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A multidisciplinary approach for the development of accurate biological markers of feed efficiency in cattle and pigs – ‘IdentiFEED’



Key external stakeholders:

Beef and pig farmers, ICBF, breed societies, AI companies, beef and pig industry, Teagasc advisory service, agri-consultants, scientific community.

Practical implications for stakeholders:

- Evaluating young beef cattle for feed efficiency on a high-energy diet indoors may not reflect the phenotypic performance later in life or on different diets.
- Feed efficiency of dairy-origin Holstein-Friesian steers was substantially inferior compared to suckler-bred Charolais steers.
- Genetic markers can be used to identify beef cattle and pigs with improved feed efficiency.

Main results:

- Feed intake and residual feed intake (RFI; a measure of feed efficiency) are moderately ‘repeatable’ traits across time/maturity, stages of production and different diet types in beef cattle; however, it is evident that some animal re-ranking occurs, suggesting the existence of a genotype × environment interaction for the traits. In comparison, daily live weight gain and other measures of feed efficiency were not repeatable.
- Feed efficiency of dairy-origin Holstein-Friesian steers was up to 50% poorer than suckler-bred Charolais steers.
- Differentially expressed genes identified in pigs divergent in feed efficiency, once validated, can be used as genetic markers to identify animals with improved feed efficiency.
- Significant SNPs associated with feed efficiency in beef cattle are incorporated onto the IDBv4 of the national genotyping chip and utilised for delivery of the national genomically-assisted selection breeding programme.

Opportunity / Benefit:

- Insight into the biology of feed efficiency in beef cattle and pigs was provided, which can inform current breeding programmes and form the basis of molecular biomarker discovery for genomic-assisted selection for feed efficiency.
- The Irish beef and pig farming industry will benefit from enhanced animal breeding programmes resulting in more economically and environmentally sustainable animal production.

Collaborating Institutions:

UCD, NUIG, Irish Cattle Breeding Federation (ICBF) and University of Alberta

Teagasc project team:	Dr. Mark McGee (PI) Dr. David Kenny Dr. Sinead Waters Dr. Paul Cormican Dr. Claire Fitzsimons
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1. Project background:

Feed efficient animals are central to profitable, sustainable and efficient Irish beef and pig production. A major constraint to genetic progress in feed efficiency is the difficulty and enormous expense of measuring it directly. Therefore, robust cost-effective molecular-based biomarkers of feed efficiency are necessary. Although there are many different approaches to measuring feed efficiency, residual feed intake (RFI) has increasingly become the measure of choice. Defined as the difference between an animal's actual and predicted feed intake (based on weight and growth), RFI is conceptually independent of growth and body size. Low-RFI animals are feed efficient and High-RFI animals are feed inefficient. Clearly, if feed efficiency is to be included as an economically-important trait worthy of consideration in selection programmes, an animal's status for the trait must be repeatable across the various phases and physiological states of its productive life, as well as across different dietary regimens. High repeatability for a trait is also important in breeding animals where predictions of performance can only be made early in life. Furthermore, genotype x environment interactions are particularly relevant if estimates of genetic merit for improved productivity or feed efficiency are derived under conditions different from that under which progeny are reared. Worldwide, performance testing of beef breed bulls is usually carried out using high-energy, concentrate-based diets, whereas the majority of beef cattle are largely produced on predominantly forage-based diets, which have very different intake characteristics.

2. Questions addressed by the project:

- Determine the repeatability of feed efficiency measures in growing beef cattle.
- Determine if genotype x environment interactions for feed efficiency exist for growing beef cattle offered contrasting diets, sequentially.
- Identify key gene expression profiles and biological pathways underlying improved feed efficiency in both cattle and pigs.
- Discovery of robust DNA-based biomarkers for feed efficiency in beef cattle and pigs.

3. The experimental studies:

- Bovine and porcine models to examine divergence in feed efficiency were developed in Teagasc Grange and Lyons Estate UCD, respectively.
- The bovine model tested the existence of genotype x environment interactions and the repeatability of feed efficiency measures in growing beef cattle, both within and across diet. Suckler-bred Charolais (¾-bred or greater) and dairy-origin Holstein-Friesian steers, were offered a series of diets in the following sequence: High-concentrate diet + 10% roughage (dry matter basis); high-nutritive value grass silage plus mineral & vitamins; zero-grazed grass only; grazed grass only (i.e. grazing period) and finally, a high-concentrate diet + 10% roughage (dry matter basis). Each of the dietary feeding periods comprised a minimum of 70 days preceded by an adaptation period of 21-days. The cattle were approximately one-year old at the end of the first high-concentrate dietary phase and two-years old at the end of the second high-concentrate (final) dietary phase.
- Correspondingly, two experiments measuring feed efficiency in male pigs from 30 to 95 kg were completed.
- Individual animal intake and performance was measured, and feed efficiency (primary emphasis on RFI) was determined. Cattle and pigs with the best and worst feed efficiency were identified. Detailed supporting measurements and samples were undertaken to further characterise the animals, with a particular focus on those with extremes in feed efficiency. In both species, blood and key metabolic tissue samples were harvested at appropriate times for molecular characterisation.

4. Main results:

Repeatability of feed efficiency measures in beef cattle:

For both cattle breed types, feed dry matter intake was moderately correlated ($P < 0.05$) between the two high-concentrate dietary phases, between the grass silage and zero-grazed grass dietary phase, and

between the zero-grazed grass and (second) high-concentrate dietary phase. Residual feed intake (RFI) was low-to-moderately correlated ($P < 0.05$) between the two high-concentrate dietary phases and between the grass silage and zero-grazed grass dietary phase, but correlations were low ($P < 0.10$) between the zero-grazed grass and (second) high-concentrate dietary phase. In contrast, daily live weight gain and other measures of feed efficiency, namely Feed Conversion Ratio (FCR, feed:gain), Feed Conversion Efficiency (FCE: gain:feed), Residual Intake and Body Weight Gain (RIG), and Residual Body Weight Gain (RG) were not correlated ($P > 0.10$) between dietary phases. In terms of RFI re-ranking between selected dietary phases; when categorised as low-, medium- and high- RFI (i.e. terciles), approximately 50% of both breeds maintained efficiency classification between the two high-concentrate dietary phases, and 40% maintained efficiency classification between the zero-grazed grass and (second) high-concentrate dietary phase. When based on quintiles, approximately two-thirds of both breeds either maintained or re-ranked only one position between the two high-concentrate dietary phases, whereas between the zero-grazed grass and (second) high-concentrate dietary phase half of Charolais and two-thirds of Holstein-Friesian steers either maintained or re-ranked only one position. Overall, RFI was the most (only) repeatable measure of feed efficiency.

Feed efficiency of suckler-bred Charolais compared to dairy-bred Holstein-Friesian steers:

The Holstein-Friesian steers were 24 days older than the Charolais, which reflects the mean calving dates of the national dairy and suckler cow herds. For the zero-grazed grass phase, Holstein-Friesian were 80 kg lighter and had a 70 g/day lower growth rate compared to Charolais. Despite this, Holstein-Friesian consumed 4% more grass dry matter daily resulting in a 10% poorer feed conversion ratio (FCR - kg dry matter intake/kg live weight gain) than Charolais. Likewise, in the finishing phase, the older, lighter, slower-growing Holstein-Friesian steers consumed 10% more feed dry matter resulting in a 20% inferior FCR than Charolais. At slaughter, carcass fat score was similar (9.9, scale 1-15) for both breed types but kill-out proportion (6 percentage units lower), carcass weight (84 kg lighter) and carcass conformation score (6 units poorer, scale 1-15) were considerably inferior for Holstein-Friesian compared to Charolais. Due to the lower kill-out proportion and lower estimated meat proportion in the carcass of Holstein-Friesian compared to Charolais, during the finishing phase Holstein-Friesian consumed approximately 33% and 50% more feed dry matter/kg carcass and meat gain, respectively, than Charolais.

Genomic and transcriptomic investigations into feed efficiency phenotypes:

Beef Cattle:

Liver gene expression: Analysis of the RNA-Seq data generated resulted in the identification of differentially expressed genes and enriched biological pathways. For Charolais steers a total of 322 differentially expressed genes were identified across three dietary phases investigated (i.e. high-concentrate phase 1, zero-grazed grass and high-concentrate dietary phase 2), whereas for Holstein-Friesian steers 33 genes were differentially expressed. There were no shared differentially expressed genes across the three dietary phases for either breed. RNA-Seq analysis also identified a total of two and ten enriched biological pathways for Charolais and Holstein-Friesian steers, respectively. Of these twelve pathways, eleven were associated with variation in immune function. Using gene expression data generated, a systems biology approach was utilised to identify key genes regulating RFI. A total of 349 hub genes were identified and ten were differentially expressed in the RNA-Seq analysis carried out and 37 have been previously associated with feed efficiency in livestock, particularly in relation to protein turnover and mitochondrial efficiency amongst other physiological processes.

Muscle gene expression: Effects of RFI on gene expression in muscle of beef cattle were not consistent across breed type or dietary phase. There was no consistent biological mechanism governing variation for RFI in muscle tissue across Charolais and Holstein-Friesian cattle breeds sequentially offered different diets. Within each diet there appears to be different biological mechanisms governing RFI in muscle, demonstrating the possibility of a genotype x environment interaction at least at the level of muscle metabolism. An altered immune response may be contributing to variation in RFI in Holstein-Friesian animals while consuming fresh forage.

Genome-wide association study (GWAS):

Additionally, GWAS was completed in a multi-breed reference population of 1,492 Irish beef cattle. Individual GWAS were carried out for Aberdeen Angus, Charolais, Simmental, Limousin and Belgian Blue cattle. Individual GWAS summary statistics were combined using meta-analysis. A total of 24 SNPs reached significance following meta-analysis. Following GWAS meta-analysis expression quantitative trait loci (eQTL) analysis was carried out in order to gain insight into functional effects of GWAS associated SNPs. One SNP was associated with expression of GFRA2 in liver tissue. This was the first reported eQTL for RFI in beef cattle. Population structure and breed composition analyses was conducted using 50K IBD chip genotype information of 450 and 884 animals from TEAGASC and ICBF, respectively, with a Canadian reference population of 7723 animals. After merging and data cleaning (quality control), 23,872 SNP markers remained for analyses. Results showed both Irish populations clustering together. Comparing with the Canadian populations, some individuals clustered around purebred Charolais animals, while others spread towards the

Canadian crossbreed animals. On average similarities for TEAGASC and ICBF animals were mainly 27% and 34% Angus, respectively, and other breeds that were not represented in the reference. The genomic heritability estimation of RFI for the combined population was 0.2.

Pigs:

Gene expression profiles from tissues including liver, muscle, duodenum, jejunum, ileum and hypothalamus were evaluated using technology such as RNA-sequencing and Nanostring nCounter analysis software. In pigs divergent in feed efficiency, a total of 6463 genes were identified as being differentially expressed in muscle, while 964 genes were identified as being differentially expressed in liver. Genes that were commonly differentially expressed between muscle and liver ($n = 526$) were used for multi-tissue analysis. These 526 genes were associated with protein targeting to membrane, extracellular matrix organisation and immune function. In the muscle-only analysis, genes associated with RNA processing, protein synthesis and energy metabolism were down regulated in Low-RFI animals, whereas in the liver-only analysis, genes associated with cell signalling and lipid homeostasis were up regulated in the Low-RFI animals. Interestingly differences in the transcriptome segregated on pig RFI value rather than the genotype/farm of origin.

A further study evaluated the basal colonic innate immune response and also following an ex vivo lipopolysaccharide challenge. The alterations in gene expression profiles indicate that more efficient Low-RFI pigs have an upregulated basal colonic inflammatory state and a heightened response to an LPS challenge compared to inefficient pigs. Further transcriptomic analysis of the small intestine increased gene expression of nutrient transporters such as SGLT1, GLUT2 and FABP2 and the enzyme Sucrase-Isomaltase. In relation to appetite control, the gut peptide GLP-1R was increased in the High-RFI pigs; however, no changes in the expression of hypothalamic neuropeptides were identified.

While alterations in the transcriptome are key drivers of feed efficiency another potentially important factor is the impact of the microbiome. 16s rRNA sequencing analysis established that differences in bacterial diversity were different between the farm of birth rather than RFI. However, despite variation between farms, interesting taxonomic differences were identified between RFI groups. Within the phylum Bacteroidetes, the Low-RFI pigs had increased abundance of two families BS11 and increased Bacteroidaceae relative to the High-RFI group. This suggests that while farm of birth has a substantial influence on microbial diversity in the pig colon, a microbial signature indicative of feed efficiency status was evident.

3. Opportunity/Benefit:

- Differentially expressed genes identified in pigs divergent in feed efficiency, once validated, can be used as genetic markers to identify animals with improved feed efficiency.
- Significant SNPs associated with feed efficiency in beef cattle are incorporated onto the IDBV4 of the national genotyping chip and utilised for delivery of the national genomic selection breeding programme.

4. Dissemination:

PhD (2) & M.Agr.Sc. (1) Theses; Peer-reviewed scientific papers; Proceedings of scientific conferences (e.g. ASAS, ISNH, BSAS, EAAP, IPVS, DPP), National and international technical meetings (e.g. ASA, ICBWM, AFBI-initiated Fall Forum, World Charolais Technical Congress); Teagasc BEEF Open Days; Teagasc Beef Advisory Newsletter; Teagasc In-service Training; QQI Ruminant Nutrition courses; Lectures/ seminars.

Main publications:

Higgins M.G., Kenny, D.A., Fitzsimons, C., Blackshields, G., Coyle, S., McKenna, C., McGee, M., Morris, D.W. and Waters, S.M. (2019) 'The effect of breed and diet type on the global transcriptome of hepatic tissue in beef cattle divergent for feed efficiency' *BMC Genomics* 20: 525.

Vigors, S., O' Doherty, J.V., Bryan, K. and Sweeney, T. (2019) 'A comparative analysis of the transcriptome profiles of liver and muscle tissue in pigs divergent for feed efficiency' *BMC Genomics* 10: 1186.

Kenny, D.A., Fitzsimons, C., Waters S.M. and McGee, M. (2018) 'Invited Review: Improving feed efficiency of beef cattle – the current state of the art and future challenges' *Animal* 12: (9), 1815–1826.

Popular publications:

Coyle, S., Fitzsimons, C., Kenny, D.A., Kelly, A.K. and McGee, M. (2017) 'Feed efficiency correlations in beef cattle offered zero-grazed grass and a high-concentrate diet' *Advances in Animal Biosciences* 8: 1, 121.

Coyle, S., Fitzsimons, C., Kenny, D.A., Kelly, A.K. and McGee, M. (2016) 'Repeatability of feed efficiency in beef cattle offered grass silage and zero-grazed grass' *Journal of Animal Science* 94: 5, 719.

Coyle, S., Fitzsimons, C., Kenny, D.A., Kelly, A.K. and McGee, M. (2016) 'Repeatability of feed efficiency in steers offered a high concentrate diet' *Journal of Animal Science* 94: 5, 719.

McGee M. and Kenny D. (2019) 'Charolais more efficient' [Feed efficiency of suckler-bred Charolais compared to dairy-bred Holstein-Friesian steers]. *Teagasc Beef Advisory Newsletter*, March 2019.

5. Compiled by: Dr Mark McGee