estimate material density. MRI relies on the proton content of tissues and is commonly used to show the 3D anatomy of soft tissue structures, since it allows to distinguish between different soft tissue material e.g. muscle and connective tissue. Normal MRI is very sensitive to motion, however there are novel sequences developed that will allow the assessment of structures in motion. Both MRI and CT work well for cadaver studies, but require sedation or general anaesthesia in live animals and are severely restricted in what size of animals or parts of animals can be imaged. They are also much more expensive than radiographs and ultrasound and require specialist training to use.

Scintigraphy is the only imaging modality which portrays the metabolism of the system being studied, rather than the anatomy (the anatomic resolution of scintigraphy is rather poor). Scintigraphy is an imaging process which detects and depicts gamma radiation emitted by the patient after injection of a radiopharmaceutical. There is a large range of possible labels - including antibodies, erythrocytes, leukocytes - and it is possible to specifically image the liver, kidneys, brain, heart, thyroids or skeleton in animals.

Watch them move: an overview of motion assessment in animals

T Pfau

Royal Veterinary College, North Mymms, Hatfield, Herts, UK *Email: tpfau@rvc.ac.uk*

In animals, similar to humans, objective gait analysis helps us to conduct a range of basic and applied research activities into the limitations of the musculoskeletal system. A limited number of specialized gait laboratories exist around the world that provide a range of technological devices that allow detailed quantification of the kinetics (forces, pressures etc) and kinematics (movement patterns) of quadrupedal animals. In this presentation we will give a brief overview of (some of) the available techniques, their strengths and weaknesses and present example applications in horses. In the first part we will cover the 'traditional' gait laboratory based techniques, while in the second part we will present information about 'novel' sensor based gait analysis approaches that now allow us to perform gait analysis under more practical conditions, e.g. in the riding arena rather than on the treadmill.

Traditional Techniques

Kinetics

Force plates measure the forces acting on the horse's body in standing and during locomotion: the ground reaction forces. Multiple force sensors per plate resolve direction, magnitude and location of force application. Plates are either semi-permanently embedded in a runway or integrated into an equine treadmill (vertical force). When embedded into a runway, horses have to perform multiple runs to collect a sufficient number of clean foot strikes (only one foot in contact with the plate).

Pressure mats measure the vertical forces/pressures at multiple points under the hoof during ground contact and can identify individual pressure points. They are however restricted to vertical force, however plates can be moved around more freely since no permanent mounting is required. Specialised pressure mats are also available to measure the force acting between horse and rider.

Kinematics

Standard and high speed video cameras are good qualitative record keeping tools. Quantitative assessment is more difficult, generally requiring calibration, and fixed cameras (filming a moving object).

Optical motion capture systems combine the views of two or more cameras into a three dimensional view. These are for example used in the movie industry to create animated characters based on the movements of people or animals. Calibrated areas (the area covered by at least one pair of cameras) directly depend on the number of (expensive) cameras available and the movement of reflective markers attached to the animals can be assessed with sub mm accuracy. Measurements can be achieved over ground (within the calibrated area.), new systems can also be used for measurement in day light (rather than inside laboratory conditions) but it is easier to collect a sufficient number of strides on the treadmill.

Novel Techniques

Inertial sensors come in a variety of configurations (shapes, sizes, number of individual sensors and their specs). Accelerometers measure linear (translational) acceleration of the body part they are attached to, while gyroscopes give a read-out of the rotational (angular) velocity. Both measure in the sensor's own coordinate system that constantly changes with the orientation of the body during locomotion. In a general case, due to sensor drift and lack of integration constants, it is not straight forward to integrate from linear accelerations to velocity or position. During locomotion, we can make use of the periodicity of the signal (stride cycle!) to achieve this task with full six degree of freedom sensors integrating 3-axial accelerometers, 3-axial gyroscopes and 3-axial magnetometers. These are now the methods of choice to assess movement under non-laboratory conditions. However rapid changes in limb movement characteristics between swing, impact and stance still provide us with challenges when trying to resolve 3D limb movement.

Behaviour and welfare of captive animals with reference to the domestic horseJ CooperUniversity of Lincoln, Lincoln, UK *Email: jcooper@lincoln.ac.uk*

There are broadly three approaches to assessing quality of life in captive animals; these being relationship to natural habitat, biological functioning in the captive context, and the animal's psychological state. Classically biologists have considered the animals' capacity to adapt to environmental conditions including behavioural responses as being related to the long-term functional benefits to the individual, such as survival or reproductive fitness. This approach may also be seen in a veterinary or animal production scientist's approach to animal welfare which focuses on the animal's ability to grow, reproduce and maintain good health in captive conditions. In captive species, including the domestic horse, this level of explanation is limited as for the most part they are housed in conditions that differ markedly from those in which they have evolved and because an individual's reproductive fitness is largely determined by man, rather than its own behavioural strategies. Nevertheless understanding the original function of adaptive responses will help to explain why animals perform apparently novel or functionless activities in the conditions they are kept, whilst poor health, growth or fertility may be a consequence of failing to cope with captive conditions. In investigating the behaviour of domestic animals, animal welfare scientists have a tendency to concentrate on the proximate causes of behaviour when explaining responses to environmental challenges, such as the animals' capacity to physiologically respond to conditions and its capacity to learn new responses to those challenges. Attention to control and development of behaviour is relevant to the welfare of captive animals and the interpretation of behavioural responses to captivity. An integrated approach involving consideration of both behavioural and physiological responses to environmental challenges is a robust approach to understanding the captive animal's adaptive mechanisms. In this context, behavioural responses to captivity, irrespective of whether they appear normal or abnormal to us, are not only a useful practical indicator of specific environmental deficiencies, but may also serve the animal as an adaptive response to these deficiencies in an "abnormal" environment. These principles will be illustrated in situations where horses appear able to show some adaptation to conditions such as the stable environment and conditions where the animal's capacity to cope may be severely compromised such as long distance transport in meat trade.

Hyperinsulinaemia = Laminitis : Truth or Fiction?

P Harris

WALTHAM Centre for Pet Nutrition, Leicestershire, UK Email: pat.harris@effem.com

Laminitis is a systemic condition that manifests in the foot and results in varying degrees of pain, lameness and debilitation. The most straightforward definition of laminitis is failure of the bond between the inner hoof wall and the distal phalanx. Although the term 'laminitis' was not used until the 18th century it has in fact been recognised for well over 2000 years. The link with carbohydrate intake was even appreciated by Aristotle, who referred to it around 350 BC as 'Barley Disease'. Various causes and pathophysiological pathways have been proposed since then and today laminitis may best be regarded as a syndrome rather than a single disease entity because of these multiple inciting causes (Harris 2011). The term *equine metabolic syndrome* (EMS) is currently used to describe horses and ponies with an insulin resistant phenotype (OBESE) linked with laminitis susceptibility (Frank *et al.*, 2010), while *Endocrinopathic laminitis* is sometimes used to describe EMS-associated laminitis as well as that associated with pituitary pars intermedia dysfunction (PPID, equine Cushing's disease). It has been argued that hyperinsulinaemia is the unifying factor in endocrinopathic and pasture-associated laminitis (McGowan, 2008). High concentrations of insulin (with a variable rate infusion of 50% glucose solution to prevent hypoglycaemia) have been shown to result in laminitis independent of any gastrointestinal trigger in ponies and standardbreds (Asplin *et al.*, Vet J 2007, de Laat *et al.*, 2010) and very recently a more moderate level of hyperinsulinaemia (induced through glucose infusion) over 48 h apparently induced pathological changes within the laminae of healthy standardbred horses (De Laat *et al.*, 2011).

Could differences in insulin response to the ration being fed and/or the background level of insulin resistance/plasma insulin concentrations therefore explain some or all of the differences in risk for laminitis – at least pasture associated laminitis? And if so, what could be the link between insulin and laminitis? BUT how robust is the evidence? and if it is involved how high/how long/how often does the hyperinsulinaemia have to be in-order for laminitis to be initiated? These and other questions will be considered in this talk.

References

- Asplin KE, Patterson-Kane JC, Sillence MN, Pollitt CC., McGowan M. Histopathology of insulin-induced laminitis in ponies. *Equine Veterinary Journal* 2010; 42, 700-706.
- De Laat MA, McGowan CM, Sillence MN., Pollitt CC. Equine laminitis: induced by 48 h hyperinsulinaemia in Standardbred horses. *Equine Veterinary Journal* 2010; 42, 129-135.
- De Laat MA, Sillence MN, McGowan CM., Pollitt CC. Continuous intravenous infusion of glucose induces endogenous hyperinsulinaemia and lamellar histopathology in Standardbred horses. *Veterinary Journal* 2011a (Epublication ahead of print).
- Frank N, Geor RJ, Bailey SR., Durham AE., Johnson PJ. Equine metabolic syndrome. *Journal of Veterinary Internal Medicine* 2010; 24, 467-475.
- Harris P. Laminitis after 2000 years: Adding bricks to our wall of knowledge. *Veterinary Journal* 2011 (in press): doi:10.1016/j.tvjl.2011.09.014
- McGowan C. The role of insulin in endocrinopathic laminitis. *Journal of Equine Veterinary Science* 2008; 28, 603-607.

Healthy food for healthy animals, the role of companion animalsM CobbUniversity of Nottingham, Nottingham, UK *Email: malcolm.cobb@nottingham.ac.uk*

The One Health concept is a worldwide strategy for expanding interdisciplinary collaborations and communications in all aspects of health care for humans, animals and the environment which it is hoped will advance health care by accelerating biomedical research discoveries, enhancing public health, expanding the scientific knowledge base, and improving veterinary and medical education and clinical care. While the main veterinary focus of this strategy is on the farmed species, many people believe that they and others derive physiological, psychological and social benefits from relationships with their pets, and numerous studies support this contention. In terms of benefits for human health, companion animals have been demonstrated to improve cardiovascular health, reduce stress, loneliness and depression, and facilitate social interactions among people who choose to have pets.

There is currently much debate about the suitability of commercial processed pet foods currently fed as complete diets to cats and dogs in particular and the increasing popularity of feeding raw food diets and natural pet treats to companion animals has the potential to expose the companion animal population and their co-habiting owners to zoonotic organisms. Additionally, as a consequence of this risk, many terminally ill, pregnant, or immunocompromised people are encouraged to relinquish their pets and this may have a detrimental effect on their overall health. In the context of the One Health strategy, human health and veterinary professionals may be able to contribute to the welfare of their patients by encouraging the human-animal bond and this presentation will discuss the impact of companion animals on public health and will examine in particular the companion animal as a link between food produced from farmed species and pet owners.

Nutritional approaches for parasite control in ruminants

S Athanasiadou

SAC, Easter Bush, Edinburgh, UK Email: spiridoula.athanasiadou@sac.ac.uk

Background Gastrointestinal parasitism is a major challenge for the health and the welfare of ruminants; helminth parasitism in particular impairs health by causing inappetence, diarrhoea, anaemia and, in severe cases, death. In addition, it impairs productivity by causing poor growth and reproductive performance. Ruminants are constantly exposed to gastrointestinal parasites through grazing. They eventually acquire immunity to the parasites and successfully regulate them, although their immunity may break down during certain stages of their lives. For the times when they are susceptible to gastrointestinal parasites, control of parasitism has traditionally depended on the use of anthelmintic drugs. However, the worldwide occurrence of resistance to anthelmintics (Jackson and Coop, 2000) and the increased awareness of the environmental impact of drug residues (McKellar, 1997) lead to an urgent requirement to re-evaluate the use of drugs, minimise their excessive prophylactic use and combine it with non-chemical means to achieve sustainable control of parasites. Here it is discussed how the nutritional environment can help the robustness of the host and its ability to cope with a helminth challenge and ways of how to incorporate host's nutrition into strategies to improve parasite control are considered.

Consequences of host's nutrition on parasite populations Host nutrition can affect parasite populations in two major ways. Firstly, it can directly affect the parasite fitness, either through the ingestion of compounds that are toxic for the parasite or through altering the conditions in the micro-environment from beneficial to detrimental and even toxic for their survival. Secondary plant compounds originating from conventional grazing forages but also medicinal plants from ethno-veterinary sources, such as tannins, alkaloids and saponins have been shown to have direct anthelmintic properties (Athanasiadou *et al.*, 2008). Secondly, host nutrition can indirectly affect parasites by improving the resistance of the host, i.e. the ability of the host to regulate establishment, development, reproduction and survival of the parasite. Host resistance is regulated and mediated through immunity and the consumption of certain nutrients, such as amino acids, fatty acids and minerals have been shown to affect host's immunity in rodent models and ruminants (Athanasiadou and Houdijk, 2010). Furthermore, the consumption of certain plant secondary metabolites, such as glycosides, alkaloids and saponins has also resulted in immune-modulatory activity (Athanasiadou and Houdijk, 2010). Depending on the stage of the infection and immune response, parasitized host benefit to a different degree by the direct and indirect effects of nutrition. For example, parasitized hosts can benefit from the direct effects of nutrition during the initial establishment of the infection, i.e. the acquisition of immunity and once the infection has become patent, and immunity has been acquired. On the other hand, it has been proposed that the resistance of parasitized hosts can only benefit from the indirect effects of nutrition once immunity to parasites has been acquired (Coop and Kyriazakis, 2001). As a consequence, the expectation is that the strategic combination of direct and indirect effects of nutrition during the course of a parasitic infection, alone or with other non-chemical treatments can greatly contribute to achieving sustainable parasite control.

Interactions between nutritional and other strategies for sustainable parasite control The investigation of the interactions between the nutritional and other sustainable approaches to control helminth infections only recently started receiving attention and are not yet well described. To date, experimental outcomes are encouraging but not always consistent (Burke *et al.*, 2007; Martinez Ortiz de Montelano *et al.*, 2007). It has become clear that in order to maximise the benefit from the incorporation of nutritional approaches for parasite control, we need to acquire knowledge at the underpinning mechanisms of how nutrition affects parasitism not only at phenotypic level, but also at molecular and cellular level (Athanasiadou and Huntley, 2008). Once these mechanisms have been described, and we have become aware of the sequence of events nutrition orchestrates and the pathways it affects, it will become easier to precisely characterise the nutrition - parasite interactions and maximise the benefits. The latter will contribute towards explaining the inconsistencies reported in the activity of the nutritional interventions and towards identifying the best implementation strategies for optimum parasite control.

References

- Athanasiadou, S., Houdijk, J.G.M., and Kyriazakis, I. 2008. *Small Ruminant Research*, 76, 2-11.
- Athanasiadou, S. and Huntley, J.F. 2008. *Parasite Immunology*, 30, 101-111.
- Athanasiadou, S., and Houdijk, J.G.M. 2010. In: *Dietary components and immune function-prevention and treatment of disease and cancer* (edited by RR Watson and V. Preedy), p. 63-74
- Burke, J.M., Terrill, T.H., Kallu, R.R., Miller, J.E., and Mosjidis, J. 2007. *Journal of Animal Science*, 85, 2753-2761.
- Coop, R.L., and Kyriazakis, I. 2001. *Trends in Parasitology* 17, 325-330.
- Jackson, F., and Coop, R.L. 2000. *Parasitology* 120, S95-S107.
- Martinez Ortiz de Montellano, C., Vargas-Magana, J.J., Aguilar-Caballero, A.J., Sandoval-Castro, C.A., Cob-Galera, L., May-Martinez, M., Miranda-Soberanis, R., Hoste, H., Camara Sarmiento, R., Torres-Acosta, J.F., 2007. *Veterinary Parasitology* 146, 66-76.
- McKellar, Q.A. 1997. *Veterinary Parasitology* 72, 413-426.