THE OPPORTUNITIES AND CHALLENGES OF INTEGRATING GENOMICS IN A BROILER BREEDING PROGRAM

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SUMMARY

Cobb-Vantress is one of the leading global suppliers of broiler breeding stock, with products distributed in more than 100 countries. Cobb has continually invested in new technologies to consistently deliver genetic improvement that provide a competitive advantage in the market place. Recently, Cobb has made significant investments to implement a genomic selection program to complement the traditional breeding program. In addition to genomic selection, opportunities such as causative mutation detection, parentage testing and simple trait selection have been successfully implemented in various breeding programs within Cobb. There are many challenges involved in implementing these genomic technologies, including a simple but complex effort toward the logistics of sample collection and management from multiple pure-line populations at different geographical locations. The current state of the genome sequence presents some barriers to the successful use of these technologies in some instances; however there is currently some significant effort toward its improvement. We believe that genomic technologies are beneficial technologies to improve the genetics of our broilers.

INTRODUCTION

Cobb-Vantress, Inc. (Cobb) is the world's oldest broiler breeding company. Since 1916 Cobb-Vantress has contributed to the dynamic growth of the global poultry industry that has transformed chicken into a popular, affordable and healthy protein choice. Cobb maintains a pedigree program, ensuring continual genetic progress for a production pipeline where it creates multiple parent stock targeted toward the production of multiple products with different performance profiles, ranging from highly efficient, to high yielding, and slow growing broilers. These products are successfully produced in very diverse environmental, management and regulatory production systems globally. There are several challenges facing the poultry industry requiring the production of alternative broiler solutions for future markets. Some of these challenges include;

- Volatile global grain prices emphasizing the need for continual improvement in feed conversion and use of alternative feed products.
- Emerging market opportunities emphasizing the need for diversified products for new environments.
- Welfare and customer requirements driving the need for innovative products such as antibiotic free chicken.
- Governmental and regulatory changes requiring the need for unique breeds (such as slow growing lines) or management practices.

Genomics is a technology being investigated to help Cobb create broiler solutions to tackle some of these industry challenges. To date, Cobb has successfully integrated genomic technologies such as genomic selection, parentage testing, identification and elimination of deleterious alleles, and single gene tests. This paper will address some of the challenges and opportunities that Cobb has identified through its genomic program.

CHICKEN BREEDING AT COBB

Over 2 million pure line chicks are hatched at Cobb annually. All chicks hatched on one of our seven pedigree farms are individually identified, and individually phenotyped for over 50 traits, including;

- broiler traits such as weight, feed conversion and breast meat percentage,
- reproduction traits such as hatch of fertile and egg production
- welfare and health traits such as skeletal defects, foot pad dermatitis and liveability

Less than 5% of hatched chicks are retained as future breeders based on selections using both phenotype and BLUP breeding values. It is estimated that one selected female pedigree breeder makes a genetic contribution over 3 million broilers (which are a four-way line cross). Given our ability to pedigree millions of birds and maintain high selection intensities we can make genetic gains very quickly. Figure 1 illustrates the progress made in the final broiler product (a cross of 4 pure-lines) over a 20 year period.

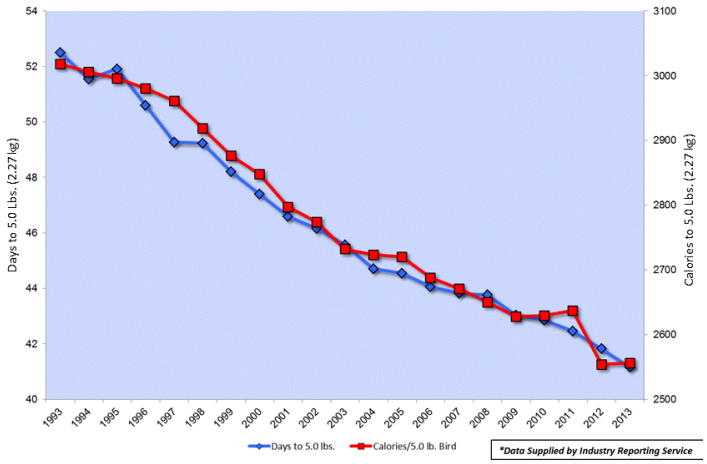

Figure 1. Days to 5.0 Lbs. (2.27 kg) and Calories/5.0Lb for broiler between 1993 and 2013

GENOMIC TOOLS FOR CHICKEN BREEDING

The chicken genome sequence was made available by the international chicken genome consortium in 2004 (Hillier *et al*. 2004) and has been revised three times (2006, 2011 and 2013). The chicken genome is just over a third the size of a typical mammalian species, being only 1.2 Bbp. Similar to other livestock species the genome sequence has been used to create a variety of public genotyping tools such as the Illumina 60K chip (Groenen *et al*. 2011) and the Affymetrix high-density chip (Kranis *et al*. 2013), and the additional development of company specific arrays.

Opportunities

There are a variety of opportunities afforded to chicken breeding through genomic technologies such as high-throughput genotyping and sequencing. Two such opportunities described in this paper include genomic selection and identification of DNA variations explaining deleterious phenotypes.

Genomic selection

In boiler production, the gains of genome selection are made through the improvement of accuracy of selection, and through the introduction of new traits that could not otherwise be incorporated into the breeding program, rather than reducing generation interval. In Cobb, the

current analytical tool used to estimate genomic breeding values is single-step genomic BLUP (ssGBLUP) (Aguilar *et al*. 2010; Christensen & Lund 2010) using BLUP90IOD (Aguilar *et al.* 2011; Tsuruta *et al*. 2011). This methodology is amenable to our program due to the simple and fast calculation of genomic breeding values.

In order to calculate the impact of genomic selection on our pedigree traits, both traditional and genomic evaluations are computed and compared. The accuracy of each evaluation is determined by correlating the corrected phenotype with the predicted breeding values (either traditional BLUP or ssGBLUP) when the phenotype is not included in the analysis. These estimates of accuracy indicate that the improvement in breeding value accuracy due to genomic selection is highly variable and dependent on the heritability and the number of birds with genotypes for the trait(s) in question.

The key to the improvements in accuracy of breeding values for ssGBLUP is the increase in accuracy of estimated relationships between genotyped individuals. Estimated genomic relationships between full-sibs ranges from 0.266-0.701 with a mean of 0.483 while half-sib relationships range from 0.050-0.547 with a mean of 0.239 (Figure 2).

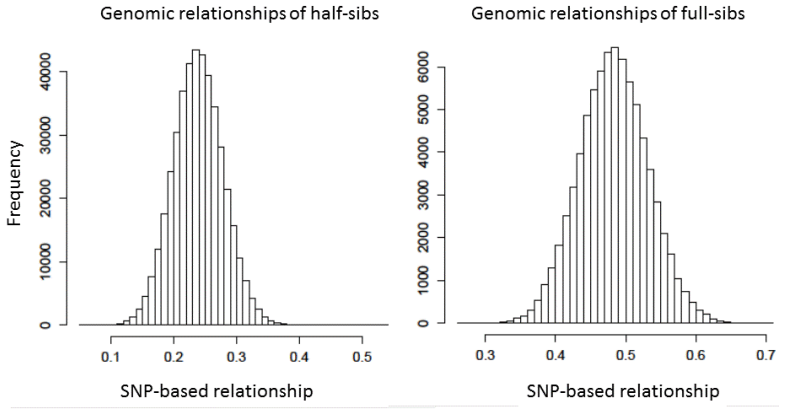

Figure 2. Histogram of genomic relationships among half-sibs (left) calculated from 431778 half sib pair combinations, and full sibs (right) calculated from 78352 full sib pair combinations.

The improvements in accuracy of selection of traits measured in our pedigree program represent the first step in the application of genome selection to a chicken breeding company. We anticipate that the largest gains for genomic selection will be for the incorporation of new traits that can only be measured on chickens outside the pedigree facility (such as disease challenge, and broiler performance in commercial environments as a four-way cross broiler).

DNA variations explaining deleterious phenotypes

High-throughput sequence analyses can be utilised to identify causal or predictive mutations for particular phenotypes. One such effort was toward the identification of causal or predictive alleles for a phenotype specific to one of our pure line breeds. This phenotype was termed 'wiry down', where affected chicks appeared wet and lethargic, and in most cases died soon after hatch. Pedigree analyses of affected families indicated that this phenotype was likely the result of a genetic mutation that occurred in one sire, seven years prior to the phenotype becoming obvious at our hatchery. In order to identify the mutation for this genetic disease, high-throughput genome sequencing was completed on pooled samples representing affected and unaffected individuals. Allele frequencies were compared between pools which highlighted a 10Mb region on chromosome 4 associated with the phenotype. Subsequent fine mapping of this region identified a

single SNP that was 100% predictive of the phenotype. This SNP is now being used to eliminate the condition from our population.

Similar efforts to identify predictive mutations for broiler phenotypes have not all been successful. Some of these efforts have utilised the same pooling approach as above and some have utilised an individual sequencing approach. The incomplete genome sequence, inaccuracies of phenotype recording and the complex nature of some of these phenotypes impact the successful identification of predictive tests for all traits.

Challenges

There are a variety of challenges that impact the utility of genomic tools in Cobb-Vantress.

- The chicken genome sequence is currently incomplete. In spite of the continual improvement of the chicken sequence, it is estimated that the current build is missing ~20% of the total genome (Warren 2014). Some of these missing sequences are due to missing micro-chromosome sequences (9 completely missing, and one other is poorly covered) and approximately 30,000 gaps in the available sequence (W. Warren *pers. comm*.). More importantly for the success of our genomic selection program, this missing sequence is estimated to contain between 5% and 20% of the expressed genes. This presents a difficult challenge in our ability to completely scan the chicken genome for genetic elements contributing the expressions of phenotypes.
- Current sequencing technologies are unable to sequence the GC-rich micro-chromosomes. Therefore tools such as genotyping-by-sequencing, or low coverage genome sequence for use in genomic selection will also fail at scanning the entire genome for contributions toward trait expression.
- The development of 'stable' and multiple-line genotyping tools (like SNP chips) is complicated by the massive allele frequency differences both between pure line populations, and the rapid changes in allele frequencies between generations of the same line.
- Logistics is one of the greatest challenges for the implementation of genomic selection. Examples of obstacles to overcome are:
    - The timing of sampling; genomics is simply not cheap enough to sample and process every chick at hatch; therefore, sampling has to be completed strategically.
    - Genotype processing time; the available time between sampling and selection age for the calculation of genomic breeding values is very short.
    - Sample collection and management; thousands of samples are collected and processed on a weekly basis from a number of pedigree farms both in the US and Europe.

While many challenges exist for implementing genomics, the opportunities and potential gains for a chicken breeding program are large.

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