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Investigating the basis of variable bird performance

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Executive Summary

Variability in broiler bird performance can cause management issues for producers. Cost savings could be made if all birds grew at an even rate and used energy efficiently. There are a range of biological, physical, environmental and behavioural factors that could influence bird performance. This project investigated two aspects of bird biology; gene expression in the gut and bacterial populations in the gut. Comparisons were made between birds with high and low apparent metabolisable energy (AME) measurements looking for correlations between AME and gene expression or bacterial population structures.

A single animal trial was carried out in which a number of productivity measures where made on a single group of birds (n=96) reared in a system modelling typical production parameters. After being raised on the floor in a single group the birds were transferred to metabolism cages for measurement of individual feed conversion ratios (FCR) and AME. The 24 birds at each extreme of the FCR distribution were sampled for gene expression and bacterial analysis. Although our primary interest was in AME readings the FCR values were used to select birds for sampling because the FCR values were available immediately whereas it takes some time to process samples for AME determination and hence results aren't available at the time of necropsy and tissue sampling. It was found that FCR did not correlate well with AME so the distribution of AME values in our sampled birds was not as extreme as we had hoped. Low FCR birds had a tight distribution of AME values whereas the high FCR birds had more widely distributed AME values.

A new, highly advanced, microarray platform (Nimblegen) was used to study gene expression in the sampled guts. By comparing gene expression in high AME and low AME birds it was possible to define a large number of genes that were differentially regulated between the two groups. The differentially expressed genes were widely distributed across a range of biochemical functions and pathways and included genes encoding proteins with key functions in nutrient transport and metabolism. Few genes showed levels of regulation of more than 4-fold between groups. There are clear differences in gene expression in the duodenum between birds with high and low AME values.

Previous CRC sponsored investigations of gut bacterial populations have relied on terminal restriction fragment length polymorphism (T-RFLP) for characterization. In this project we have applied the latest, most advanced, metagenomics based bacterial profiling method to quantitatively characterise the major bacteria present in the caeca of chickens with high and low AME values. This method utilizes recent advances in Next Generation high throughput DNA sequencing technology to provide detailed microbial characterisation of samples. The bacterial population structure in the birds was diverse with many birds having grossly similar populations but a few birds with quite different bacterial populations. Beyond the broadly similar bacterial populations in the trial birds there were a few bacterial groups that showed statistically different population levels between the high and low AME groups.

The results of this 4 month study show that there are clearly defined differences in chicken gene expression and bacterial population structure that correlate with differences in AME values. The challenge now will be to determine the causal links between these differences and develop strategies by which this knowledge can be effectively used to help optimise bird performance.

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Introduction

In a production environment the growth performance of individual broiler birds can be quite variable despite the fact that all birds within a production flock have similar genetics and are raised together in an optimal environment with access to the same feed and water. For the producer it is desirable that bird performance is as even as possible and that the feed supplied is used as efficiently as possible.

This project aimed to investigate two aspects of bird biology, gene expression in the gut and gut microbial populations, to determine if there are correlates with bird performance. The identification of correlated aspects of bird biology may indicate ways in which we could intervene to reduce variation in bird performance and enable more birds to perform at an elite level.

The structure and function of the gut is directed by the genes that are expressed. During embryogenesis the cellular differentiation and formation of the various gut tissues is directed by changing patterns of gene expression. In the mature gut a wide variety of genes must be expressed to provide the metabolic and catabolic functions of the gut as well as the constant renewal of gut tissue. Analysis of differences in gene expression may indicate aspects of gut biology which vary between different groups of birds and may provide some explanation for the differences observed. The aim of the current study was to determine if there were gene expression differences in birds with different apparent metabolisable energy (AME) levels. The subsequent goal, to be addressed in a more comprehensive study, would be to determine why these differences in gene expression become established (e.g. is it a result of genetic differences, is it established by small environmental differences, or simply stochastic variation, etc.). Chicken microarrays have been available for sometime and have been used to investigate gene expression in a variety of tissues. Some focused arrays have been constructed for analysis of gene expression in the gut (van Hemert et al., 2003; Kim et al., 2008) but with the availability of the whole chicken genome (Int. Chick. Seq. Consort., 2005) it is now relatively straight forward to construct chicken microarrays containing probes for virtually all genes and thus monitor the expression of the entire gene complement. The use of such genomics tools to study the relationship between gene expression and diet has produced a new field of study called "nutrigenomics". There are now several hundred publications in the broad field of nutrigenomics however there are only a few relevant publications related to chickens, for example de Greff et al. (2009) studied the effect of an organic diet compared to a conventional diet on gene expression in the jejunum. Given the high importance of the diet in the profitability of chicken production it will be valuable to direct all possible technologies available at a greater understanding of the interaction between diet and bird biology.

One possible influence on gene expression in the gut and on overall gut performance is the resident microbial population. The intestinal microflora of chickens is very complex with over 600 different bacterial species identified (Apajalahti et al., 2004).Various experimental systems in animals and humans have been used to show that resident microflora can play key roles in gut and immune development (Kelly and Conway, 2005; Umesaki et al., 1999). It has been hypothesised that the normal healthy gut microflora can exclude colonisation by bacterial pathogens by actively filling ecological niches (Nurmi et al., 1992) and by production of antimicrobial compounds (Brisbin etal., 2008). The gut microflora facilitates food digestion and nutrient assimilation to the benefit of the host but the microflora also uses a considerable proportion of the nutrients ingested by the host and thus there is a balance between the beneficial and detrimental effects of the native microflora. Previous work in chickens has demonstrated differences in microbial populations feed two closely related barley based diets, one with and one without enzymes (Torok et al. 2008); our interest for the current study was to determine if differently performing birds fed the same diet carried different microbial populations.

Microbial populations have traditionally been characterised by culturing recovered bacteria on artificial media but it is clear that the vast majority of bacteria from environmental samples are not culturable and hence these traditional methods vastly underestimated the complexity of natural gut

bacterial populations. In the last decade molecular techniques have been used to expand our understanding of complex microbial populations. Methods directed at characterising 16S ribosomal RNA genes have refined our ability delve deeper into the bacterial populations present. These methods have included denaturing gradient gel electrophoresis (DGGE) (Muyzer et al., 1993), terminal restriction fragment length polymorphism (T-RFLP) (Liu et al., 1997) and construction and sequencing of extensive 16S clone libraries (Sekeguchi et al., 1998). Recent technological advances in DNA sequencing have further revolutionised the way that complex microbial populations can be characterised and have opened up new opportunities in this broad area of research (Petrosino et al., 2009). The power of these new technologies is gaining wide recognition, for example there is now a very large effort directed towards characterising the human microbiome (http://nihroadmap.nih.gov/hmp/) using these new techniques. We are now able to apply these advanced methodologies to chicken biology to assist in the characterization, monitoring and understanding of the structure and role of the chicken microbiome. These detailed studies of the gut microflora may indicate favourable microbial populations that could be used to establish optimal gut conditions to produce elite performing birds.

Objectives

- Investigate the biological basis of variable bird performance.
- Identify gene expression differences that correlate with differences in apparent metabolisable energy measurements.
- Evaluate the utility of deep sequencing of 16S rDNA amplicons for the identification of changes in gut bacteria population structure that correlate with differences in apparent metabolisable energy measurements.

Materials and Methods

Birds and diet

Male Cobb 500 broilers (Baiada Hatchery, Willaston, SA, Australia) were raised in a rearing pen in a temperature-controlled room until the commencement of the apparent metabolisable energy (AME) study period. All birds were given *ad libitum* access to a broiler grower diet (Table 1) which met or exceeded National Research Council guidelines for broiler chickens (NRC, 1994).

Table 1. Chicken rearing diet composition

Ingredient	**g/kg**
Wheat	444
Barley	150
Peas	50
Soybean meal	170
Canola meal	100
Meat meal	32
Tallow	30
Limestone	10
Salt	3.5
Lysine HCl	2.5
DL-methionine	2.3
Threonine	0.7
Vitamin and mineral premix †	5

† Included xylanase and phytase enzyme products

All procedures were approved by the Animal Ethics Committees of the University of Adelaide and the Department of Primary Industries and Resources South Australia. Birds were exposed to the following lighting schedule; Day 0-3, 23 hours; Day 4, 21 hours; Day 5, 18 hours; Day 6, 15 hours; Day 7-25, 12 hours.

Apparent metabolisable energy study

At 13 days post-hatch, 96 chickens were transferred in pairs to 48 metabolism cages located in a temperature-controlled room (23-25°C). Birds were placed in pairs for an initial acclimation period to minimise stress associated with isolation. Birds continued to have free access to food and water prior to, and during, the experimental period. On day 15, birds were placed individually in 96 metabolism cages.

Apparent metabolisable energy (AME) values were determined in a classical seven-day AME study between days 15-22. Body weight was recorded at the beginning and end of the seven-day period. The first three days enabled the chickens to adapt to solitary confinement in the metabolism cages. During the following four days, all excreta was collected daily and dried at 80°C. Feed intake was recorded during the adaptation and collection phases of the study period. Dry matter (DM) contents of feed were measured.

On day 22, all birds were weighed and were retained in individual cages until day 25. Feed conversion ratio (FCR; g feed eaten/g weight gain) was calculated for each bird and the 24 birds with the highest FCR and 24 birds with the lowest FCR were identified. On day 25 post-hatch the 24 high FCR and 24 low FCR birds were killed one at a time by cervical dislocation, alternating between high and low FCR birds.

From all birds, a 1cm segment of tissue from the midpoint of the duodenum, jejunum and ileum was collected, rinsed in PBS and stored in a 5ml tube containing 2ml of RNAlater. Samples were stored on ice and later refrigerated (4°C) for gene expression analysis by whole chicken genome array. One caecum was cut open and a 1cm segment from the tip was also rinsed in PBS and collected in RNAlater for gene expression analysis. The contents of this caecum were collected in a 5ml tube and stored on ice and later frozen for microbial analysis by high throughput DNA sequencing (Roche/454). A 5cm length of jejunum (directly distal to the segment collected for gene expression analysis) was rinsed gently in PBS and the mucosa was collected in a 5ml tube by gentle scraping with a glass slide. The mucosal scraping was collected for microbial analysis of mucosa-associated bacteria.

Also from all birds, the remaining caecum and 3 cm sections of tissue and associated digesta from the midpoints of the jejunum and ileum (immediately distal to previously collected segments) were removed and kept at 4°C until frozen and stored at -20°C for microbial profiling by terminal-restriction fragment length polymorphism (T-RFLP) analysis if required in future.

Gross energy values of feed were measured with a Parr isoperibol bomb calorimeter (Parr Instrument Company, Moline, IL, USA). Gross energy values of dried excreta were measured from the 48 birds selected for tissue collection (n=24 high FCR birds and 24 low FCR birds). AME values (in MJ/kg dry matter basis) were calculated as follows;

AME_{diet} = [(GE_{diet} x g feed consumed) - ($GE_{excreta}$ x g dry excreta)] /g feed consumed /DM feed

Sample storage and transport

Samples for gene expression profiling and microbial analysis by 454 sequencing were transported to CSIRO Livestock Industries (Geelong, Vic, Australia). Samples for gene expression profiling were transported chilled whilst samples for microbial analysis were sent on dry ice.

Samples collected for T-RFLP analysis were stored at SARDI-PPPI (Roseworthy, SA, Australia) for future freeze-drying and T-RFLP analysis if required.

Gene expression analysis

Selection of samples for analysis

Gene expression was measured in duodenum samples collected from the animal trial. Samples were chosen according to their AME values and separated into two groups- high and low AME respectively. Details of the samples used and their AME values are shown in Table 2.

Each sample was independently hybridised to a sub array on the 12-plex chicken microarray. The gene expression profiles of high and low AME groups were compared to obtain an overall differential gene expression pattern.

Table 2. Details of samples used in microarray experiment

Sample number	AME value	High/Low AME value
H13	15.8546	High
H12	15.1278	High
H09	15.1232	High
H14	14.9459	High
H20	14.9120	High
L11	14.8957	High
H10	14.3429	Low
L08	14.2807	Low
L24	14.2137	Low
H08	14.1823	Low
H11	14.0425	Low
H17	13.7690	Low

Microarray design and hybridisation

Total RNA was isolated from each duodenal sample using the Meridian total RNA isolation kit (Cartagen). Three micrograms of total RNA was reverse transcribed into cDNA and indirectly labelled with Cy3 using the NimbleGen one-colour DNA labelling kit (Roche). The quality of each sample was verified by evaluation on a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies). Labelled samples were then concentrated in a Speed Vac (Savant Instruments) and resuspended in sample tracking control. Whole genome chicken arrays were incubated with labelled probe in hybridisation solution, using components from a NimbleGen Hybridisation Kit (Roche), for 72 hours at 42 °C. Post hybridisation the array was washed as per NimbleGen's protocol (Roche NimbleGen, 2009).

A custom designed Nimblegen 12x135K chicken high-density microarray was used in this experiment. This microarray contained both a set of 20,460 long oligos (65-75nt) designed at the Roslin Institute based upon chicken Ensembl gene transcripts and other genomic information supplied by various research groups around the world (http://www.ark-genomics.org/microarrays/byspecies/chicken) and set of 33,383 chicken UniGene sequences (60nt) designed at CSIRO Livestock Industries. Each gene was represented by at least two independently designed probes and each probe was present in

duplicate on the array. The Nimblegen Maskless Array Synthesis (MAS) technology was used to fabricate the microarray, combining photo-deposition chemistry with digital light projection. Each fabricated glass slide contained 12 identical arrays that could be independently hybridized.

Microarray analysis

After hybridization and washing all arrays were scanned and gene expression signals captured using a GenePix (Axon Instruments) laser scanner. NimbleScan Software (Roche) was used to extract and pre-process the data. Pre-processing consisted of background correction, normalization, final summarization and quality control and was performed using the Robust Multichip Average method (Bolstad et al., 2003; Irizarry et al. 2003 and 2004; Wu et al, 2004). This method is the preferred method for high-density oligonucleotide arrays as it puts each chip's values in the context of a set of similar values (Irizarry, 2002). Statistical tests were carried out using Genowiz Version 4.0.5.3 (Ocimum Biosolutions) to determine all genes greater than two-fold differentially regulated ($p = 0.01$) between the high and low AME groups. Gene ontology and pathway analysis was also performed in Genowiz.

Microbial profiling

DNA preparation from gut samples

The gut samples, either caecal content or luminal gut scrapings, were diluted 1:1 with phosphate buffered saline. Total DNA from these samples was then isolated using the method of Yu and Morrison (2004). Briefly, a sample was transferred to a tube with lysis buffer (500mm NaCl, 50mM Tris-HCl pH8.0, 50 mM EDTA and 4% sodium dodecyl sulfate) and sterile zirconium beads and then homogenized using a Qiagen TissueLyser at maximum speed for 3 minutes. Following centrifugation the supernatant was collected and nucleic acid was precipitated out following the addition of ammonium acetate and isopropanol. After centrifugation the pellet was resuspended in Tris-EDTA buffer and digested with DNase-free RNase and proteinase K to remove RNA and protein. The DNA was finally purified on a QIAamp column (Qiagen) according to the manufacturer's instructions. DNA quantity and quality was measured on a NanoDrop ND-1000 spectrophotometer.

PCR amplification of 16S ribosomal RNA gene sequences

DNA derived from the gut bacteria of the 12 birds at each extreme of the AME distribution was processed. DNA was amplified using Bio-Rad iProof DNA polymerase. Each PCR reaction contained 2 µl of template DNA, 20 µl of iProof 2X master mix (containing buffer, nucleotides and iProof enzyme), 2 µl of each primer (final concentration 0.5 µM), and 14 µl of water. The primers used were designed to amplify the V2-V3 region of the 16S rRNA gene (forward primer, 5' AGAGTTTGATCCTGG 3'; reverse primer, 5' TTACCGCGGCTGCT 3'). Each primer also included sequences to facilitate the sequencing of products in the Roche/454 system and the reverse primers consisted of a related set of primers which differed in "barcode" sequences; specific sequences introduced into the primers to allow tagging of individual samples in a multiplex sequencing system. PCRs were performed in an Eppendorf Mastercycler using the following conditions: 98°C for 60 seconds then 25 cycles of 98°C for 5 seconds, 40°C for 30 sec, 72°C for 30 sec; elongation at 72°C for 10 min then hold at 5°C. The efficiency of PCR amplification of each sample was assessed by running 10 µl of the PCR mix on a 1.2% agarose gel.

High throughput sequencing and analysis of 16S amplicons

The amplified 16S samples from each bird were pooled using approximately equal amounts of each PCR product. The pooled sample was sequenced using the Roche/454 FLX Genome Sequencer and the latest Titanium chemistry. Twenty-four pooled caecal samples were sequenced on half a PicoTitre plate. The output sequence file was analysed using a number of publically available software packages

and databases. Bacterial classification was first carried out via the Ribosomal Database Project (RDP) Release 10 database using the "Classifier" tool (http://rdp.cme.msu.edu/classifier/classifier.jsp). We also used MG-RAST (http://metagenomics.nmpdr.org/) to interrogate the greengenes database (http://greengenes.lbl.gov/). Finally, detailed analysis was carried out using the MOTHUR program (Schloss et al., 2009) and the SILVA database (http://www.arb-silva.de/).

The bacterial classification system has the following hierarchy; phylum, class, order, family, genus, species.

Results

Apparent metabolisable energy study

Ranking by FCR

When birds were ranked based on high and low FCR, significant differences were observed in FCR and BWG ($p<0.0001$; Table 3). No significant differences were observed in AME and FI between high and low FCR birds ($p>0.05$).

Table 3. Performance data from AME study when birds are ranked by FCR

	BWS (g)	BWG (g)	FI (feed/bird/day)	FCR (g feed:g gain)	AME (MJ/kg)
Low	473 ± 11	530 ± 10	102 ± 2	1.34 ± 0.01	14.57 ± 0.04
High	481 ± 8	479 ± 9***	104 ± 2	1.52 ± 0.01***	14.70 ± 0.10

Data are expressed as mean ± SEM (n = 24 birds per group). *** indicates significant difference between high and low FCR birds ($p<0.001$). BWS, body weight start; BWG, body weight gain; FI, feed intake; FCR, feed conversion ratio; AME, apparent metabolisable energy.

Ranking by AME

When birds were ranked based on high and low AME values, significant differences were observed in AME ($p<0.001$) and BWS ($p<0.05$) between high and low birds (Table 4). FCR, BWG and FI was not significantly different between high and low AME birds ($p>0.05$).

Table 4. Performance data from AME study when birds are ranked by AME

	BWS (g)	BWG (g)	FI (feed/bird/day)	FCR (g feed:g gain)	AME (MJ/kg)
Low	464 ± 8	508 ± 9	103 ± 1	1.43 ± 0.02	14.38 ± 0.04
High	490 ± 10*	501 ± 13	102 ± 2	1.43 ± 0.02	14.88 ± 0.07***

Data are expressed as mean ± SEM (n = 24 birds per group). * indicates $p<0.05$, *** indicates $p<0.001$. BWS, body weight start; BWG, body weight gain; FI, feed intake; FCR, feed conversion ratio; AME, apparent metabolisable energy.

Gene Expression Analysis

A comparison of gene expression in the duodenum of birds in the high and low AME groups found a total of 2272 genes that were differentially expressed at a level of two-fold or greater using a p-value of 0.01. A number of the genes differentially expressed between high and low AME are known to be involved in metabolism and nutrient transport. In addition to this, a number of genes that participate in key metabolic pathways have been identified as differentially regulated (Table 5).

Table 5: Selected metabolic pathways that have genes differentially expressed in high and low AME birds.

	High	Low	Total
Carbohydrate Metabolism			
Glycolysis / Gluconeogenesis	2 / 43	2 / 43	4 / 43
Starch and sucrose metabolism	1 / 25	4 / 25	5 / 25
Inositol phosphate metabolism	2 / 44	4 / 44	6 / 44
Pyruvate metabolism	0 / 32	2 / 32	2 / 32
Propanoate metabolism	0 / 24	3 / 24	3 / 24
Butanoate metabolism	1 / 23	1 / 23	2 / 23
Amino sugar and nucleotide sugar metabolism	1 / 35	3 / 35	4 / 35
Pentose phosphate pathway	1 / 21	0 / 21	1 / 21
Ascorbate and aldarate metabolism	1 / 8	2 / 8	3 / 8
Galactose metabolism	0 / 25	2 / 25	2 / 25
Fructose and mannose metabolism	2 / 26	2 / 26	4 / 26
Pentose and glucuronate interconversions	1 / 12	1 / 12	2 / 12
Energy Metabolism			
Oxidative phosphorylation	1 / 108	5 / 108	6 / 108
Lipid Metabolism			
Sphingolipid metabolism	2 / 33	0 / 33	2 / 33
Glycerophospholipid metabolism	1 / 51	5 / 51	6 / 51
Arachidonic acid metabolism	0 / 29	1 / 29	1 / 29
Glycerolipid metabolism	0 / 37	3 / 37	3 / 37
alpha-Linolenic acid metabolism	0 / 15	1 / 15	1 / 15
Ether lipid metabolism	0 / 27	2 / 27	2 / 27
Linoleic acid metabolism	0 / 16	1 / 16	1 / 16
Fatty acid metabolism	1 / 27	2 / 27	3 / 27
Androgen and estrogen metabolism	0 / 18	1 / 18	1 / 18
C21-Steroid hormone metabolism	0 / 6	1 / 6	1 / 6
Amino Acid Metabolism			
Alanine, aspartate and glutamate metabolism	0 / 25	2 / 25	2 / 25
Arginine and proline metabolism	0 / 35	3 / 35	3 / 35
Glycine, serine and threonine metabolism	1 / 24	1 / 24	1 / 24
Cysteine and methionine metabolism	1 / 30	3 / 30	4 / 30
Phenylalanine, tyrosine and tryptophan biosynthesis	0 / 5	1 / 5	1 / 5
Lysine degradation	0 / 32	2 / 32	2 / 32
Valine, leucine and isoleucine degradation	2 / 36	3 / 36	5 / 36
Histidine metabolism	0 / 18	1 / 18	1 / 18

Tyrosine metabolism	2 / 30	2 / 30	4 / 30
Phenylalanine metabolism	1 / 15	1 / 15	2 / 15
Tryptophan metabolism	0 / 33	2 / 33	2 / 33
beta-Alanine metabolism	0 / 18	2 / 18	2 / 18
Selenoamino acid metabolism	1 / 19	0 / 19	1 / 19
Transport and Catabolism			
ABC transporters	1 / 31	3 / 31	4 / 31
Endocytosis	6 / 158	3 / 158	9 / 158
Lysosome	3 / 87	0 / 87	3 / 87
Endocrine System			
Insulin signaling pathway	1 / 105	5 / 105	6 / 105
GnRH signaling pathway	2 / 75	4 / 75	6 / 75

The data indicate the number of genes differentially expressed compared to the total number of genes in the particular pathway, e.g. for the starch and glucose metabolism pathway there are a total of 25 genes in the pathway of which 5 are differentially regulated in the AME experiment, with 1 being more highly expressed in the high AME birds and four genes more highly expressed in the low AME birds.

We next looked beyond the differentially expressed genes to investigate the ontology of all the genes that were expressed at a detectable level above background. Amongst the biological functions that we investigated the proportion of genes that were detectable was consistently around 80%. Relating the differentially expressed genes back to broad biological classification showed that genes involved in carbohydrate metabolism, amino acid metabolism and membrane transport processes were the most over-represented with 15.6%, 12.3% and 13.3% of the genes in each class with detectable expression levels being differentially expressed between the high and low AME groups (Table 6). Interestingly when we compared gut gene expression in the birds at the extreme of the FCR distribution we found only a single biological function that was heavily overrepresented, this was membrane transport in which 35% of the genes with detectable levels of expression were differentially expressed (data not shown).

Table 6: Expression of genes associated with particular biological functions.

Biological Function	**Total genes detectable**	**Total genes on array**	**% of genes detected**	**No. of genes differentially expressed**	**% detected genes differentially expressed**
Carbohydrate Metabolism	243	331	73	38	15.6
Energy Metabolism	93	116	80	7	7.5
Lipid Metabolism	233	296	79	21	9.0
Sphingolipid metabolism	28	32	88	2	7.1
Nucleotide Metabolism	132	170	78	6	4.5
Amino Acid Metabolism	220	284	77	27	12.3
Metabolism of Other Amino Acids	68	82	83	3	4.4
Glycan Biosynthesis and Metabolism	149	206	72	12	8.1
Metabolism of Cofactors and Vitamins	100	121	83	5	5.0
Transcription	106	134	79	7	6.6
Translation	49	56	88	3	6.1
Membrane Transport	30	40	75	4	13.3
Signal Transduction	701	838	84	53	7.6
Signaling Molecules and Interaction	370	443	84	30	8.1

Transport and Catabolism	186	245	76	13	7.0
Cell Motility	127	155	82	13	10.2
Cell Growth and Death	166	211	79	13	7.8
Cell Communication	310	377	82	33	10.6
Circulatory System	97	118	82	8	8.2
Endocrine System	356	439	81	30	8.4
Immune System	114	144	79	2	1.8
Total	3878	4838	80	330	8.5

Bacterial populations in the caecum

The Roche/454 sequencing run produced almost half a million sequence reads with approximately 20,000 reads from each bird sampled. The simplest analysis of the data, using the RDP classification tool which classifies sequences down to the genus level, revealed that there were a few bacterial groupings that were represented at statistically significantly different levels between the high AME birds and the low AME birds (Table 7).

Analysis of the complete set of samples that were sequenced showed that there were two dominant bacteria phyla; the Bacteroidetes represented 14.3% of all sequences and the Firmicutes were present at 60.6%. 25.1% of sequences were unable to be assigned a phylum using the RDP classifier. Within the Bacteroidetes most of the sequences could be classified down to the family level Bacteroidaceae. The Firmicutes were more diverse; approximately a third were of the class Bacilli, predominantly in the family Lactobacillus, and half were in the class Clostridia, distributed amongst the families Ruminococcaceae, Lachnospiraceae and unclassified Clostridia. A more detailed description of the Clostridia is shown in Table 7. There was a statistically significant difference in the number of Clostridia sequences seen in the high and low AME birds with the high AME birds having on average well over twice as many Clostridia.

Table 7. Analysis of sequences classified to the class Clostridia in six selected birds from each of the high and low AME classes.

Classification	**Low AME average**	**Low AME SD**	**High AME average**	**High AME SD**	**T-test P-value**
» » » » class "Clostridia"	3834.67	1831.16	9068.67	4402.66	0.03
» » » » » order Clostridiales	3399.50	555.08	8573.50	4142.16	0.05
» » » » » » family Incertae Sedis XIII	1.83	3.54	3.17	4.08	0.82
» » » » » » » genus Anaerovorax	1.67	2.83	2.67	3.30	0.91
» » » » » » family "Ruminococcaceae"	1281.50	818.69	2676.17	2196.80	0.20
» » » » » » » genus "Ruminococcaceae Incertae Sedis"	2.67	2.16	7.67	4.46	0.19
» » » » » » » genus Faecalibacterium	356.33	721.46	796.00	1683.15	0.57
» » » » » » » genus Anaerotruncus	56.33	71.20	88.17	119.21	0.76
» » » » » » » genus Subdoligranulum	79.67	144.31	132.00	137.59	0.68
» » » » » » » unclassified_"Ruminococcaceae"	784.33	494.88	1651.17	1045.33	0.11
» » » » » » family "Lachnospiraceae"	603.00	612.13	1867.00	1463.17	0.08
» » » » » » » genus Syntrophococcus	0.50	0.71	2.17	2.22	0.36
» » » » » » » genus Roseburia	10.83	6.52	81.00	84.09	0.12
» » » » » » » genus "Lachnospiraceae Incertae Sedis"	98.17	104.04	507.00	408.22	0.04
» » » » » » » genus Hespellia	2.00	2.00	3.83	2.28	0.33
» » » » » » » unclassified_"Lachnospiraceae"	485.67	556.54	1431.83	1150.29	0.11

The average numbers of sequences to a particular classification are shown as well as the standard deviation (SD) across each sample set. A Student T-test was performed to compare the high AME and low AME birds.

The most detailed analysis of the bacterial populations was performed using the MOTHUR program. MOTHUR was able to classify all sequences to at least the phylum level and most to at least the genus level. This program is challenging to use as it is still in relatively early development and so we had to overcome a number of bugs. To process the large dataset that that we had developed it was necessary to use an advanced computer cluster at the Queensland Facility for Advanced Bioinformatics (QFAB) and even that facility was challenged by the computer time required to analyse the half million sequences that we obtained. A significant advantage of the MOTHUR program is that its deep analysis goes down to the species level, when possible, and compares all the new unknown sequences and clusters them into distinct operational taxonomic units (OTUs). An indication of the diversity found in our samples is given by the wide range of classifications that MOTHUR made (Table 8).

Table 8. Phylogenetic categories found to be present using the MOTHUR program

Phylogenetic depth	OTUs	Genus/Species	Phylogenetic depth	OTUs	Genus/Species
2	0.1.1	Bacteroidetes-Chlorobi	9	0.1.2.1.2.2.1.1.1.1	Streptococcus_minor
3	0.1.1.1	Bacteroidetes	4	0.1.2.1.3	Mollicutes
4	0.1.1.1.1	Bacteroides-Prevotella	6	0.1.2.1.3.1.1	Staphylococcus_aureus_et_rel.
5	0.1.1.1.1.1	Bacteroides	8	0.1.2.1.3.1.1.1.1	Staphylococcus_warneri
6	0.1.1.1.1.1.1	Bacteroides_fragilis	7	0.1.2.1.3.1.1.2	Staphylococcus_cohnii_et_rel.
7	0.1.1.1.1.1.1.1	Lactobacillus_delbrueckii_et_rel.	8	0.1.2.1.3.1.1.2.1	Staphylococcus_equorum-linens_
8	0.1.1.1.1.1.1.1.1	Lactobacillus_crispatus_et_rel.	4	0.1.2.1.4	Staphylococcaceae
9	0.1.1.1.1.1.1.1.1.1	Lactobacillus_gallinarum_et_rel.	5	0.1.2.1.4.1	Staphylococcus
9	0.1.1.1.1.1.1.1.1.2	Lactobacillus_kefirofaciens	3	0.1.2.2	Clostridia
9	0.1.1.1.1.1.1.1.1.3	Lactobacillus_ultunense	4	0.1.2.2.1	Desulfotomaculum_et_rel.
8	0.1.1.1.1.1.1.1.2	Lactobacillus_fermentum_et_rel.	5	0.1.2.2.1.1	Desulfotomaculum_alkaliphilum_et_rel.
9	0.1.1.1.1.1.1.1.2.1	Lactobacillus_igluviei-theromtolerans	6	0.1.2.2.1.1.3	Clostridium_xylanovorans
9	0.1.1.1.1.1.1.1.2.2	Lactobacillus_mucosae	7	0.1.2.2.1.1.4.1	Clostridium_nexile
9	0.1.1.1.1.1.1.1.2.3	Lactobacillus_panis_et_rel.	7	0.1.2.2.1.1.4.2	Dorea
9	0.1.1.1.1.1.1.1.2.4	Lactobacillus_pontis	9	0.1.2.2.1.1.5.1.1.2	Eubacterium_ruminantium
9	0.1.1.1.1.1.1.1.2.5	Lactobacillus_reuteri	7	0.1.2.2.1.1.6.1	Eubacterium_oxidoreducens
8	0.1.1.1.1.1.1.1.3	Lactobacillus_inners-johnsonii-gasseri	8	0.1.2.2.1.1.6.2.1	Eubacterium_ramulus_et_rel.
9	0.1.1.1.1.1.1.1.3.1	Lactobacillus_inners	7	0.1.2.2.1.3.3.2	Eubacterium_eligens_et_rel.
6	0.1.1.1.1.1.2	Bacteroides_thetaiotaomicron	8	0.1.2.2.1.3.3.2.1	Eubacterium_eligens
5	0.1.1.1.1.2	Prevotella	8	0.1.2.2.1.3.3.2.2	Lachnospira_pectinoschiza
6	0.1.1.1.1.2.1	Prevotella_brevis-ruminicola	5	0.1.2.2.2.1	Clostridium_pasteurianum_et_rel.
7	0.1.1.1.1.2.1.1	Prevotella_brevis_et_rel.	6	0.1.2.2.2.2.5	Parasporobacterium_et_rel.
9	0.1.1.1.1.2.1.1.1.1	Prevotella_brevis	6	0.1.3.1.1.2.1	Prevotella_melaninogenica_et_rel.
4	0.1.1.1.2	Runella	7	0.1.3.1.1.2.1.1	Prevotella_veroralis
3	0.1.1.2	Clostridiales	6	0.1.3.1.2.1.2	Lactobacillus_perolens
4	0.1.1.2.1	Acetobacterium_et_rel.	5	0.1.3.1.2.2	Enterococcus_phoeniculicola
5	0.1.1.2.1.1	Acidaminobacter_et_rel.	5	0.1.3.1.3.1	Spiroplasma_et_rel.
6	0.1.1.2.1.1.1	Mogibacterium_et_rel.	6	0.1.3.1.3.1.1	Spiroplasma_citri_et_rel.
7	0.1.1.2.1.1.1.1	Eubacterium_infirmum-minimum	4	0.1.3.1.4	Paenibacillus
8	0.1.1.2.1.1.1.1.1	Eubacterium_infirmum	5	0.1.3.1.4.1	Paenibacillus_chibensis_et_rel.
4	0.1.1.2.2	Caloramator_et_rel.	6	0.1.3.1.4.1.1	Paenibacillus_chibensis
5	0.1.1.2.2.1	Clostridium_proteolyticum_et_rel.	7	0.1.3.2.1.1.1.1	Eubacterium_sp.
4	0.1.1.2.3	Johnsonella_et_rel.	5	0.1.3.2.1.2	Clostridium_difficile
5	0.1.1.2.3.1	Clostridium_colinum-piliforme	5	0.1.3.2.1.3	Peptostreptococcus_anaerobius
5	0.1.1.2.3.2	Clostridium_propionicum_et_rel.	5	0.1.3.2.1.4	Tissierella_et_rel.
6	0.1.1.2.3.3.1	Anaerostipes_et_rel.	6	0.1.3.2.1.4.1	Soehngenia
6	0.1.1.2.3.3.2	Bryantella_et_rel.	5	0.1.3.2.2.2	Oxobacter
7	0.1.1.2.3.3.2.1	Bryantella	7	0.1.3.3.3.3.2.3	Hespellia_et_rel.
7	0.1.1.2.3.3.2.2	Clostridium_bolteae-clostridiforme	2	0.1.4	Firmicutes
7	0.1.1.2.3.3.2.3	Clostridium_sphenoides_et_rel.	3	0.1.4.1	Bacillales_Mollicutes

8	0.1.1.2.3.3.2.3.1	Clostridium_sp.	4	0.1.4.1.1	Erysipelothrix_et_rel.
7	0.1.1.2.3.3.2.4	Clostridium_symbiosum_et_rel.	6	0.1.4.1.1.1.1	Bacillus_benzoevorans
6	0.1.1.2.3.3.3	Dorea_et_rel.	5	0.1.4.1.2.1	Enterococcus_hermanniensis
7	0.1.1.2.3.3.3.1	Clostridium_scindens_et_rel.	6	0.1.4.1.2.1.1	Enterococcus__avium_et_rel.
7	0.1.1.2.3.3.3.2	Ruminococcus_torques	7	0.1.4.1.2.1.1.1	Enterococcus_avium
7	0.1.1.2.3.3.4.1	Catonella_et_rel.	7	0.1.4.1.2.2.4.1	Pediococcus_damnosus-inopinatus-parvulus
8	0.1.1.2.3.3.4.1.1	Acetitomaculum_et_rel.	5	0.1.4.1.3.1	Acholeplasmataceae
9	0.1.1.2.3.3.4.1.1.1	Eubacterium_halli	6	0.1.4.1.3.1.1	Phytoplasma
7	0.1.1.2.3.3.4.2	Clostridium_fimetarium	7	0.1.4.1.3.1.1.1	Phytoplasma_ash_et_rel.
8	0.1.1.2.3.3.4.3.1	Syntrophococcus	8	0.1.4.1.3.1.2.1.1	Lactobacillus_nageli
6	0.1.1.2.3.3.5	Roseoburia_et_rel.	6	0.1.4.1.3.2.2	Spiroplsma_diminutum_et_rel.
6	0.1.1.2.3.3.6	Ruminocoocus	7	0.1.4.1.4.1.1.2	Phytoplasma_peach_et_rel.
7	0.1.1.2.3.3.6.1	Ruminococcus_obeum	5	0.1.4.1.4.2	Mycoplasma_pneumoniae_et_rel.
7	0.1.1.2.3.3.6.2	Ruminococcus_productus	4	0.1.4.2.1	Acidaminococcaceae
7	0.1.1.2.3.3.6.3	Ruminococcus_schinkii	5	0.1.4.2.1.1	Allisonella-Dialister
4	0.1.1.2.4	Ruminococcus_et_rel.	6	0.1.4.2.1.1.1	Dialister_sp.
5	0.1.1.2.4.1	Acetanaerobacterium_et_rel.	5	0.1.4.3.1.4	Filifactor_et_rel.
6	0.1.1.2.4.1.1	Linmingia_et_rel.	6	0.1.4.3.1.4.1	Clostridium_sticklandii
5	0.1.1.2.4.2	Acetivibrio_et_rel.	3	0.1.5.1	Desulfurella-Hippea
6	0.1.1.2.4.2.1	Clostridium_thermocellum_et_rel.	4	0.1.5.1.1	Desulfurella
5	0.1.1.2.4.3	Anaerofilum-Faecalibacterium	6	0.1.5.1.2.1.1	Anaeroplasma
6	0.1.1.2.4.3.1	Faecalibacterium	6	0.1.5.3.2.1.1	Clostridium_pascui-peptidovorans
7	0.1.1.2.4.3.1.2	Faecalibacterium_prausnitzii	2	0.1.6	Epsilonproteobacteria
5	0.1.1.2.4.4	Eubacterium_desmolans	3	0.1.6.1	Campylobacteriaceae_2
5	0.1.1.2.4.5	Eubacterium_siraeum	4	0.1.7.1.1	Bacillus_cereus_et_rel.
5	0.1.1.2.4.6	Papillibacter_et_rel.	5	0.1.7.1.1.1	Sporolactobacillus_dextrus
6	0.1.1.2.4.6.1	Clostridium_viridae_et_rel.	4	0.1.7.1.2	Bacillus_circulans_et_rel.
7	0.1.1.2.4.6.1.1	Clostridium_orbiscindens_et_rel.	5	0.1.7.1.2.2	Lactococcus-Streptococcus
6	0.1.1.2.4.6.2	Oscillospira	6	0.1.7.1.2.2.1	Streptococcus
6	0.1.1.2.4.6.3	Papillibacter-Termitobacter	7	0.1.7.1.2.2.1.1	Streptococcus_suis_et_rel.
5	0.1.1.2.4.7	Ruminococcus_bromii_et_rel.	8	0.1.7.1.2.2.1.1.1	Streptococcus_minor_et_rel.
6	0.1.1.2.4.7.1	Clostridium_leptum	4	0.1.7.1.3	Coprobacillus_et_rel.
5	0.1.1.2.4.8	Ruminococcus_callidus	5	0.1.7.1.3.1	Clostridium_ramosum_et_rel.
2	0.1.2	Beta_Gammaproteobacteria	5	0.1.7.1.3.2	Coprobacillus
3	0.1.2.1	Betaproteobacteria	4	0.1.7.2.1	Syntrophomonas_et_rel.
4	0.1.2.1.1	Neisseriales	5	0.1.7.2.1.1	Syntrophomonas_sp.
5	0.1.2.1.1.1	Neisseria_et_rel.	6	0.1.7.2.1.3.1	Filifactor
6	0.1.2.1.1.1.1	Alysiella-Simonsiella			
6	0.1.2.1.1.1.2	Lactobacillus_salivarius_et_rel.	4	1 representative	
8	0.1.2.1.1.1.2.1.1	Lactobacillus_ruminis	5	30 representatives	
8	0.1.2.1.1.1.2.1.2	Lactobacillus_salivarius	6	62 representatives	
6	0.1.2.1.1.1.3	Paralactobacillus	7	70 representatives	
5	0.1.2.1.1.2	Lactobacillaceae	8	31 representatives	
9	0.1.2.1.1.2.1.1.3.1	Lactobacillus_johnsonii	9	41 representatives	
4	0.1.2.1.2	Lactobacillales	10	10 representatives	
6	0.1.2.1.2.2.1	Lactobacillus_buchneri_et_rel.			
8	0.1.2.1.2.2.1.1.1	Lactobacillus_hilgardii-vermiformis			

The first and fourth columns define how far down the phylogenetic hierarchy the sequences have been classified. The species assignment is at level 6-9, depending on the complexity of a particular arm in the phylogenetic tree. The second and fifth columns show the OTU designation which is the classification tree constructed during MOTHUR analysis and the third and sixth columns give the phylogenetic name at the level defined. The last seven entries summarize the many distinct classifications of the large number of sequences which represent currently unnamed or unknown bacteria, so at level 5 of the taxonomy there were 30 different groups identified but not assigned to known phylogenetic names, similarly at level 6 there were 62 distinct unclassified groups, etc.

There are a variety of ways to present the results of the large scale sequencing. Measurements of the diversity and quantity of bacteria within each sample can be used to construct a tree indicating the relationship between each sample (Figure 1). This method of examining the data is informative because it indicates that the samples are not readily separated into low AME and high AME groups but rather the samples are mixed with no higher branching point exclusively containing high or low AME birds. Some birds, such as AL2, AH2 and AH11 are outliers that are quite different to the other samples; when the individual bacterial profiles were examined it was found that these birds did not carry any bacteria of the phylum Bacteroidetes. When considered in the context of the initial statistical analysis, showing significant differences in the carriage of some particular bacterial classes, the tree analysis shows that such differences are subtle and do not dominate the overall structure of the bacterial populations.

A heat map (Figure 2) demonstrates the diversity of operational taxonomic units (OTUs) that have been defined. Common bars across multiple samples at the top of the map show OTUs common to most samples whereas the region at the bottom of the map consists mainly of OTUs found in one or a few samples. The OTU designation is a surrogate for a species designation. With the new molecular approaches to identifying bacteria there are a large number of 16S sequences which cannot be assigned to currently known species, the OTUs are closely related groups of sequences.

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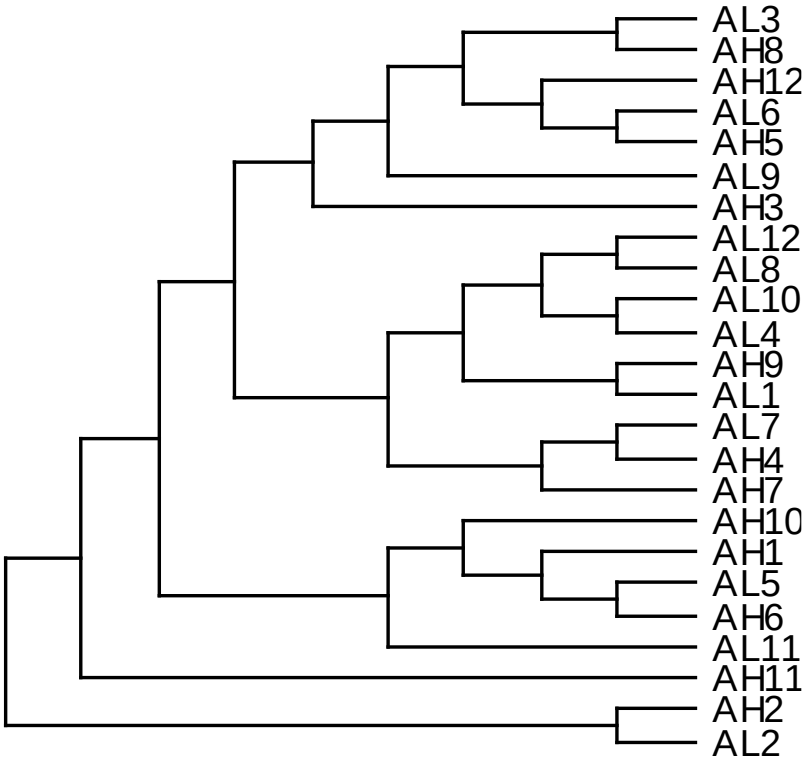

Figure 1. Braycurtis tree of sample relatedness (distance 0.03). The labels refer to the sample numbers, with AL1 being the bird with the lowest AME value and sample AH1 being the bird with the highest AME value.

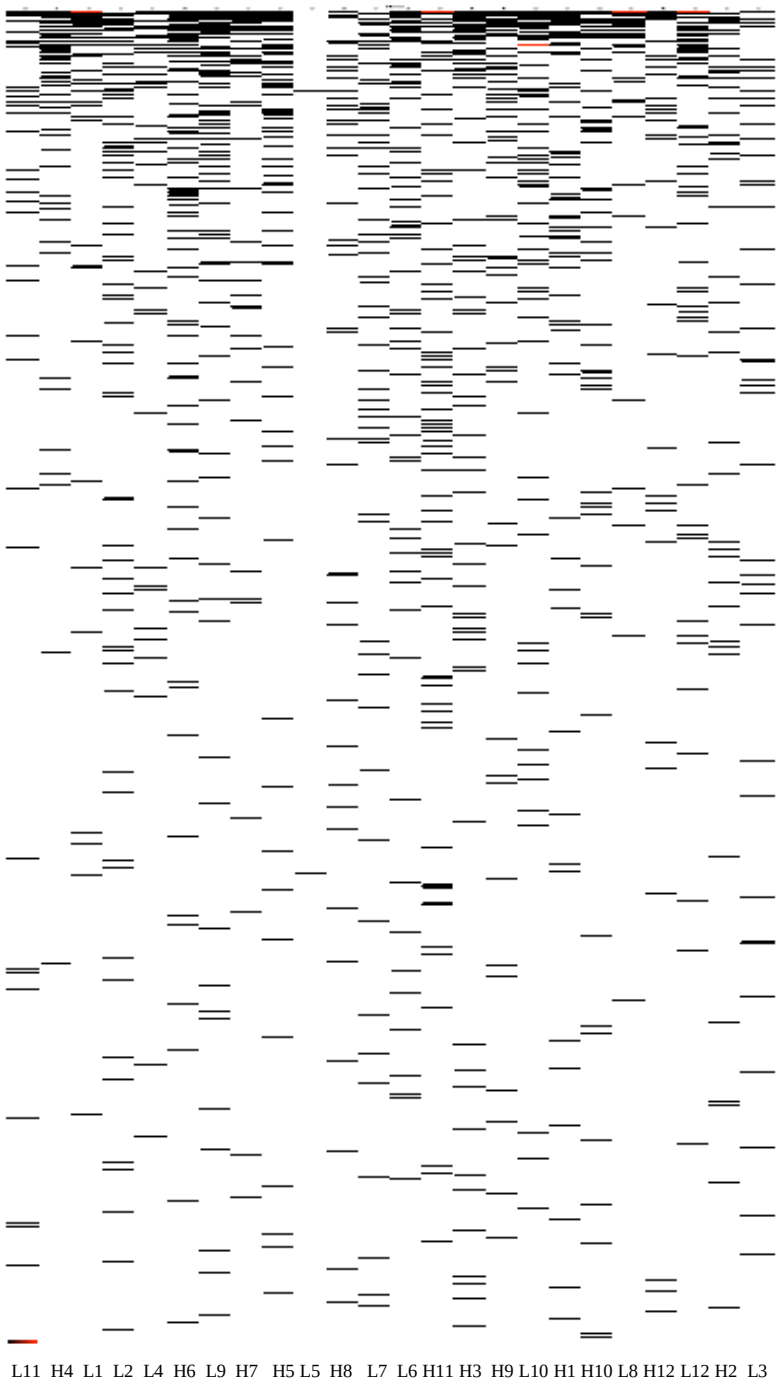

Figure 2. Heat map of OTUs (90% similarity) found in caecal samples. Each line represents an OTU and the thickness gives an indication of the abundance of the OTU. The labels at the foot of the figure refer to the sample numbers, with L1 being the bird with the lowest AME value and sample H1 being the bird with the highest AME value.

Bacterial populations in the jejunum

A preliminary analysis of bacterial samples recovered from the surface scraping of the jejunum (from a limited number of birds) has shown that the microbiota associated with the mucosal surface of the jejunum is dominated by *Lactobacillus* species. The tree analysis (Figure 3) indicates that the ileum samples from high and low AME birds may be more distinct than the caecal samples, as there is a clear separation of the two groups in the tree presented. Statistical analysis revealed that the low AME

birds carried more *Lactobacillus reuteri* than the high AME birds (p-value 0.007). It is also likely that *Lactobacillus johnsonii* is more abundant in the low AME birds.

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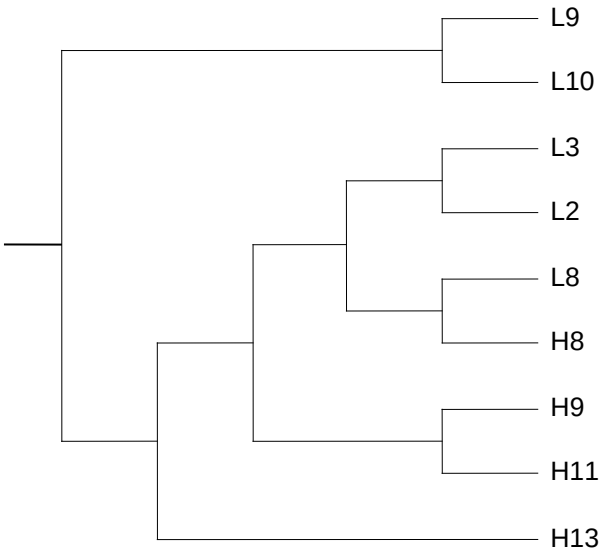

Figure 3. Jest tree of sample relatedness (distance 0.03). The labels refer to the sample numbers, L prefixes indicate birds from the low AME group and the H prefix indicates birds from the high AME group.

Note: We have an extensive dataset characterising the microbiota of each bird we used in the study. Within the limits of this report we have only presented a sample of the data and analysis that has been carried out.

Discussion

Different cohorts of birds were ranked in the high and low groups when AME and FCR were used as the primary determinant for separation. When birds were ranked by FCR, the AME for the corresponding high and low FCR birds was not significantly different. Likewise, when birds were segregated based on AME, there was no significant difference in FCR between high and low AME birds. The correlation between FCR and AME was poor ($r^2 = 0.04$, $p>0.05$), this is likely to be due to the nature of these two parameters. AME is a measure of the energy available to the bird for metabolism (Farrell 1999), and is often used for the evaluation of feedstuffs and subsequent feed formulation (Farrell 1999). AME is the difference between the energy consumed via the feed and the energy lost via the excreta. This AME value is therefore indicative of the energy available to the bird for all necessary processes, including maintenance, energy requirements of the microbiota, and growth. AME however does not predict how effectively this energy will be utilised by the bird (Farrell 1999), nor does it account for the quality of other essential components in the diet and their relative availability to the host. This can include amino acid profiles, vitamins and minerals. Therefore a high AME value for a bird may not necessarily indicate that the bird will perform to a high standard. Factors including intestinal villus/crypt structure, microbial composition and microbial activity can also influence AME (Hughes 2001). Feed conversion ratio however, is a direct indication of how efficiently a bird is converting feed consumed into body weight gained and is therefore a more accurate measure of bird performance.

In the current study, which involved high replication and a single diet, the AME value provided an indication of the energy available to each bird, and when combined with performance data such as FCR, was indicative of how effectively a bird partitioned this energy towards growth. For example, a

bird with high AME and poor FCR would indicate high energy expenditure in processes other than growth, whilst a bird with low AME and a good FCR would indicate that the bird was effective at partitioning available energy towards growth and has lower energy expenditure in other areas. When birds were ranked based on FCR, there were no significant differences in AME evident between the two groups of birds. This indicates that whilst the energy available to these birds was comparable, the low FCR birds may have been more effective at partitioning this energy towards growth-related processes. Whilst, when birds were ranked by AME, the FCR between the two groups was numerically identical. Therefore, whilst the high AME birds appeared to have more energy available, the low AME birds may have converted this energy into tissue more effectively than the high AME birds.

An analysis of gene expression in the duodenum of birds at the extremes of the AME distribution showed that there are a large number of fairly subtle differences in gene expression. The differentially expressed genes encompassed a wide range of functions, mapping to many different biochemical pathways and cellular functions. Key genes involved in metabolism, nutrient and ion transport, growth, gut health and gut hormone action have been identified. No genes were highly differentially expressed (> 10-fold), suggesting that the differences seen are modulating the activity of pathways rather than turning whole pathways on or off. A large number of the genes expressed in the gut encode proteins involved in basic cellular growth and metabolism and have key roles in the ongoing rapid tissue renewal which is a prominent feature of the gut. In future analysis it may be useful to focus in more detail on those genes which encode proteins involved in direct nutrient scavenging and use. This would include sampling and analysing other organs, such as the pancreas, that are producing key digestive enzymes.

Differences in gene expression may result from induction or repression, brought about by changes in the local cellular environment, or may result from intrinsic differences in expression of different alleles of the same gene. Allelic differences in expression are the result of sequence differences. The differential expression data could be used to direct the search for single nucleotide polymorphisms (SNPs) that are associated with genotypes that have favourable production characteristics.

The composition of the gut microbiota has been analysed by metagenomic analysis of 16S rRNA genes and has identified differences between the high AME and low AME birds. In the caecum the class Clostridia bacteria are more abundant in the high AME birds. This difference is due to a number of OTU groups of unknown species and also members of the genus *Lachnospiraceae*. The preliminary analysis of jejunum samples showed that *Lactobacillus* species dominate this niche. *L. reuteri* and *L. jonhsonii* are clearly more abundant in the low AME birds. These results lay a solid foundation for the ongoing analysis of the structure and function of the gut microbiota in nutritional studies. One long-term goal is to use such information to identify individual bacterial isolates or groups of isolates that could be targeted for use as inoculation cultures to help improve energy usage in birds.

The very large number of 16S sequences that can now be generated by the Roche/454 Titanium sequencing technology allows in depth analysis of bacterial samples but requires powerful computing resources. We have used internal computing resources as well as the computer cluster at the Queensland Facility for Advanced Bioinformatics (QFAB). Even with these resources the complete bioinformatic analysis of the dataset is very time consuming and limited. We are investigating the use of the supercomputer facilities at the High Performance Computing and Communication Centre (a joint facility of the Bureau of Meteorology and CSIRO) and awaiting the upgrade of the QFAB cluster as well as evaluating new software analysis packages that are just becoming available.

The limited nature of the current study has not allowed an evaluation of the cause and effect relationship between differences in gene expression, microbial populations and AME values. This is likely to be a complex issue to dissect and inevitably there will be a range of interactions which establish the exact conditions and performance of the chicken gut. The aim of future work should be to identify the most consistently correlated measurements and focus in on those factors which are likely to be most informative and/or amenable to manipulation.

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Implications

This study has shown that there are differences in gene expression in the duodenum of birds with high and low AME values and also differences in the resident microbial populations. It can be seen that particular metabolite transporters and enzymes in key metabolic pathways, as well as a range of other proteins, are potentially involved in determining the efficiency of energy usage and bird performance. Similarly we have also found correlations between AME values and the carriage of particular bacterial classes. Such a brief study inevitably leads to more questions - it will now be important to establish how general these correlated findings are – how many of these differences will be found in repeated experiments and how many will be maintained over a variety of diets and environmental conditions (e.g. changes in litter, water quality, bird line, hatchery conditions, temperature, etc.)? An essential point to address is the causal relationship between differences in energy usage and gene expression and microbial populations as an understanding of this will allow us to direct efforts to utilise the information to improve broiler performance. This project has established a foundation for continuing work aimed at identifying ways to manipulate gene expression and microbiota for the benefit of the industry.

Recommendations

This study has shown that there are two key elements of bird biology, gut gene expression and micriobiota, which are amenable to detailed study and show differences which can be correlated to differences in bird performance. Further detailed study has the potential to advance our understanding of the basis of variable bird performance. This single trial has shown that the methods that have been applied are appropriate and are capable of producing detailed gene expression and microbial profiles. The priorities now should be to:
(1) Extend the study to determine how reproducible the findings are and how variable the expression and microbiota profiles are when different diets and environments are used.
(2) Production of a dataset that demonstrates which genes are reproducibly differentially expressed between high and low performance birds should be a priority. Such a dataset would be of value to poultry breeders looking for ways to focus in on genes (and alleles) important in bird performance.
(3) Determine if elements of the differentially present microbiota can be cultured and characterised with a view to developing beneficial cultures for inoculation of young birds to improve performance.
(4) Investigate non-invasive methods of microbiota sampling, via faeces, to allow longitudinal studies on individual birds to follow colonisation and microbiota development over time.
(5) Investigate statistical methods that will allow the multi-variant co-analysis of expression and microbiota data to determine the extent of influence exerted by the interaction of these two elements.

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Plain English Compendium Summary

Project Title:	
Project No.:	09-20
Researcher:	Dr Robert Moore
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Objectives	• Investigate the biological basis of variable bird performance. • Identify gene expression differences that correlate with differences in apparent metabolisable energy measurements (AME). • Evaluate the utility of new technology high throughput deep sequencing of 16S rDNA amplicons for the identification of changes in gut bacteria population structure that correlate with differences in AME measurements.
Background	In a production environment the growth performance of individual broiler birds can be quite variable despite the fact that all birds within a production flock have similar genetics and are raised together in an optimal environment with access to the same feed and water. For the producer it is desirable that bird performance is as even as possible and that the feed supplied is used as efficiently as possible. This project aimed to investigate two aspects of bird biology, gene expression and microbial populations in the gut, to determine if there are correlates with bird performance. The identification of correlated aspects of bird biology may indicate targets for intervention to reduce variation in bird performance and enable more birds to perform at an elite level.
Research	A single animal trial was carried out in which productivity measurements were made on 96 birds. There was a low correlation between the key productivity measurements of feed conversion ratio and AME. Samples from birds at the extremes of the AME distribution were analysed for global gene expression in the duodenum and for the structure of the microbial populations in the caecum and jejunum. Gene expression was measured using a newly designed NimbleGen microarray. Over 2000 genes were found to be differentially expressed between high and low AME birds. The differentially expressed genes were widely distributed across a range of biochemical functions and pathways and included genes encoding proteins with key functions in nutrient transport and metabolism. Roche/454 DNA sequencing technology was used to characterise the microbial populations present in the gut of birds. Half a million sequence reads were generated and the bioinformatic analysis of these sequences revealed particular classes of bacteria that differed in abundance between high and low AME birds.
Outcomes	The new technologies that we have applied to the study of key aspects of bird biology are powerful and appropriate. We found both gene expression differences and microbial population differences between birds at the extremes of the AME distribution.
Implications	The project has established a foundation for continuing work aimed at understanding some of the key drivers of variable bird performance and subsequently identifying ways to manipulate gene expression and microbiota for the benefit of the industry.
Publications	In preparation