



Intestinal Microbiota Associated with High Feed Conversion Efficiency in Chickens

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Introduction

- Antibiotics have been used as growth promoters in commercial poultry production over many decades.
- The industry is interested in the development of strategies that avoid antibiotics but deliver the productivity benefits.
- Low dose antibiotics modify the gut microflora and this is believed to be the basis of at least some of the beneficial effects of antibiotic use.
- Can the gut microflora be modified in other ways to optimise animal performance (efficiency of food conversion, energy retention)?

Linkage between performance and microbiota

- *A priori* reason to think microbiota will be important.

The microbiota is a dynamic “organ” – a metabolic powerhouse that provides the functionally limited host with an extensive array of enzymes and substrates.

The host + microbiota is a “**meta-organism**”

- Studies in mice and humans have demonstrated that gut microbiota can influence energy retention and can predispose to obesity.

The importance of microbiota in the gut

- Bacteria play an important role in:
 - digestion of food
 - mobilisation of inaccessible nutrients
 - absorption, storage, energy harvest – obesity
 - renewal of gut epithelial cells
 - breakdown of toxins
 - fight off and exclude pathogens
 - stimulate the immune system
 - bile acid metabolism → endocrine activity

Aims

- Our aim is to understand the impact of gut microbiota on animal performance.
- Long term vision is to be able to manipulate the gut microbiota to achieve optimal gut health and performance.
- Develop well targeted and rigorously validated alternatives to antibiotics, e.g, Prebiotics, Probiotics, Management Practices, etc.



Experimental Approach

- To understand the effects of microbiota on performance we have taken a different approach to most other published studies.
- Rather than have experimental groups in which diet, additives, environment, etc. have been varied to give animals with different performance values we have used a single uniform treatment group and then selected birds at the extremes of the performance continuum for careful analysis.

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Typical 2 x 2 x 2 Design

Diet A Additive, Yes Environment A	Diet B Additive, Yes Environment A
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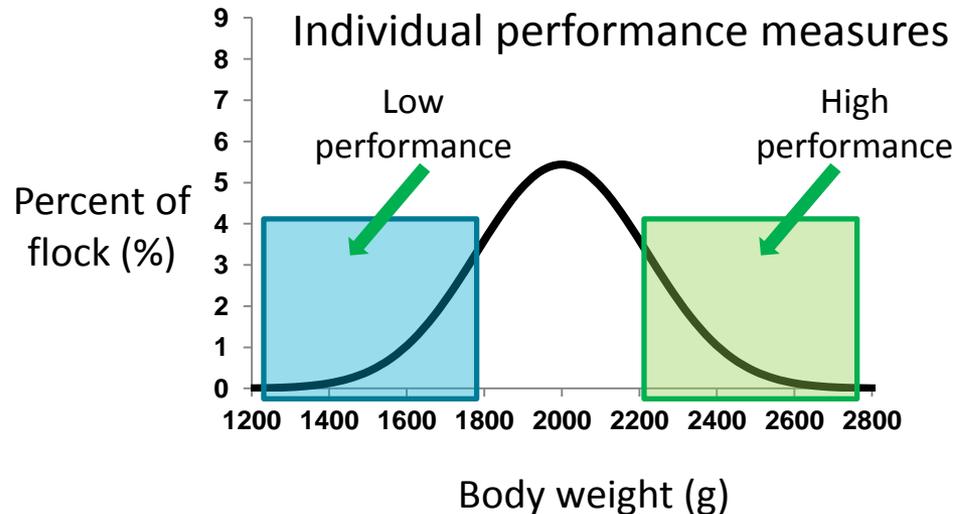
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Our approach - Single group Individual performance measures



What are the potential causes of performance variation?

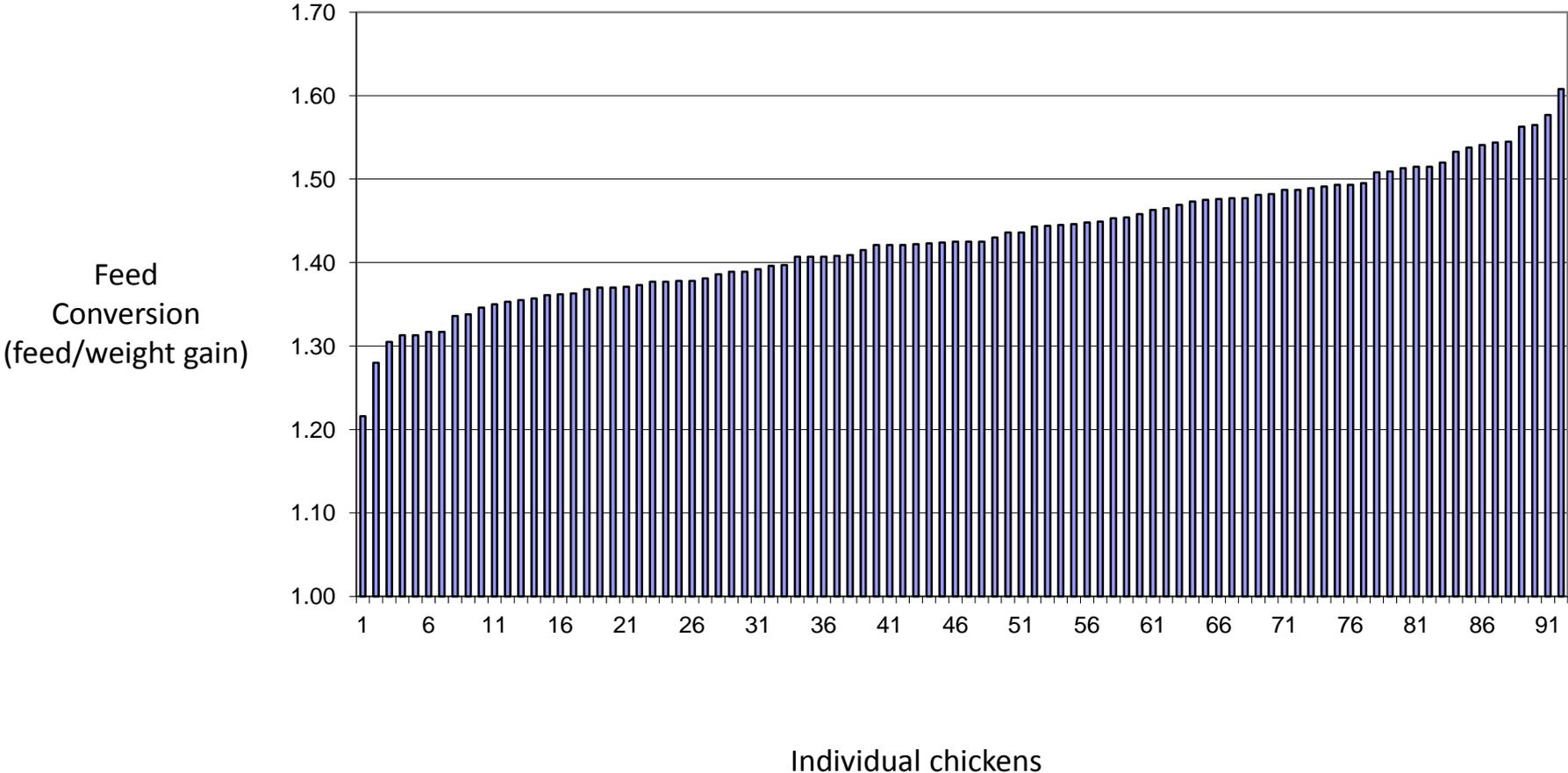
- Differences in genetics
- Behavioral differences
- Micro-environmental differences
- Differences in gene expression
- **Differences in microflora**
- Stochastic variation in identical birds

Experimental Design

- 96 male Cobb chickens.
- Initially housed in group on floor, ad lib. food and water.
- At day 13 transferred into metabolism cages, in pairs, to acclimatize, then at day 15 moved to individual cages.
- After 3 days further acclimatization individual feed intake and excreta was measured for 4 days to determine Apparent Metabolisable Energy (AME).
- Feed consumption and weight gains monitored to determine Feed Conversion Ratio (FCR).
- Analysis carried out on birds at extremes of AME and FCR distributions.



Variable Performance Data



Variable Performance Data



Performance data of high and low FCR birds

	Body Weight Start (g)	Body Weight Gain (g)	Feed Intake (feed/bird/day)	Feed Conversion Ratio (g feed:g gain)
Low	473 ± 11	530 ± 10	102 ± 2	1.34 ± 0.01
High	481 ± 8	479 ± 9***	104 ± 2	1.52 ± 0.01***

*** indicates significant ($p < 0.001$) difference between high and low FCR birds.
One-way analysis of variance using the general linear model procedure of
SAS ver. 9.1 . N=24

All birds eat the same amount of feed but the best performing birds (low FCR) put on more body weight – they use 0.2 kg less food per kilogram of body weight.

Gut Microflora from Extreme Birds



Jejunum surface

Ceecal content

Bacterial analysis

How we characterise the microbiota

Sample of interest (e.g. caecal content)



Extract total microbial DNA



PCR amplify variable region of 16S rRNA genes



High throughput sequencing of amplicons (454 FLX Sequencer)



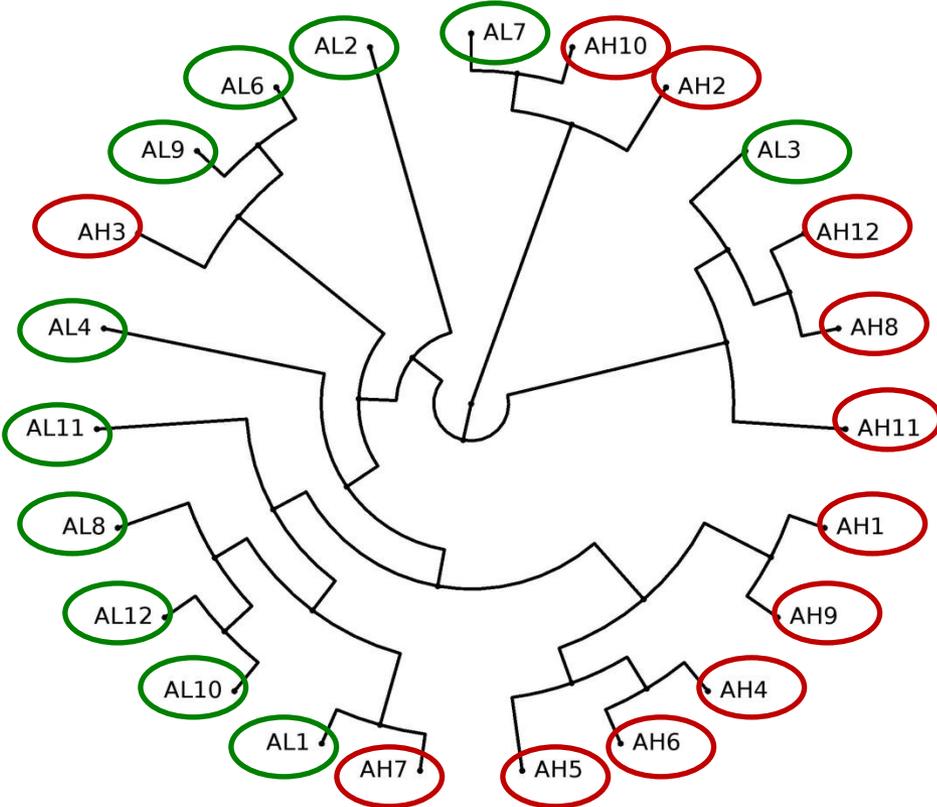
Bioinformatic analysis of sequences to group into Operational Taxonomic Units (fairly equivalent to species), quantify, and align with known bacterial taxonomy

General findings from first experiment

- 746 Operational Taxonomic Units (OTUs) were identified (OTUs are equivalent to species)
- The caecal populations are more complex than the jejunal
- The jejunal mucosa is dominated by *Lactobacillus* species
- The caecal microbial populations were dominated by bacteria in the phyla Bacteroidetes and Firmicutes

The high and low AME birds have distinctive microbiota

Sample relationship tree



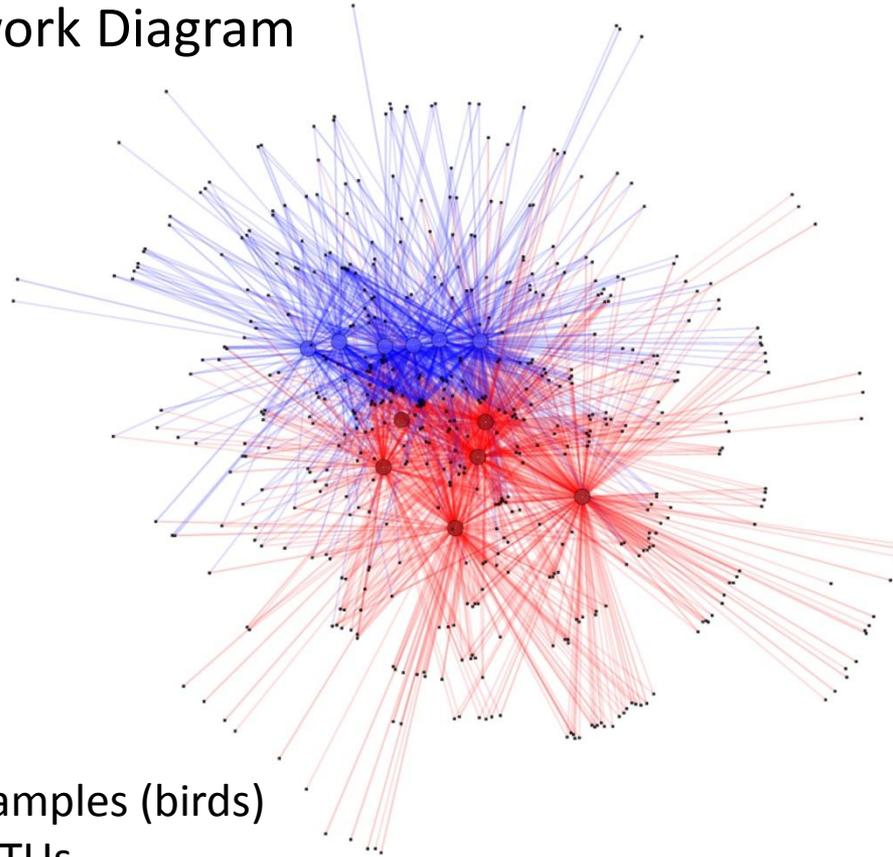
○ = Low AME bird

○ = High AME bird



The high and low AME birds have distinctive microbiota

Cytoscape Network Diagram



Large nodes are samples (birds)
Small nodes are OTUs
Blue = High, Red = Low

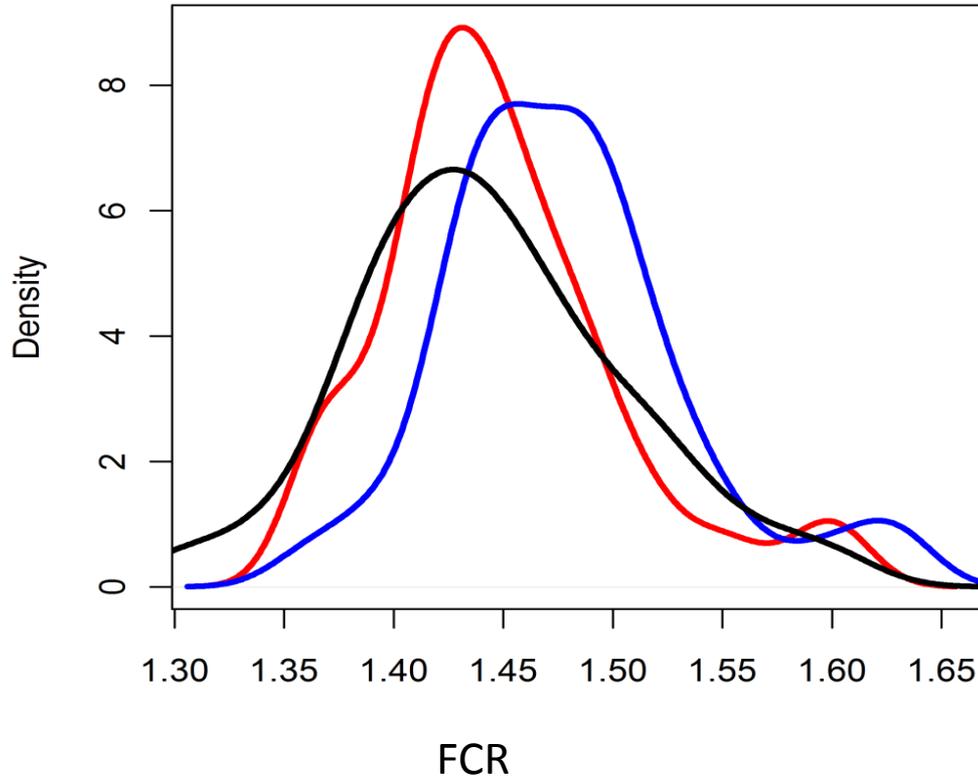
Microbiota differences in the caecum

- High AME birds carried twice as many bacteria of the class Clostridia.
- FCR birds had 26 OTUs that were differentially abundant between high and low performance birds. 22 were more abundant in the low, 4 were more abundant in the high.

Reproducibility of results?

- Before pursuing the possibility of changing performance by manipulating these differentially abundant OTUs we were interested to know if they always come up as important in differentiating high and low performance birds.
- Correlation vs. Causation
- Three replicate trials were performed and analysed in the same way.
 - All conditions were maintained as constant as possible.
 - Only known variable was time of year.
 - Analysed 48 samples from each of three sources (caecal, faecal, ileum).

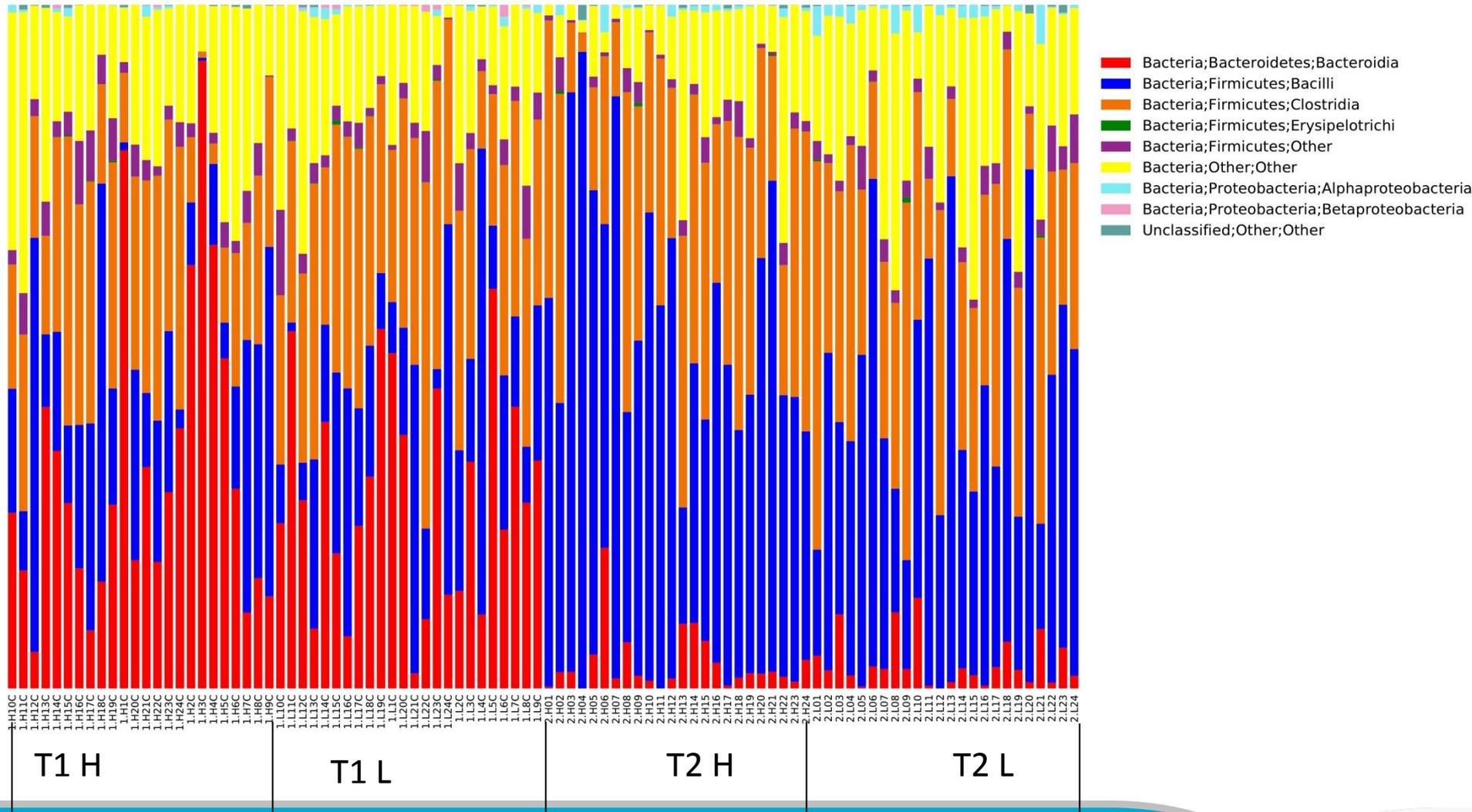
Reproducibility of results?



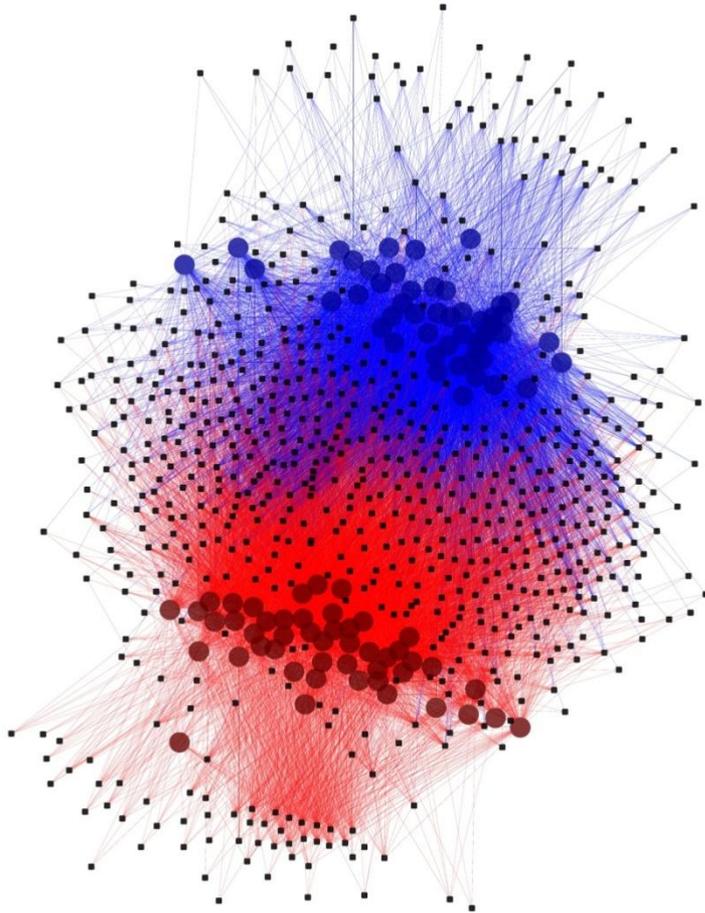
- EXP1 – black
- EXP2 – red
- EXP3 – blue

Clearly the FCR profile is different in the three trials

Comparison of Trials 1 and 2 – Class Level

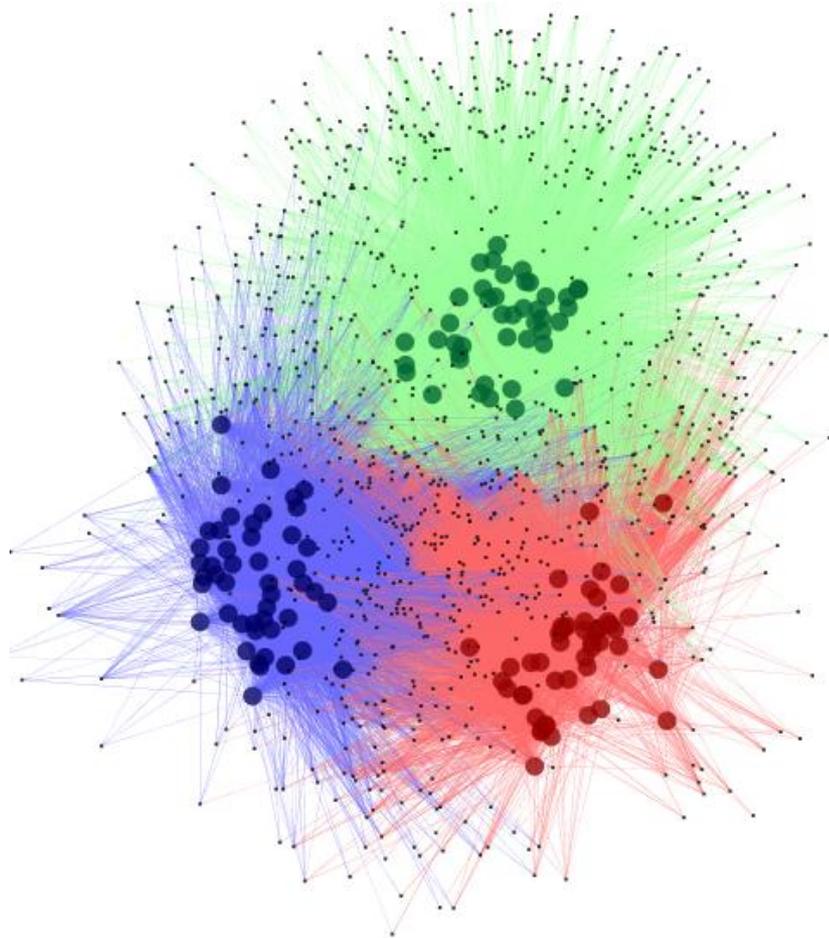


Comparison of Trials 1 and 2 – Class Level



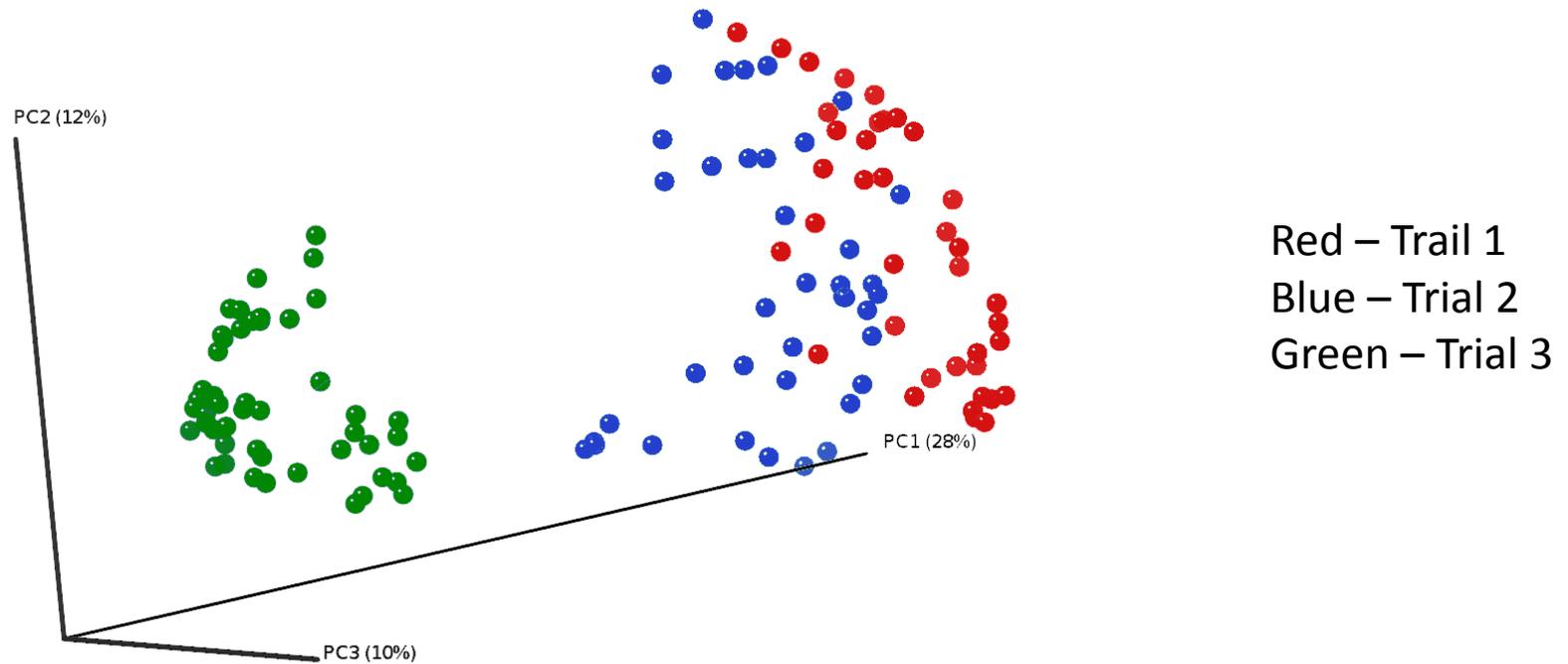
Red – Trial 1
Blue – Trial 2

Comparison of Trials 1, 2 and 3 – Network



Red – Trial 1
Blue – Trial 2
Green – Trial 3

Comparison of Trials 1, 2 and 3 – PCoA Plot



Trials 1 and 2 are more similar than trial 3

Candidate OTUs driving performance

The OTUs differential in at least two of the tree trials include:

- CEACAL SAMPLES
 - *Ruminococcus*
 - Clostridiales RF5
 - Unknown Clostridium
 - *Faecalibacterium prausnitzii*
 - Cultured unclassified butyrate producer
 - *Oscillospira*
 - *Eubacterium siraeum*
 - Ethanoligenes
- FEACAL SAMPLES
 - Uncultured *Lactobacillus* (similar to *L. johnsonii*)
- Poor performance birds have greater microbial diversity – lots of rare OTUs

Conclusions from replicate trials

- Each batch of birds had its own unique microbiota structure.
- There were large bird to bird variations in microbiota structure within a batch.
- Each batch of birds had a different performance profile.
- Within each batch of birds there were differentially abundant OTUs in the high and low performance sub-groups.
- No differentially abundant OTUs were found across all three trials.
- The most consistent microbiota difference between high and low performance birds was greater complexity (more rare OTUs) in the poor performing birds.

How might we improve performance?

- Reduce complexity of microbiota.
- Ensure favourable conditions for proliferation of OTUs found to be correlated with performance (e.g. prebiotics, probiotics).
- Stabilize microbiota structure so that there is less batch to batch variation.

What is driving the difference in microbiota?

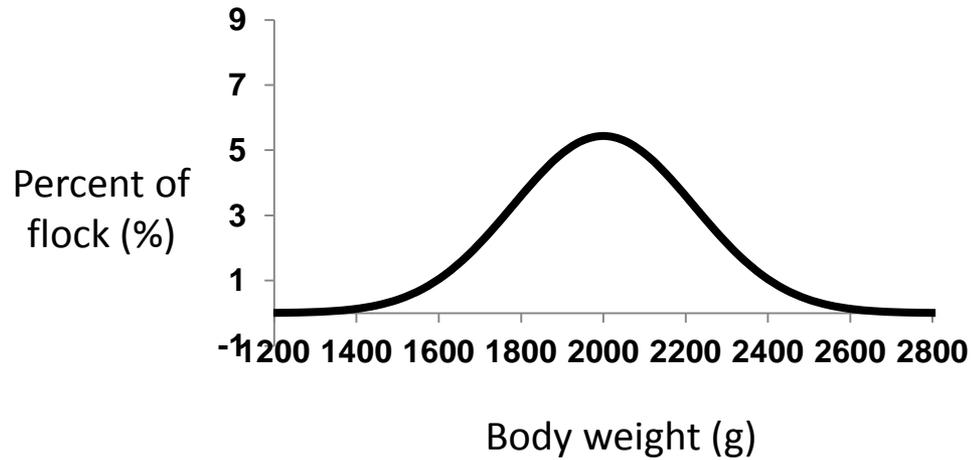
We hypothesize that high hygiene in modern commercial hatcheries causes very variable microbial colonization of the gut.

- Newly hatched chicks are not exposed to the natural microflora from the hens and nesting environment.
- Rather, they are exposed to a largely random collection of microbes from the environment, e.g. from the hatching environment, transport boxes, feed, handlers, etc.
- Therefore colonization can vary, depending on the extraneous sources and timing.

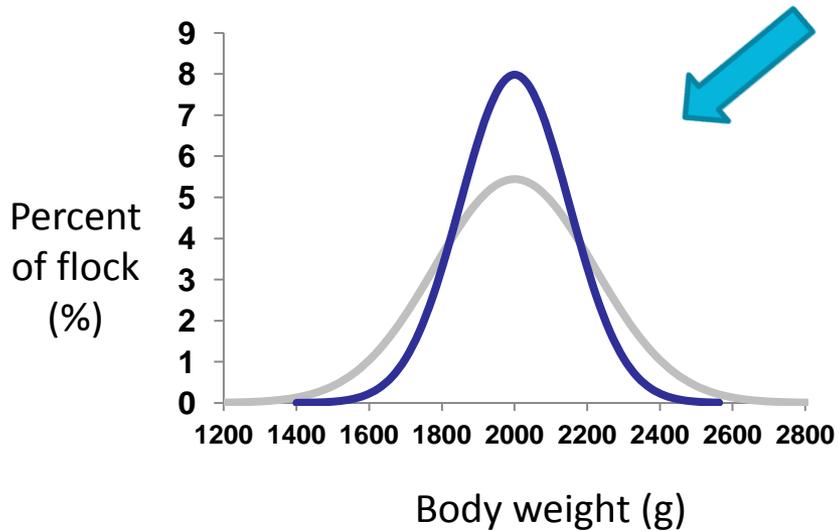
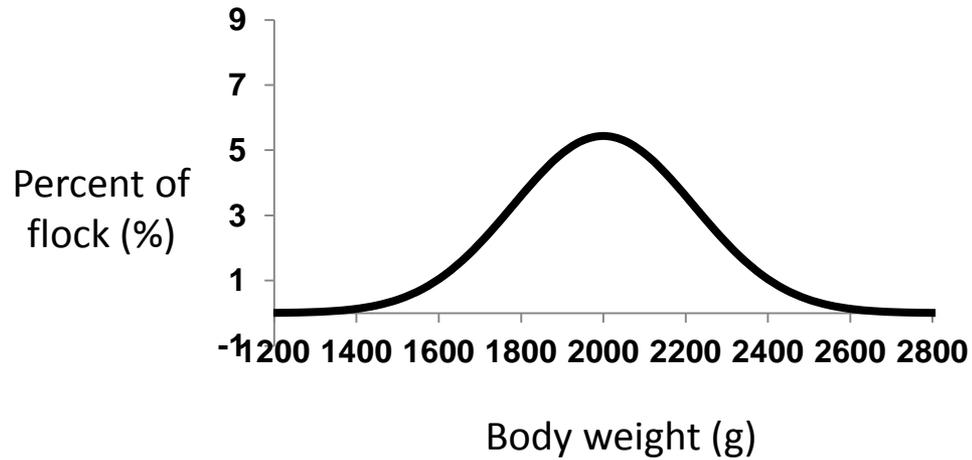
Can controlled colonization reduce variability?

- Can a consistent, healthy, beneficial microbiota be established by “inoculating” birds with an ideal microbiota?
- Is it necessary to use a whole microbiota or can a select few strains be used?
- We anticipate that such treatments will not only reduce flock to flock variation but also variation within a flock, thus producing a more even homogenous flock in terms of performance.
- We are working to test these hypotheses and produce a performance enhancing/equalising microbiota mix.

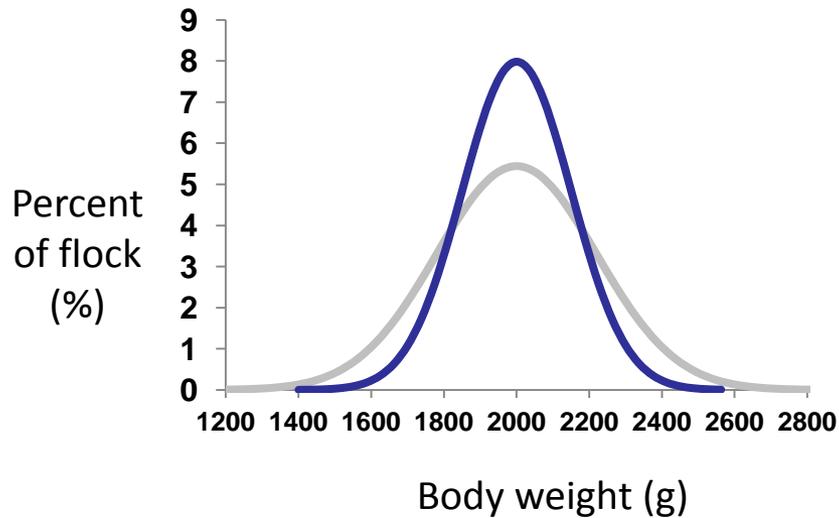
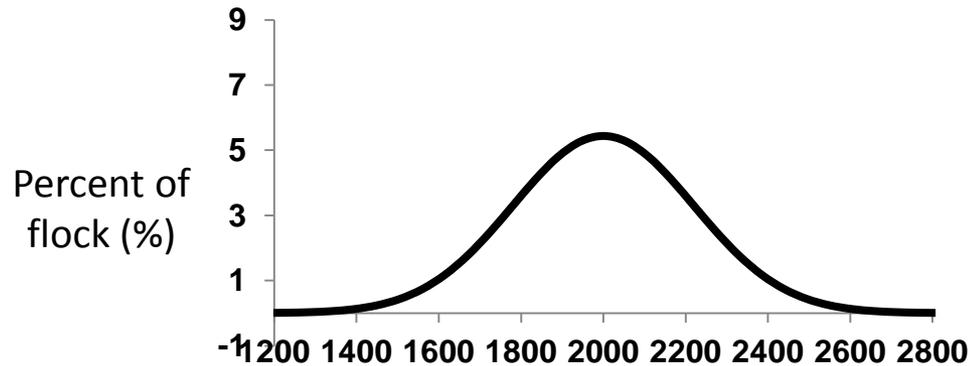
Controlling batch performance



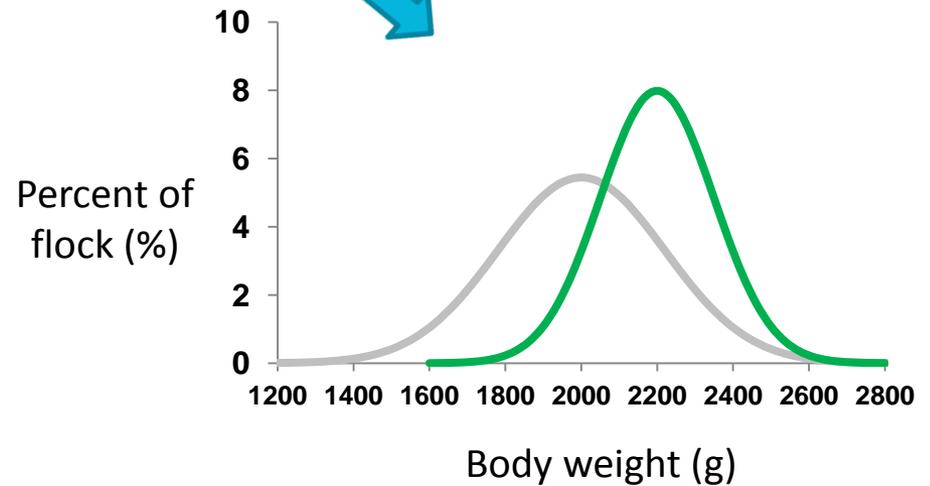
Controlling batch performance



Controlling batch performance



Body weight (g)



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