

Nov 08–10, 2023

Leonardo Hotel
Hannover Airport



XII. European Symposium
on Poultry Genetics **2023**

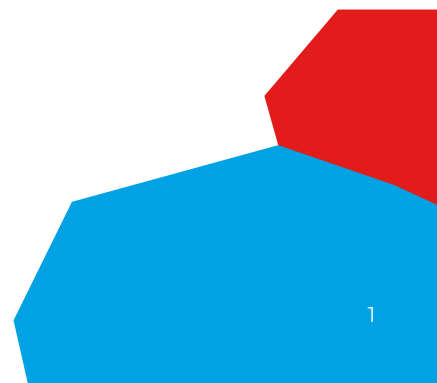
PROCEEDINGS



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Dear Colleagues

It is our great honor and pleasure to welcome you to the 12th European Symposium on Poultry Genetics, which will be held in Hannover, Germany, from 08 to 10 November, 2023.


The symposium has a long tradition of bringing together scientists from academia and industry. After the merger of the Poultry Breeders Roundtable in Western Europe and the AVIAGEN Symposium in Eastern Europe under the umbrella of the WPSA in 1999, the first European Symposium on Poultry Genetics was held in 1999 in Neustadt-Mariensee, Germany, just 30 kilometers from here. We, the German branch of WPSA in collaboration with Working Group 3 'Breeding and Genetics' of the European Federation of WPSA are proud to host the 12th Symposium again in Germany.

We are pleased to have been able to invite internationally renowned keynote speakers for six sessions of the symposium. They will report on current research in the areas of genetic engineering applied to chickens, genetic resources, and biological limits in breeding and genetic approaches to improve welfare as well as new phenotyping methods in poultry breeding and genomic developments in other bird species. In addition, the many contributions that you have submitted will make the symposium an outstanding event. We are particularly pleased to welcome many young scientists, three of whom we have invited to present their work at our special PhD Award session.

We would like to thank all members of the scientific committee for their support in the preparation of the program. In addition, we are grateful to the European Federation of WPSA, the Dutch Foundation for the Promotion of Poultry Science, the breeding companies Lohmann Breeders, Aviagen Group and Hendrix Genetics, and the journal MDPI Poultry for their support. Not least, we would like to thank the team of Eventlab for the technical preparation of the symposium and the excellent cooperation.

We hope that you will take home many new scientific insights and personal impressions from this symposium, so that your stay in Hannover will get an unforgettable professional and personal experience.

With our best wishes for a successful and enjoyable meeting in Hannover



Steffen Weigend
Chair of Working Group 3



Michael Grashorn
President of German Branch of WPSA



PROGRAM

— WEDNESDAY, 08. NOVEMBER 2023

13:00–13:15	OPENING Steffen Weigend, Friedrich-Loeffler-Institut, Germany
SESSION 1: GENETIC ENGINEERING APPLIED IN CHICKENS Chairs: Rudolf Preisinger & Kellie Watson	
13:15–13:45	Genetic modification of Z-Chromosome to avoid embryonic development of male chicks Yuval Cinnamon, The Volcani Center, Israel
13:45–14:15	Enhancing Chicken Immunity: Unleashing the Power of Gene Editing Benjamin Schusser, Technische Universität München, Germany
14:15–14:45	Chicken Primordial Germ Cells – Challenges and Opportunities in a Genebank Context Stefanie Altgilbers, Friedrich-Loeffler-Institut, Germany
14:45–15:00	General discussion
SHORT ORAL PRESENTATIONS (selected from abstracts)	
15:00–15:15	Production of genetically altered chickens in a Specified Pathogen Free (SPF) context: challenges and recent advances Dominique Meunier, University of Edinburgh, UK
15:15–15:30	PCR analysis for in-ovo sexing of chicken embryos Carla van der Pol, HatchTech, Netherlands
15:30–15:45	Improved technology of CRISPR/CAS9-mediated gene knock-out and gene editing in chicken as an approach to chicken resistance to viral diseases Jiri Hejnar, Czech Academy of Sciences, Czech Republic
15:45–16:00	General Discussion
16:00–16:30	Coffee break
POSTER SESSION Chairs: Steffen Weigend & Ahmad Reza Sharifi	
16:30–18:30	24 Posters to presented; 3 minutes each
18:30–21:30	POSTER VIEWING AND WELCOME RECEPTION

— THURSDAY, 09. NOVEMBER 2023

SESSION 2: GENETIC RESOURCES FOR BREEDING IN ORGANIC AGRICULTURE Chairs: Steffen Weigend & Pavel Trefil	
09:00–09:30	Requirements for and possibilities of breeding for organic farming Thomas Schneider, Bundesministerium für Ernährung und Landwirtschaft, Germany Dirk Hinrichs, Universität Kassel, Germany
09:30–09:45	Discussion
SHORT ORAL PRESENTATIONS OF CASE STUDIES	
09:45–10:00	Genetic management of French local breeds and their use in alternative farming systems Romuald Rouger, SYSAAF, France
10:00–10:15	Innovative Ways of Regional Sustainable Use of Animal Genetic Resources in Domestic Chickens Steffen Weigend, Friedrich-Loeffler-Institut, Germany
10:15–10:30	Establishing a breeding program for dual purpose chickens under certified organic farming conditions in Germany – A case study Christiane Keppler, Gallicon Poultry Consulting, Germany
10:30–10:45	General Discussion
10:45–11:15	Coffee break



PROGRAM

— THURSDAY, 09. NOVEMBER 2023

PHD AWARD SESSION

Chairs: Michael Grashorn & Steffen Weigend

11:15–11:35	Genomic prediction of commercial layers' bone strength across furnished cages and floor housings Mohammed Sallam, Swedish University of Agricultural Sciences, Sweden
11:35–11:55	The heritability of maxillary beak shape traits and their relationship to production and egg quality traits in a White Leghorn pure line population Sarah Struthers, Scotland's Rural College, UK
11:55–12:15	Is it possible to breed for optimized gut microbiota composition to improve efficiency in poultry? Valentin Haas, University of Hohenheim, Germany
12:15–12:30	General discussion
12:30–13:30	Lunch Break

SESSION 3: BIOLOGICAL LIMITS BREEDING

Chairs: Elisabeth Duval & Martino Cassandro

13:30–14:00	Breast muscle myopathies – do they limit broiler breeding? Avigdor Cahaner, The Hebrew University of Jerusalem, Israel
SHORT ORAL PRESENTATIONS OF CASE STUDIES	
14:00–14:15	Transcriptional impact of the naked neck genotype on the development of the Musculus complexus in naked neck broiler chickens Alexander Mott, Georg-August-University Göttingen, Germany
14:15–14:25	General discussion
14:25–14:55	Skeleton integrity & Laying performance / Keel Bones Mike Toscano, Universität Bern, Switzerland
SHORT ORAL PRESENTATIONS OF CASE STUDIES	
14:55–15:15	Developing a practical phenotype for laying hen bone quality & Tibial X-ray density measurement in a White Leghorn pure line population Ian Dunn, The University of Edinburgh, UK Björn Andersson, Lohmann Breeders, Germany
15:15–15:30	General discussion
15:30–16:00	Coffee break

SESSION 4: GENETIC APPROACHES TO IMPROVE WELFARE AND RESILIENCE

Chairs: Michele Boichard & Olivier Demeure

16:00–16:35	Behaviour genetics to help improve chicken welfare Julie Collet; Nicolas Bédère, INRAE, France
16:35–16:45	Discussion
SHORT ORAL PRESENTATIONS OF CASE STUDIES	
16:45–17:00	Genetic background of natural and induced antibody response in layer chickens Anna Wolc, Iowa State University, USA
17:00–17:15	The microbiome as a feature of multi-environment selection Richard Bailey, Aviagen Ltd., UK
17:15–17:30	General discussion
17:30–19:00	WG3 BUSINESS MEETING Chair: Steffen Weigend
20:00–22:00	GALA DINNER – HOTEL LEONARDO



PROGRAM

— FRIDAY, 10. NOVEMBER 2023

SESSION 5:

NEW PHENOTYPING METHODS IN POULTRY BREEDING

Chairs: Avigdor Cahaner & Yuval Cinnamon

09:00–09:40 New phenotyping methods in poultry breeding
Pauline Willemsen & Yves Jégo, Institut de Sélection Animale, Hendrix Genetics, Netherlands

09:40–09:50 Discussion

SHORT ORAL PRESENTATIONS OF CASE STUDIES

09:50–10:05 Genetic parameters for lifetime locomotor activity in group-housed broilers recorded using a radio frequency identification system
Esther Ellen, Wageningen University and Research, Netherlands

10:05–10:20 Genetics of gait score in broilers
Dagmar Kapell, Aviagen Limited, UK

10:20–10:35 General discussion

SHORT ORAL PRESENTATIONS OF CASE STUDIES

09:50–10:05 Genetic parameters for lifetime locomotor activity in group-housed broilers recorded using a radio frequency identification system
Esther Ellen, Wageningen University and Research, Netherlands

10:05–10:20 Genetics of gait score in broilers
Dagmar Kapell, Aviagen Limited, UK

10:20–10:35 General discussion

10:35–11:00 Coffee break

SESSION 6:

GENOMIC DEVELOPMENTS IN OTHER POULTRY SPECIES

Chairs: David Cavero & Milan Tyller

11:00–11:30 Latest development in Turkey breeding
Valentin Kremer, Speaker from Aviagen Turkeys Ltd., UK

11:30–12:00 Latest development in Duck breeding
Julien Fablet, ORVIA, Gourmaud Selection, France

12:00–12:15 Discussion

SHORT ORAL PRESENTATIONS OF CASE STUDIES

12:15–12:30 Main challenges for red partridge mating in cage-free system: reproduction efficiency, pedigree recording, and management of genetic diversity
Sophie Brard-Fudulea, SYSAAF, France

12:30–12:45 Genomic diversity and population structure of twelve Italian local turkey (*Meleagris gallopavo*) populations
Medhat Saleh, Bari University, Italy

12:45–13:00 General discussion

13:00–13:10

CLOSING

Steffen Weigend, Friedrich-Loeffler-Institut, Germany

WPSA WORKSHOP

13:30–15:15 Limits of genetic intervention:
Solving problems of cage-free housing
Michael Toscano, University of Bern, Switzerland



ABSTRACTS



WEDNESDAY, 08. NOVEMBER 2023

S-01 | Genetic engineering applied in chickens

Rudolf Preisinger
Kellie Watson



A GENETIC MODEL TO ELIMINATE MALE CHICK CULLING IN THE EGG INDUSTRY

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Keywords: Primordial Germ Cells, Transgenic chickens, Animal welfare

Male chicks, an undesirable outcome of the table egg industry, face a bleak fate due to their inability to lay eggs and unsuitability for meat production. Each year worldwide, approximately seven billion male chicks are sorted and culled shortly after hatching. This practice not only constitutes severe harm to animal welfare but also has significant environmental and economic consequences. Despite numerous efforts to address this issue, such as sorting technologies, sex ratio biasing, and creating dual-purpose breeds, no viable solution is available. Nevertheless, global opposition to this practice is gaining momentum, resulting in its progressive prohibition. We propose a genetic approach that inhibits the embryonic development of male chicks only, allowing females to hatch normally. This groundbreaking method ensures both the layer hens and the table eggs produced remain genetically identical to those currently used and produced by the industry (Figure 1), thus offering a promising strategy to end the culling of day-old male chicks.

In chickens, males are homogametic possessing two Z sex chromosomes, while females are heterogametic possessing W and Z chromosomes. Thus, the sex of the offspring is determined by the segregation of the maternal sex-chromosomes (Figure 1). This allows to selectively pass on a sex-linked trait, solely to male embryos (Figure 1, red-marked Z), leaving the genome of female embryos unmodified. By targeting a sex-linked trait that interferes with the early stages of embryogenesis, we can effectively stop the development of male embryos. However, for this trait transfer to be hereditary, it is essential that the activation of the gene will be controllable.

For this purpose, we designed a genetic sequence directed to integrate to the Z chromosome. This construct, which we term the Holy Grail (HG, Figure 2A), contains a single promoter that sequentially drives the expression of three elements, separated by polyadenylation sites (PA). The activity of each gene is determined by its proximity to the promoter. The first element to be expressed is the Green Fluorescence Protein (GFP), which serves as a “safe-lock” (SL) mechanism preventing unintended activation of the downstream elements. This element is flanked by Flippase Recognition Target (FRT) sites, which are targeted by the Flippase (Flp) recombinase enzyme to excise the SL element from the genome. Upon excision, the promoter drives the expression of the second element (Figure 2B, HG-SL), which serves as a light-inducible molecular switch based on Optogenetics. The optogenic system comprises of two proteins that dimerize upon blue-light illumination. Each of them is fused in-frame to an inactive half of the Cre-recombinase enzyme. Upon dimerization, the Cre is activated and excises the genomic regions flanked by loxP recognition sites. In the HG, loxP sites are flanking the optogenic system itself; thus, following blue-light illumination, the optogenes are excised from the genome and the promoter drives the expression of the third element, the Noggin gene (figure 2C). Noggin is an antagonist of the Bone Morphogenetic Proteins (BMP) signaling pathway, which is critical for early embryonic development. The BMP pathway plays fundamental roles in regulating cell differentiation and patterning during the early stages of embryogenesis. Thus, Noggin expression inhibits the BMP pathway and male embryogenesis.

We have generated two transgenic chicken lines. The first has directed insertion of the HG construct into the Z chromosome, and the second expresses the FlpO recombinase. These lines were created by genomic modification of cultured chicken Primordial Germ Cells (PGCs), which are embryonic cells that give rise to the gametes in adult chickens. Utilizing Homology-Directed Repair (HDR) mechanism and CRISPR-Cas9, the integrations were guided into the Z chromosome. Cross-breeding these chicken lines, males with Z^{FlpO}/Z^{HG-SL} genotype were produced, the GFP safe-lock element was excised, resulting in HG-SL (HG minus SL) genotype with an active optogenetic system. Crossing these males with WT females led to the generation of W/Z^{HG-SL} hens, which we termed “Golda”. Blue light-illuminated Golda breeding eggs are expected to yield WT female with no male hatchlings.

Indeed, from illuminated eggs obtained from crossing Golda hens with WT males, 310 WT females hatched with no male hatchlings. The WT females reached to sexual maturity and are laying table eggs, for which no males were culled.

Our genetic approach, presented here, has been design to seamlessly integrate with the current industry workflow. Based on genetic segregation of the sex chromosomes, as expected it is 100% accurate, and can save energy and space of hatcheries. This solution is applicable to any chicken strain, or egg types and colors but most importantly, if adopted by the industry, it will put an end to the practice of sorting and culling billions of day-old male chicks globally.

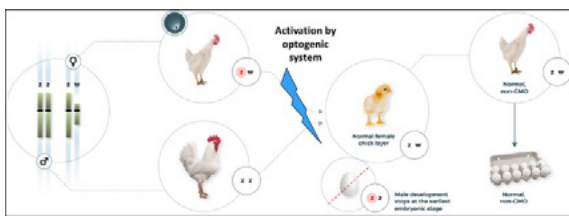


Figure 1

Eliminating male chick development through a maternal Z chromosome sex-linked, light-inducible genetic trait.

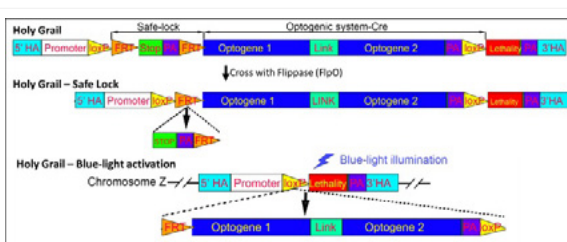


Figure 2

The structure and molecular mechanism of the Holy Grail construct.



SESSION 1

— Genetic engineering applied in chickens

ENHANCING CHICKEN IMMUNITY: UNLEASHING THE POWER OF GENE EDITING

Theresa von Heyl¹, Romina Klinger¹, Dorothea Aumann¹, Mohammed Alhussien¹, Antonina Schlickerrieder¹, Kamila Lengyel¹, Hanna-Kaisa Vikkula¹, Teresa Mittermair¹, Hicham Sid¹, Benjamin Schusser^{1,2}

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Keywords: gene editing, T cells, adaptive immune response

Genetically modified animals have significantly contributed to our understanding of immunity, infectious diseases, neurology, behavior, and developmental biology. Understanding the immune system represents a crucial branch of science that continues providing humans and animals with new health solutions. This branch has been revolutionized by the CRISPR/Cas9 system that allowed the introduction of site-specific gene modifications in various animal species.

The chicken represents an essential source of protein worldwide and a valuable model for studying developmental biology in vertebrates. In comparison to mammals, difficulties were always associated with the generation of genetically modified chickens due to the complex structure of the chicken zygote. The ability to genetically modify PGCs and their subsequent reintroduction into the embryonic vasculature facilitated the production of transgenic chickens. Our recent data explained the role of T cells on chickens' health and revealed the importance of different T cell subpopulations. $\gamma\delta$ T cells are unique effector lymphocyte population in the adaptive immune system with innate-like features. They are assumed to have essential contributions to various innate and adaptive immune responses, such as ab T cell priming and resistance against infections, and participate in tissue homeostasis. However, their exact role in mammals and chickens is still poorly understood. Chickens harbor a comparatively high percentage of $\gamma\delta$ T cells, which makes them an intriguing research model. A substantial and detailed understanding of this T cell subpopulation will, therefore, help to develop more suitable and optimized therapeutic and prophylactic approaches to prevent diseases in mammals as well as in birds.

So far, no model has been developed to study T cell subpopulations in chickens. To investigate the role of ab and $\gamma\delta$ T cells in birds, we generated chickens lacking these T cell populations. This was achieved by genomic deletion of the constant region of the T cell receptor β or γ chain, leading to a complete loss of either ab or $\gamma\delta$ T cells. In TCR $\gamma\delta$ ^{-/-} chickens, the CD8⁺αβ T cell population significantly increases in the caecum, spleen, and cecal tonsils, indicating that these cells compensate for the absence of the $\gamma\delta$ T cells in the gut. Surprisingly, our results showed that a deletion of ab T cells but not $\gamma\delta$ T cells resulted in a severe phenotype in chicken, as demonstrated by granulomas in the comb, leg, and beak and inflammatory reactions in the spleen and the proventriculus of ab T cell knockout (KO) chickens. The immunophenotyping revealed a significant increase in monocytes and the absence of CD4⁺ T cells and FoxP3⁺ regulatory T cells compared to wild-type chickens. Also, in the ab T cell KO chickens, we observed a significant decrease in immunoglobulins, B lymphocytes, and associated changes in the bursa morphology.

Additionally, we found changes in the thymus- and spleen structure of the TCR β ^{-/-} chickens, and we showed that the lack of ab T cells impacts the number of species and the diversity of the gut microbiome. Our data reveals the consequences of T cell knockouts in chickens for the first time and provides new insights into avian T cell functions. In the future, genetically modified chickens will pave the way for the understanding of T cell biology in birds and how they may interact with other immune cells and with different infectious pathogens. This will also affect the development of new preventive strategies that will consider the features of the chicken immune system to achieve optimal health.



CHICKEN PRIMORDIAL GERM CELLS – CHALLENGES AND OPPORTUNITIES IN A GENE BANK CONTEXT

Stefanie Altgilbers, Claudia Dierks, Sabine Klein, Wilfred Kues, Steffen Weigend, Claudia Klein

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Due to the peculiarities of avian reproductive physiology, the methods used for mammals are not sufficient to ensure genetic diversity in chickens. Ex situ preservation of germ cells in chickens is mainly limited to sperm, with the consequence that female W-chromosomal and mitochondrial genes are not preserved, or the progeny produced can only retain the variations in mitochondrial DNA of the recipient population.

In chickens, the use of germ cell precursors, so-called primordial germ cells (PGCs), can close this gap. PGCs circulate in the embryonic chicken blood and migrate from there into the embryonic gonads to later differentiate into sperm or oocytes^{1,2}. Collection and ex situ conservation (genebank) of female and male PGCs offers the possibility of long-term conservation of the entire genetics of threatened and locally adapted breeds. Sperm preservation is a non-invasive procedure and artificial insemination is simple and well-established. However, sperm preservation usually requires several backcrosses to recreate the breed^{3,4}. Since PGCs can multiply in vitro without losing their differentiation potential and reinjection into the sterile chicken embryo produces PGC progeny in only one generation⁵, extensive genetic and phenotypic analyses can be performed on these cells or animals without losing the material for the gene bank. In breeds with low fertility, especially with low sperm concentration or poor sperm quality, PGCs may be an alternative option to preserve genetic material for long-term storage. This is due to the fact that PGCs can be enriched in vitro at any time and tolerate both slow freezing protocols and vitrification⁶.

Conserving genetic diversity thus allows the integration of selected genetic traits of certain breeds into commercial breeding programs for future use of potentially important traits such as diversified genetics, better use of alternative and more resource-efficient feed sources, disease and stress resistance and climate-specific traits. The possibility of applying genome editing to PGCs also helps, in terms of basic research, to uncover gene functions quickly and precisely in a causal manner⁷. In this context we established a CRISPR/Cas9-mediated genome editing protocol for the genetic modification of PGCs derived from chickens with blue or white egg color. The sequence we targeted is a provirus (EAV-HP) insertion in the 5'-flanking region of the *SLCO1B3* gene on chromosome 1 in Araucana chickens, which is supposedly responsible for the blue eggshell color. We succeeded in generating, on the one hand, a deletion of this provirus in Araucana PGCs⁸ and, on the other hand, the integration of the entire provirus sequence (4.2 kb) on chromosome 1 in PGCs derived from white layer chicken. Subsequent experiments with injections of the genetically modified primordial germ cells into recipients will reveal whether this provirus is responsible for the blue egg phenotype. Besides all these advantages, the in vitro culture of PGCs, is still difficult and expensive, and only a few laboratories have established it so far. Obtaining PGCs from poultry breeds at risk of extinction and from locally adapted breeds that are less selected for reproductive traits may be less efficient than for commercially exploited breeds. Moreover, the culture conditions do not yet seem to optimally reflect the in vivo conditions, especially for female PGCs⁹. The establishment of proliferating PGCs from embryonic gonads or blood is quite time consuming. If the cells are genetically modified and subjected to selection processes, they sometimes remain in the in vitro culture for weeks or even months, which in turn seems to be one reason why their competitive ability against the endogenous PGCs, when reinjected into a recipient embryo, is reduced¹⁰. Single-cell RNA sequencing of male and female PGCs cultured over different time periods can provide clues as to whether the transcriptome changes in a way that affects competitive ability, gonadal colonization ability, or proper differentiation into sperm or oocytes. These problems do not seem to occur when injecting PGCs into sterile recipients, such as the iCaspase9 chicken⁵. However, apart from the legal hurdles regarding GMOs, not every institute has the ability to establish or maintain this genetically modified chicken line with inducible sterility. This is why further research in this area is beneficial to optimize the use of PGCs in non-sterile recipients and to gain further insights into the biology of PGCs and the associated reproductive processes in chickens.

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SESSION 1

— Genetic engineering applied in chickens

PRODUCTION OF GENETICALLY ALTERED CHICKENS IN A SPECIFIED PATHOGEN FREE (SPF) CONTEXT: CHALLENGES AND RECENT ADVANCES

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Keywords: National Avian Research Facility, Specified Pathogen Free (SPF), Genetically altered chickens

The National Avian Research Facility (NARF) at the Roslin Institute is a large repository of conventional and Specified Pathogen Free (SPF) lines of inbred, outbred, and transgenic reporter chickens available to the international research community. NARF is also a leader in genetic engineering of the chicken genome. It has recently implemented a highly efficient pipeline for creating genetically altered (GA) chickens, capitalising on strategies developed in the McGrew lab, in particular the ability to precisely edit avian primordial germ cells (PGCs) [1] and the creation of the iCaspase9 sterile surrogate host [2]. Many new lines of GA chickens have already been created within NARF conventional unit using this pipeline.

To broaden this approach and offer the research community the opportunity to create GA birds of known health status, NARF has recently implemented this pipeline in its SPF unit, which houses unvaccinated flocks certified free from 17 specified pathogens. GA chickens created in this SPF context are particularly suitable for studying the immune system.

The challenges associated with transposing this gene-editing pipeline to a highly biosecure SPF unit, and the lines successfully created so far, will be discussed.

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SESSION 1

— Genetic engineering applied in chickens

PCR ANALYSIS FOR IN-OVO SEXING OF CHICKEN EMBRYOS

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Keywords: in ovo sexing, male culling, gender identification in the hatching egg

Sexing chicken embryos in the egg (in-ovo sexing) can eliminate the practice of day-old male chick culling in layer hatcheries, reduce animal welfare concerns and promote sustainable poultry production. DNA based methods are advantageous because sex differentiation is independent of embryonic developmental stage. Genes on the W chromosome can be used to differentiate males (ZZ) from females (ZW)^{1,2}. Fresh allantoic fluid (AF) is adequately high in DNA content, and low in inhibitory substances, for easy PCR analysis. Sampling AF is harmless from embryonic day 9 onward, and without ethical concerns till day 12, as lack of brain activity up until that point shows that the embryo cannot yet experience pain³. Results from commercial sexing of chicken embryos show that it can be done in a high throughput industrial setting with high accuracy (>99%). The wealth of available data enabled simplification of protocols. For example, the given 50:50 male:female ratio allows for testing without housekeeping genes or separate controls. To conclude, PCR analysis has proven to be a valuable tool for in-ovo sexing. It is an accurate, affordable, non-invasive, and fast solution that meets the demands of the poultry industry.

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SESSION 1

— Genetic engineering applied in chickens

IMPROVED TECHNOLOGY OF CRISPR/CAS9-MEDIATED GENE KNOCK-OUT AND GENE EDITING IN CHICKEN AS AN APPROACH TO CHICKEN RESISTANCE TO VIRAL DISEASES

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Keywords: avian leukosis virus type J, gene editing in chicken, resistance to virus infection

Introduction

Gene editing technology is becoming an established part of genetics, immunology, and developmental biology of chicken. In virology, it could be useful in studying virus-cell interactions by manipulation the host dependence genes or anti-viral restriction factors. Cell-surface receptors for virus entry are the most suitable candidates for such genetic manipulation, and we demonstrate an example of artificial resistance to avian leukosis virus type J (ALV-J).

Results

ALV-J enters the cell through the Na⁺/H⁺ exchanger type 1 (NHE1). Most galliform birds are resistant to ALV-J due to a single amino acid (W38) deletion in NHE1, however, no resistant NHE1 allele has been found in chicken or turkey. Using CRISPR/Cas9 gene editing tools, we prepared a chicken line with W38 deletion within the endogenous NHE1 gene and demonstrated that this chicken line is resistant to ALV-J infection in vivo.

Conclusion

The novel methods of orthotopic PGC transplantation into adult recipients will be presented. Thanks to the sterilization of recipient roosters, we can skip the chimeric G₀ stage. We developed the system of genetic sterility in chicken, that further improves efficiency of PGC transplantation and reduces logistical complexity.



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S-02 | Genetic resources for breeding in organic agriculture

Steffen Weigend
Pavel Trefil



SESSION 2

— Genetic resources for breeding in organic agriculture

REQUIREMENTS FOR AND POSSIBILITIES OF BREEDING FOR ORGANIC FARMING

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Ladies and gentlemen, a very warm welcome to Germany from me and especially from the Federal Ministry for Food and Agriculture. I would like to pass on greetings from my Ministry. I hope that you have a very fruitful congress with an opportunity to have a personal as well as a scientific exchange.

Today it is my turn to demonstrate and explain the special requirements for poultry breeding with the target on, ecological poultry fattening and egg production. My statements are based on the view of the ecological agribusiness as well as the public and the federal ministry. I am very excited to hear the response of both my co-speaker and you, my audience.

In general, I would like to point out that the requirements for organic livestock husbandry in the field of poultry production are very multi-faceted – they are not only economic but also linked to ethical and sustainability aspects. Therefore, there is no single focus on the animals' performance for the purpose of meat or egg production. Rather than that, performance in terms of functional aspects is also important. Organic livestock husbandry in the field of poultry production aims at using dual-purpose birds, making this approach a requirement for animal breeding. However, these two performance aspects (fattening/laying) are mutually antagonistic. I am well aware of the fact that this poses a great challenge for breeding activities. Because the profitability of the fattening and laying performance continues to be a crucial factor in determining to what degree this type of dual use bird is, and will be, accepted and used by agricultural practitioners. Yet, of course, the strict requirements for animal health and animal welfare as well as the specific animal husbandry conditions in organic farming also influence the choice and prioritisation of breeding features. In this regard, I would like to appeal to all stakeholders in the area of organic livestock husbandry to lay down relevant benchmarks or standards with animal breeding experts.

In addition, there is a general call for breeding nuclei (in foundation breeding) to be kept under organic production conditions. As, in this husbandry type, the animals are kept in groups, it is particularly challenging to register (laying) performance parameters for the individual birds.

Organic livestock breeding does not, as yet, have a significant share in the poultry sector. In the German-speaking region, there is one single non-profit organic animal-breeding enterprise that was only founded in 2015. The "Ökologische Tierzucht gemeinnützige GmbH" (OTZ) carries out its breeding activities for foundation breeds under organic production conditions. Performance monitoring generally remains an important element of breeding. As a rule, pure-breed poultry originating from breeders associations or private individuals serve as a starting point for organic breeding activities. One of the challenges here is that the characteristic features of the animals in the population are very heterogeneous because they come from different breeders. In organic poultry farming, it is currently good practice to keep three indigenous breeds as foundation breeds for breeding activities and to produce commercial crossbreeds through selective pairing with productive poultry.

Key topics regarding the requirements for organic poultry breeding could be the following:

First, the robustness of the animals. This means a higher tolerance against environmental influences and a stable health condition, including an extremely well-functioning immune system that is able to react very effectively, also in unspecific cases. Poultry kept in modern housing systems with many outdoor areas should be highly resilient to minor infections. How to manage notifiable diseases like avian influenza is still an open question.

Additionally, the health of the feet and legs as well as bone stability are very important aspects with regard to modern housing systems. They show very clearly that ecological housing is something entirely different from the established types of housing in conventional systems. Poultry has to cover much longer distances in the outdoor areas or when getting back to the stable and the feeding systems. The animals must accept the open range and learn to use artificial or natural coverings to protect themselves against predators such as kite and common buzzard.

Directly related to this is the aspect of stress resistance. Animals get in contact with so many more stimuli and stressors when they are kept outside under ecological conditions. Examples include people walking along the enclosure, machines producing noise around the enclosure, and so on.

Another important point is the efficiency of feed utilisation. That means: how can the animals utilise the limited ingredients in ecological feed? Animals should not be undersupplied with essential nutrients if they are kept for egg or meat production.

The animals should be enabled to exploit or develop their full genetic potential and to deliver their best possible performance in meat and egg production under ecological conditions. Therefore, the ecological agribusiness would prefer breeding activities to be carried out in accordance with ecological requirements, too. Furthermore, German animal welfare requirements lay down special conditions, for instance that livestock is to be kept in groups. This is a challenge for livestock breeding when it comes to organising an effective breeding method and is therefore viewed critically by experts. Additionally, we should discuss possible ways of using genomic investigating methods. What is the most appropriate target? Do we need ecological lines of laying hens separated from lines of broilers/chicken intended for meat production? Or is a dual-purpose breed the best solution?

Development of dual-purpose chicken breeding in the light of classical breeding program design.

In theory animal breeding is very simple, if one takes some main points into account and include these points in a breeding program. The overall goal of every breeding program, except conservation breeding programs, is to generate genetic gain, which accumulates over time and therefore animal breeding could be seen as a sustainable tool for future activities. In every practical breeding program the first step is the definition of the breeding goal and to ensure that an effective individual animal identification system is available. The breeding goal defines the traits of interest, e.g. with respect to laying- and fattening performance. It should be noted that on the one hand the number of traits in the breeding goal defines the achievable genetic gain for every single trait and that on the other hand the genetic relationship between the breeding goal traits is also an important factor. There is a well-known antagonistic relationship on the genetic level between traits related to laying performance and fattening performance. Therefore, this is the reason for the high degree of specialisation in commercial poultry breeding programs. In addition, this genetic antagonism is the biggest challenge in the development of dual-purpose chicken. In the light of this knowledge the first thing we need is a simple, clear, and broadly expected definition about the expected genetic level of dual-purpose chicken breeds with respect to laying and fattening performance.



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In case that a clear breeding goal exists, the next step is the development of a performance test system, at least, for the breeding goal traits. Within this system phenotypes are recorded, which are the base for the next step, the breeding value estimation. For effective animal breeding it is essential that the performance test occurs in an environment which is as close as possible to the final production environment. It is quite clear, that this is a big challenge for organic production systems, e.g. it is very important that we can allocate the eggs to the hens. However, with respect to the development of effective performance test systems in breeding programs for dual purpose chicken new digital techniques could be a part of the solution for the future, e.g. the development of electronic nest boxes for the recording of laying performance under organic housing conditions.

The next step in a breeding program is the selection of the parents for the next generation based on the estimated breeding values. It should be noted, that normally more than one trait should be improved and therefore several different breeding values must be combined in a total merit index, using the selection index theory. It should be noted that especially when a new breeding program is designed the number traits for the selection decision should be limited. However, that does not mean that not more traits should be recorded, because recording several traits is the base for the estimation of genetic parameters for these traits, i.e. heritabilities and genetic correlations between different traits and a good knowledge of these parameters is the base for the design of future breeding programs.

After the selection of the parents for the next generation the next question is how to organise the mating between the selected animals. However, this question is not very important with respect to the amount of genetic gain which can be generated. Nevertheless, it is very important with respect to the management of inbreeding. If our breeding program was successful the offspring should have a higher genetic potential with respect to the breeding goal traits.

For a successful and sustainable breeding program it is important to realise that the steps described above are not independent from each other. In other words mistakes in one step of the breeding program cannot be corrected in the following steps, e.g. if the data recording in the performance test is not correct, as a consequence the estimated breeding values are not correct, because the data base of the breeding value estimation comes from the performance test. However, if the estimated breeding values are not correct as a direct consequence we select not the right animals, which leads to a reduced or no genetic gain and therefore our breeding program does not improve the genetic level of our animals. Another example is that without a clear breeding goal it is impossible to set up an accurate performance test.

Practical challenges for the development of a dual-purpose chicken breeding program

The development of a breeding program for dual-purpose chicken is a big challenge. Actually, in Germany, the development of pure lines for the production of crossbreed animals, mainly used in organic farming, has been started using two different approaches. It should be noted that also old local breeds are not dual-purpose chicken breeds. From an animal breeders' point of view these populations are unselected populations and the genetic antagonism between laying and fattening performance is also present in these populations. In addition, the performance in these populations with respect to laying- and fattening performance is unacceptable low for practical farming systems. Today, even crosses between commercial parent stocks and local breeds should not be named dual-purpose chicken, because they are either a cross with focus on laying performance or with focus on fattening performance. Performance testing should cover a wide range of traits (laying performance, fattening performance, animal welfare and animal behaviour), even if they are not directly part of the actual selection. It is important to collect phenotypes from many traits to generate knowledge about the genetic architecture, because this ensures that in the future selection decisions could be based on more traits. However, today selection should not be based on too many different traits to ensure that genetic progress could be generated for the traits in the selection decision.

During the last 15 years animal breeding has been changed dramatically for all species, due to the development and implementation of genomic selection in practical breeding programs. Genomic selection enables animal breeders to select animals on genomic information very early in life. Furthermore, genomic selection improves the selection for sex limited traits and makes a differentiation between full sibs possible. It should be noted that also for the development of dual-purpose chicken genomic selection has a high potential, e.g. for the selection of animals in nucleus herds of local chicken populations. On the one hand, the combination of genomic information with several different traits from the performance test in genome wide association studies could lead to the identification of SNPs related to important traits for the future. On the other hand, genomic information could be used for the management of inbreeding and genetic diversity. Inbreeding could be managed by genomic optimum contribution selection, a selection method which maximises genetic gain with a constrained rate of inbreeding. Especially when local breeds should be implemented in a breeding program for dual purpose-chicken the management of inbreeding is the key for a long term sustainable breeding program. Genetic management of French local breeds and their use in alternative farming systems



SESSION 2

— Genetic resources for breeding in organic agriculture

GENETIC MANAGEMENT OF FRENCH LOCAL BREEDS AND THEIR USE IN ALTERNATIVE FARMING SYSTEMS

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Keywords: Local breeds, Genetic management

The vast majority of the French local poultry breeds are made of small population constituted of loosely identified animals. As a result, 46 of the 47 French chicken local breeds are considered as threatened. The people in charge of their conservation are often amateurs, maintaining these animals for non-economic reasons (heritage, aesthetic, competition, history, localism...). However, some professional farmers use these local breeds to reach niche markets. The *Centre de Sélection de Béchanne* (CSB) hosts 12 French local breeds in order to provide professionals with chicks. Genetic management of these breeds involves pedigree selection permitting to select animals using a multi-objective criterion that includes breeding values and genetic relationship.

Farmers choose to work with local breeds because they fit their alternative farming systems: adaptation to local environmental conditions, rusticity, organoleptic characteristics, behaviour... These characteristics are however too difficult or too subjective to phenotype within an *ex situ* selection program. Concepts of *in situ* genetic management are currently being developed using *Noire de Challans* as a case study.

INNOVATIVE WAYS OF REGIONAL SUSTAINABLE USE OF ANIMAL GENETIC RESOURCES IN DOMESTIC CHICKENS

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Keywords: genetic resources, organic farming, breeding

The RegioHuhn project aims to develop an alternative approach to poultry production that takes into account the interests of organic farming in a wide range of regionally sourced products as well as supports the conservation of genetic diversity in chickens. The concept is based on crossing local chicken breeds with high-performance genotypes of layer and broiler parents. The choice of local chicken breeds took into account aspects of regionality, their potential suitability for agricultural use, and availability. The following indigenous breeds have been used: Altsteirer and Augsburgener, Bielefelder and Machelner, and Ostfriesische Möwe and Ramelsloher. Both purebred animals and their crosses with broiler and layer parent hens have been tested for their growth performance and laying performance to evaluate their suitability as a dual-purpose chicken for regional organic farming, including an economic analysis as well as an evaluation of animal welfare indicators. To assess practical relevance, the crossbreds are also included in an on-farm field trial. Nucleus flocks will be established for the local breeds, which will provide farmers with the most suitable crosses and form the basis for breeding in continuation of the project.



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ESTABLISHING A BREEDING PROGRAM FOR DUAL PURPOSE CHICKENS UNDER CERTIFIED ORGANIC FARMING CONDITIONS IN GERMANY – A CASE STUDY

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Keywords: Breeding, dual-purpose breeds, organic

Introduction

[In 2015 the German organic associations Bioland and Demeter, founded the non-profit Ökologische Tierzucht gGmbH (ÖTZ) with the long-term goal to breed dual-purpose chickens for organic conditions. Based on three pure-bred lines methods and facilities to collect individual data for performance and functional traits were established since 2017.]

Methods

[Pure-bred White Rock and New Hampshire lines established in the German Democratic Republic by Dr Götze and a newly established Bresse line are tested under certified organic conditions with 100% organic feeding in free-range barns. A wide range of animal welfare parameters were monitored using MTool for novel trait evaluation.]

Results

[Individual selection indexes were developed for all pure-bred lines to define dual purpose breeding goals combining conventional BLUP breeding values for performance (laying, egg quality, fattening) and animal welfare traits (diarrhoea, foot-pad lesions).]

Conclusion

[Crosses of pure-bred lines as well as pure-bred Bresse are actively marketed since 2019 aiming at healthy animals with moderate laying performance (200-230 marketable eggs/year) and sufficient fattening performance (1.4 - 1.7kg rooster carcass weight in week 15)]

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THURSDAY, 09. NOVEMBER 2023

PhD Award Session

Michael Grashorn
Steffen Weigend



GENOMIC PREDICTION OF COMMERCIAL LAYERS' BONE STRENGTH ACROSS FURNISHED CAGES AND FLOOR HOUSINGS

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Keywords: Commercial layers, Bone, Genomics

Introduction

Genomic prediction is a potential tool to mitigate bone damage in commercial layer hybrids. If SNP effects for bone strength in hybrids can be estimated from a modest reference population, then those estimates can be used to select within pure lines. Subsequently, the hybrids produced from these pure lines can be evaluated for genetic gain in bone strength.

Methods

Tibia bone strength phenotypes and genotypes of 50k SNPs were available for 2 white hybrids (Bovans and Lohmann LSL) in 2 housings (furnished cages and floor), resulting in 4 hybrid-housing combinations (n~218). The data of the Rhode Island Red (RIR) pure line (n=924) was also included. Each hybrid-housing combination was fitted separately via single-trait GBLUP, then simultaneously via multi-trait GBLUP: within (and across) housings and hybrids, also in combination with RIR data.

Results

The average accuracy of single-trait GBLUP was 0.29, increasing to 0.34 when combining data across hybrids. Tibia strength had a heritability estimate of 0.1-0.64.

Conclusion

The genomic prediction accuracy was relatively high given the small reference populations. Modeling the interactions of gene by environment could help to produce hybrids for specific housing systems.

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THE HERITABILITY OF MAXILLARY BEAK SHAPE TRAITS AND THEIR RELATIONSHIP TO PRODUCTION AND EGG QUALITY TRAITS IN A WHITE LEGHORN PURE LINE POPULATION

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Keywords: severe feather pecking, geometric morphometrics, welfare

Natural beak shape and size variation has been observed in laying hens and selection for blunter beak phenotypes may help reduce feather pecking-related damage [1,2]. This study examined the relationship between maxillary beak shape and egg production and quality. Maxillary beak shapes of 952 White Leghorn pure line hens were analysed using geometric morphometrics. Heritability and correlations were estimated using ASReml. Heritability estimates for beak shape and size traits ranged from 0.11 to 0.34. Negative genetic correlations were found between beak length (distance from the nares to the beak tip), beak curvature, and total egg number ($r_g = -0.56$ and -0.83). Beak depth had positive genetic correlations with feed intake ($r_g = 0.68$) and egg-breaking strengths ($r_g = 0.45$ and 0.51). Characterising maxillary beak shape phenotypes using geometric morphometrics has a moderate heritability and could be included in breeding programmes to reduce feather pecking damage; however, the correlations suggest that selecting for certain beak shapes (e.g., short and curved) may have consequences for egg production. To better understand the influence of beak shape on egg production and quality, different genetic lines should be investigated.

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IS IT POSSIBLE TO BREED FOR OPTIMIZED GUT MICROBIOTA COMPOSITION TO IMPROVE EFFICIENCY IN POULTRY?

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Keywords: Gut microbiota, Efficiency, Phosphorus utilization

Animal efficiency consists of two general components, the digestive and metabolic efficiency. Digestive efficiency reflects the ability to absorb nutrients from the gastrointestinal tract (GIT) into the bloodstream, while metabolic efficiency refers to the allocation and reallocation processes of ingested nutrients to animal products. The GIT of animals harbors a large community of microorganisms. These microbial communities can influence the digestive efficiency and thus the expression of the efficiency of animals. This study attempts to shed more light on the relationship between the host genome, gut microbiota, and efficiency traits in an F2 cross of 750 Japanese quail as model species. Phosphorus utilization and related traits were considered efficiency traits. The quail were 4k SNP genotyped and the ileum microbiota was characterized by target amplicon sequencing. We confirmed a significant genetic effect of the host on the ileum microbiota composition, identified host QTL for ileal microbiota, and inferred causal structures between different microbial classes and efficiency traits. The selective breeding to predict the microbiota-mediated part of the traits was demonstrated by microbial and genomic mixed linear models.



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S-03 | Biological limits of breeding

Elisabeth Duval
Martino Cassandro



BREAST MUSCLE MYOPATHIES – DO THEY LIMIT BROILER BREEDING?

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Keywords: selection limit, spaghetti meat, high growth rate

Since the 1950s, higher growth rate has been the main broiler breeding objective, being essential for efficient meat production, due to better utilization of feed, facilities and labor. The breeding for more rapid growth has been very successful, with broilers growing faster and faster every year, continuously reaching higher body weights at earlier ages.

Over the years, several growth-related 'defects' have emerged, related to the continuously increasing growth rate of broilers. Most important among them were excessive fat deposition, leg problems, and ascites. Due to their association with elevated growth rate, initially each defect was considered a biological limit to selection for further higher growth rate. Therefore when the elevating prevalence and severity of these defects started to reduce the overall efficiency of broiler meat production, growth-reducing diets had been suggested by the breeding companies to mitigate the negative effect of the emerging defects.

Eventually it has been shown that the tendency to develop these defects are heritable, and although rapid growth is required for the defect's expression, genetically it is independent of growth rate. Thus, identification of the genotypes prone to develop each defect allowed the breeding companies to select against it, while continuing to select for rapid growth rate, and for other economically-important traits.

In recent years, new defects - breast muscle myopathies - have been showing up: White Stripes (WS, first reported in 2007), Wooden Breast (WB, 2011), and Spaghetti Meat (SM, 2015). As with earlier defects, the emergence of these breast muscle myopathies were considered by many as indications of a biological limit to further selection for fast-growing and high-yield broilers. Consequently, the use of growth-reducing management strategies (i.e., lower-density diets) and slow-growing breeds, have been suggested to reduce the prevalence and severity of the breast muscle myopathies.

However, upon the emergence of each myopathy, broiler breeders have amended their programs with selection against the tendency to develop it. Indeed, the prevalence and severity of WS has been decreasing and currently it is very rare. Similarly, a decline is observed in the prevalence and severity of WB, with low incidence and low severity, and only among males reared to high body weights.

It appears that the prevalence and severity of SM are not declining yet. In part this could be attributed to the later emergence of SM compared to WS and WB. Moreover, the different biological nature of SM compare to WB further complicates management and genetic mitigation of this defect. Whereas the prevalence and severity of WB clearly increase with higher body weight and breast yield (hence rarely found in females), the prevalence and severity of SM are higher in females than in males, and within sex they are only lowly associated with body weight and breast size.

Moreover, the WB phenotypes observed at the slaughterhouse are those developed in the broilers during rearing, and they can be detected on live broilers. In contrast, although also SM develops in 'high-tendency' broilers during their rearing, it cannot (so far...) be measured or assessed on live broilers, and the SM observed in the slaughterhouses reflect not only the inherent tendency of each broiler, but also random or non-random effects of processing. Full comprehension of the processing effects, mainly defeathering, can reduce the prevalence and severity of SM in commercial broiler operations at present and in the near future. For the long run, aggressive defeathering can be used as a tool to better phenotype the inherent tendency of broilers to develop SM, leading to a successful genetic reduction in SM, similar to the already observed reduction in WS and WB.

Thus, to answer the title's question – the observed and expected genetically reduced prevalence and severity of breast muscle myopathies indicate that the breeding of fast-growing high-yielding broilers has not reached yet a biological limit.



TRANSCRIPTIONAL IMPACT OF THE NAKED NECK GENOTYPE ON THE DEVELOPMENT OF THE MUSCULUS COMPLEXUS IN NAKED NECK BROILER CHICKENS

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Keywords: Naked Neck, Hatchability, Transcription Factor

Introduction

The locus for naked neck (*Na*) in chickens reduces feather coverage resulting in better adaptability to heat stress. However, the *Na* genotype is linked to lower hatchability (LH) due to increased late embryonic mortality possibly related to hatching muscle (*M.complexus*) function [1]. Here we analyse the muscle transcriptome to show its potential role in LH associated with *Na*.

Methods

The *M.complexus* transcriptomes of 6 WT & 6 *Na* embryos were analysed by RNAseq.

Results

Differentially expressed genes (DEGs) were discovered linked to retinol & linoleic acid metabolism, steroid biosynthesis, as well as muscle contraction, collagen & bone formation pathways. Enrichment of binding sites for the transcription factor *PITX2* close to DEGs was also observed.

Conclusion

Enrichment of these binding sites indicates that the *M.complexus* is still undergoing development in *Na* embryos. Furthermore, retinol biosynthesis & metabolism pathways were found to be down regulated in *Na* embryos. *BMP12/GDF7* is known to suppress the development of feathers by the sensitizing action of retinoic acid [2], and as such could play a key role not only in observed reduction in feather patterning, but also in the reduced LH observed in *Na* chickens.

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SKELETON INTEGRITY & LAYING PERFORMANCE / KEEL BONES

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Keywords: keel, fracture, bone mineral

Keel bone damage is now established as a major animal welfare and production problem within the commercial laying hen industry (Harlander-Matauschek et al., 2015; Rufener & Makagon, 2020). Although historical evidence exists of damaged keels, the high rates of fractures and their occurrence within laying hens observed in commercial systems in the last 20 years have taken on added urgency given the likely implications for hen welfare and production. Unfortunately, the scientific and stakeholder communities lack much of the basic information needed to address the problem with effective solutions including an accepted understanding of the root cause for fractures. The theory which has attracted the greatest attention as to the source of fracture is that the high demands of calcium required of contemporary commercial hens for egg production induces resorption (breakdown of the bone matrix and release of contained mineral), leaving bones weak and brittle (Whitehead, 2004). While not a cause of fractures by itself, this condition leaves the hen relatively susceptible to fractures from more direct causes such as collisions with housing objects. Although the concept of high egg production draining bone mineral and weakening the hen's skeletal structure offers a convincing mechanism to explain the increase in keel fractures, variation in the appearance of fractures over the course of the laying cycle suggests involvement of other factors.

Despite this lack of understanding, progress has been made in key areas of assessment, identifying potential causes, and factors that affect the likelihood of fractures occurring. For instance, whereas fractures were initially assessed by palpation and/or dissection limiting resolution and/or longitudinal observations, multiple novel methods have been developed and validated including use of radiography, ultrasound, and micro computed tomography (T. Casey-Trott et al., 2015). Parallel to the development of assessment techniques, we have also established the effects of fractures on hen behaviour (T. M. Casey-Trott & Widowski, 2016; Rufener et al., 2019) and productivity (Eusemann et al., 2020; Rufener et al., 2018). Although the causes have not been identified with certainty, it is now clear that high and sustained egg production, while a likely major factor, is one of several likely correlated factors (Toscano et al., 2020). As the causal factors become more defined, effective interventions can be developed with more certainty. Taken together, these advances combined with the recent attention given the problem of keel damage, indicate the issue can be resolved with continued effort and dedicated research.

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DEVELOPING A PRACTICAL PHENOTYPE FOR LAYING HEN BONE QUALITY

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Keywords: Sustainability, welfare, phenotype

Introduction

When hens come in to lay there are changes to their biology associated with poor bone quality [1]. Heritability of bone quality is (~0.40 [2]) and it can be improved by genetic selection [3], but there was no practical method. We have now developed a fast, safe and reliable digital X-ray procedure for bone quality in living hens [4].

Methods
Digital x-ray was carried out at 65 kV/5mAs. Tibial density (Tibia_AUC) was measured using imageJ. The method was verified by evaluating reproducibility, correlation with post mortem measures and the effect of manipulation of bone quality were evaluated.

Results
A lateral recumbent position with minimal restraint took ~45 s to complete. Repeatability estimates were 92% and correlation of tibia_AUC with post-mortem whole bone radiographic density was from 0.62 to 0.7. Low dietary calcium and phosphorous effect was detectable after 3 weeks ($P < 0.001$).

Conclusion
We know that tibia bone quality is related to keel quality. The live bird measurement of bone density is a step towards genetic selection to reduce bone problems. An accompanying abstract will investigate the genetic estimates of the measurement in pure line hens. Funded by the Foundation for Food and Agricultural Research.

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TIBIAL X-RAY DENSITY MEASUREMENT IN A WHITE LEGHORN PURE LINE POPULATION

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Keywords: Keel Bone, Bone Quality, Laying hens

Keel bone damage is an animal welfare issue in layer hens.

Improving bone quality by genetic selection could reduce keel fractures and damage. The aim of this study was to investigate the genetic parameters for bone strength measured by a live bird X-ray.

The X-ray density measurement was performed by the method published by Wilson et al. 2023. White pure line hens (n=1000) were measured at 25, 40, 72 and 98 weeks of age. Heritabilities and genetic correlations were estimated by VCE 6 software.

Heritability of tibial density was in a range of $h^2 = 0.28$ to 0.62 , depending on age. The different age results were highly correlated ($r_g = 0.79$ to 0.88). The genetic correlation of tibial density and total egg number was low ($r_g = -0.08$ to 0.06) while it was significant for the onset of lay ($r_g = 0.23$ to 0.39). Correlations were negative for egg breaking strength ($r_g = -0.08$ to -0.21) and positive for egg weight ($r_g = 0.15$ to 0.18).

Moderate heritabilities for tibial density suggest the possibility to use this trait to improve bone quality in a layer breeding program. During the selection process, early onset of lay may need attention as it can have a negative impact on bone quality.

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THURSDAY, 09. NOVEMBER 2023

S-04 | Genetic approaches to improve welfare and resilience

Michele Boichard
Olivier Demeure



SESSION 4

— Genetic approaches to improve welfare and resilience

BEHAVIOUR GENETICS TO HELP IMPROVE CHICKEN WELFARE

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Keywords: Behavioural genetics, positive animal welfare, alternative farming systems

The definition of animal welfare, and how to improve it, has evolved over the years. Historically, animal welfare was seen as guaranteeing basic animal needs and absence of suffering: freedom from hunger, thirst, discomfort, poor health, fear and distress, and an environment that provides animals opportunities to express their natural behaviour. Since then, animal welfare is growingly focussing towards providing opportunities for animals to have positive experiences and emotions. Although satisfying farm animals' physiological needs can be quite straightforward, improving animals' emotions is a tremendous scientific and practical challenge. Behavioural genetics is crucial to help improve abilities to have positive experiences at the individual and at the group level in poultry.

Cage-free and free-range systems are more respectful of animals' needs, enabling them to express natural behaviours such as exploring, perching, scratching, dust-bathing, interacting, escaping... However, alternative farming systems challenge animals who have been selected for their performance rather than their robustness or resilience. As birds are given more opportunities to express their natural behaviours, the genetic contribution in expressing these behaviours and their trade-off with production traits need addressing. In cage-free systems, a top priority is for laying hens to lay their eggs in the provided nests. We will review recent advances in understanding hens' egg-laying behaviour in nests and show that there are some signs that nest laying could be genetically improved. Cage-free systems are also a great challenge for breeders to select their population when they freely mate on the ground. When not controlling for reproductive pairings, cage-free reproduction paves the way for uncontrolled natural selection to interact with breeders' artificial selection. Beyond very costly genetic parental assignment of all hatched chicks, we will see how understanding roosters mating behaviour could provide keys to guarantee genetic diversity in selection lines. Finally, free-range poultry is growing in popularity but individual range use behaviour and their genetic influence nevertheless needs new tools to be deciphered.

Once animals are adapted to their environment, they would still vary in their individual emotional state. By understanding animals' perceptions, emotions, and experiences, behavioural scientists are able to investigate new traits and characterise their genetic variance, but genetic contributions to emotions are very little explored yet. We will review the genetic characterisation of three traits that could improve birds' experience. First, optimism, a cognitive judgement bias that reveals positive expectations, showed some molecular basis suggesting genetic variance. Second, farm animals will always have to endure some stressful events. Emotional reactivity can be selected in quails, and may be part of a usable toolkit to improve poultry's experience. Finally, feed restriction in broiler breeders is a source of negative experience, but feeding them ad libitum causes an even more deteriorated welfare. Increasing satiation in birds, even when they are only lightly fed, may help tackle this welfare issue. Although selecting on positive experiences and emotions may improve poultry's welfare, selection on those traits can never replace providing ethical conditions to farm animals. Positive emotion traits should only be used as a supplementary tool to improve animal experiences in their lives, within an adapted environment.

Finally, all poultry species have strong social structures from their wild ancestors. However, modern conditions and density vastly differ from ancestral conditions and animals develop behaviours that may harm the welfare of the herd such as feather pecking or cannibalism. The genetic background of these harmful behaviours has been notoriously difficult to demonstrate. However, the emergence of digital tools (e.g. radio-frequency tagging, image analysis, etc.) enables individual identification and phenotyping of behavioural traits, opening up new breeding prospects for these traits. Beyond trying to suppress harmful social behaviours, scientists are now interested in understanding positive emotions coming from social experiences. For example, preferential clustering has been observed in several production systems, and its effect on animal welfare requires exploring. More generally, data on behaviours that enhance animal well-being, experiences and emotions are very scarce, as behaviours generating injuries, mortality, and lower performances have been prioritised. However, if we want to move towards improved poultry welfare, we also need to gather efforts towards phenotyping and genetically characterising behaviours that improve positive experiences in the lives of our animals.



SESSION 4

— Genetic approaches to improve welfare and resilience

GENETIC BACKGROUND OF NATURAL AND INDUCED ANTIBODY RESPONSE IN LAYER CHICKENS

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Keywords: Antibody, GWAS, Layer chickens

The level of antibodies can be used as a proxy in selection for improved disease resistance. Therefore, the objective of this study was to analyze genetic background of natural and induced antibody levels using low-pass sequencing.

The level of antibodies against KLH, OVA and PHA; and against IBD, IBV, NDV and REO after vaccination were assessed using ELISA assays. Birds represented 4 White Leghorn (WL), 2 White Plymouth Rock (WPR) and 2 Rhode Island Red lines (RIR). GWAS was performed within breeds with total number of sequenced samples ranging from 198 to 785. SP ratios were pre-adjusted for Line-Hatch-Sex. For GWAS mixed model with GRM was fitted in GCTA. SNPs with p-values below 10^{-6} were considered significant and used to identify potential candidate genes. Annotation and enrichment were analyzed in SNPEff and Panther.

In RIR, significant enrichment was found for defense/immunity protein, immunoglobulin receptor superfamily and antimicrobial response protein. In WL significantly enriched pathways included immunoglobulin receptor superfamily, defense/immunity protein and protein modifying enzyme. WPR didn't show any significant overrepresentation but some of the identified SNPs were annotated in immune relevant pathways.



SESSION 4

— Genetic approaches to improve welfare and resilience

THE MICROBIOME AS A FEATURE OF MULTI-ENVIRONMENT SELECTION

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Keywords: microbiome, selection, poultry

Poultry production is ubiquitous globally which means birds are found in a wide range of production environments; thus the commercial bird must be capable of thriving in a wide range of production settings. Multi-environment selection is used to achieve this; selection candidates are reared in an optimal environment to allow for the expression of full genetic potential, whilst siblings are reared in environments more similar to commercial poultry production. Contrasts between the environments include factors like feed quality, nutrient density, environmental parameters, stocking density and biosecurity. One key difference is the use of built up litter in the commercial like environment as a tool to select for gut and immune robustness as the birds are exposed to commercially relevant microbes. Advances in microbiome research offers the opportunity to investigate the influence of bacteria present in the birds' environments by comparing and contrasting the bacteria present in these environments. Furthermore, by comparing with the microbiome across the commercial poultry industry it is possible to ensure exposure to relevant bacterial species to further aid selection of traits associated with robustness and environmental adaptability



FRIDAY, 10. NOVEMBER 2023

S-05 | New phenotyping methods in poultry breeding

Avigdor Cahaner
Yuval Cinnamon



SESSION 5

— New phenotyping methods in poultry breeding

GENETICS OF NEW MEASUREMENTS: EXAMPLES OF IMPLEMENTING PRECISION LIVESTOCK FARMING TOOLS IN POULTRY BREEDING/ NEW PHENOTYPING METHODS IN POULTRY BREEDING

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Keywords: Poultry, genetic selection, phenotypes

In recent years, many high-tech innovations have been introduced in animal husbandry. Implementing those new technologies isn't always a smooth process. The complexity of a farm environment, the difference in scale (comparing breeding facilities with thousands of individuals versus research set-ups at universities) to identify individuals and finally the unpredictability of the animal itself in its behavior can make it challenging to quickly adopt the latest innovations in poultry breeding. The aim of breeding companies is to examine new technologies and try to understand how these concepts could enhance the field of animal genetics and poultry breeding. Open innovation and fostering collaboration with partners inside and outside the poultry sector allow animal breeders to implement techniques and technologies that are not present within the traditional way of looking at birds and recording traits.

Machine vision is one of the technologies that has been adopted in recent years in the breeding program for laying hens. This results in more accurate data on exterior egg phenotypes. Automating the egg grading process has resulted in removing the possibility for human error and subjectivity. Each external egg quality evaluation can now be completed with a high amount of measurable consistency and repeatability, thereby improving the quality of data largely, and removing the human bias. It also allows to adopt new traits which were harder to score with just making use of the human eye before.

Another technique that got implemented in poultry breeding programs is MRI. The use of MRI allows to look under the surface of the eggshell, without breaking it. The potential is high, but the major disadvantage is the high investment cost. By making use of AI affordable, fast, and versatile MRI's have been developed in recent years, allowing the tools to be implemented in hatcheries as well. By generating images for algorithms and deep learning, the current MRI tools allow for checking hatching eggs without breaking them. It allows to identify fertility prior to setting eggs, the viability of the germ disc and when performed at later stages the gender of the chick. This does not only benefit selection traits related to fertility and hatchability, but also has its direct benefits for hatchery optimization and utilization, as unfertile eggs do not need to be incubated.

IMAGEN (ANIMAL Group SENSOR) is a clear example of a multi-faceted, long-term study that combines animal breeding with advances in sensing and AI technologies to help in the transition towards sustainable livestock production. To address societies' animal welfare demands, the animal-protein sector continues to transition to "welfare-friendly systems", such as cage-free housing systems for laying hens and slower growing broilers in European countries. This change presents a challenge to understand and predict animal behavior in these relatively new environments. Advancement in vision, sensing, and AI technologies offer a crucial opportunity to develop new methods to automatically detect and analyze animal behavior. Better understanding of social interactions between animals in social groups will result in reduced instances of harmful animal tendencies such as feather pecking in laying hens. By combining sensing and AI technology with animal breeding and genetics, the IMAGEN project aims to improve the health and welfare of livestock and reduce the ecological footprint of our food production.

Another example of improving the breeding of laying hens by using innovative tracking and data analyses strategies is the HenTrack project (University of Bern, Switzerland). This project will utilize large-scale commercial facilities to conduct detailed, continuous observations of individual hens over the entire laying period. Data generated by HenTrack includes information on how animals acclimate to standard commercial stressors such as dietary changes and vaccinations, as well as variation in use of their environment, including access to outdoor areas. Information collected at the research facilities are combined with traditional breeding evaluations in small family groups.

Above are just few examples on how breeding companies are adopting "new" technologies in their breeding programs. It is evident that poultry breeding is more complex than ever before. Changing requirements that society puts on the way that poultry is kept requires sufficient focus and attention from breeding companies, to safeguard that the poultry are fit for the future. By adding new traits and technologies it remains evident that genetic variation is essential in poultry breeding, and that balanced breeding and selection is key to maintain viable populations that are fit for the future and can meet the needs of a rapidly changing society.



SESSION 5

— New phenotyping methods in poultry breeding

GENETIC PARAMETERS FOR LIFETIME LOCOMOTOR ACTIVITY IN GROUP-HOUSED BROILERS RECORDED USING A RADIO FREQUENCY IDENTIFICATION SYSTEM

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Keywords: Genetics, broilers, activity

Introduction

Commercial broilers are commonly kept in large groups and measuring health, welfare and performance traits in such systems is a challenge. Previous research suggested that individual locomotor activity levels can be used as a proxy for health, welfare and performance traits, and that a radio frequency identification (RFID) system can be applied to record individual activity levels of group-housed broilers [1]. The aim of this study was to estimate genetic parameters for individual locomotor activity collected throughout life of group-housed broilers.

Methods

In total, 387 purebred one-day-old male broilers were fitted with an RFID leg tag. Data were collected in 5 rounds, with approximately 80 birds per round kept in a pen until slaughter age. Every second the location of each bird was recorded. Using this information, the average distance moved per hour (ADM/h) was calculated as a measure of activity.

Results

The ADM/h was 9.5 (\pm 1.7) m. Heritability of ADM/h was 0.31 \pm 0.11. Furthermore, heritability of activity decreased over time.

Conclusion

Although there appears to be potential to select for increased activity in broilers, potential adverse effects of selecting for increased activity remain to be investigated.

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SESSION 5

— New phenotyping methods in poultry breeding

GENETICS OF GAIT SCORE IN BROILERS

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Keywords: leg health, gait scoring, welfare

Leg health is an important aspect of broiler welfare, and a vital component of breeding goals. It includes specific leg disorders as well as a general assessment of a bird's walking ability. Leg health traits have been included in breeding goals for many decades, but methodologies for assessing the overall walking ability are more recent.

Aviagen developed a gait score using 8 categories, with worsening gait as scores increase. This score was used to estimate heritabilities and genetic correlations with a range of production traits and leg health traits under selection in purebred commercial broiler lines.

Results show that the Aviagen gait score has a moderate heritability, ranging from 12% to 24%. An improved walking ability can be achieved either through direct genetic selection, or indirectly through the genetic correlations with other leg health traits, in the range of 0.10 to 0.60. While somewhat unfavourable genetic correlations with production traits were found (in the range of 0.20 to 0.50), previous studies have shown that trait antagonisms can be handled in broad breeding goals through a balanced selection on both production and welfare traits, thus allowing the improvement of all traits simultaneously.



FRIDAY, 10. NOVEMBER 2023

S-06 | Genomic developments in other poultry species

David Caverio
Milan Tyller



HARNESSING GENOMIC SELECTION FOR ENHANCED ACCURACY AND GENETIC PROGRESS IN TURKEY BREEDING / LATEST DEVELOPMENT IN TURKEY BREEDING

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Keywords: turkey, genomics, accuracy

Introduction

Turkey genetic progress relies on selective breeding, initiated by primary breeders at the top of the supply chain. Elite breeding lines are continuously improved through balanced trait selection driven by market demands, increasing productivity without compromising bird welfare.

Genomic selection has emerged as a key strategy to accelerate genetic progress, capturing the attention of the poultry breeding industry.

Challenges

The seminal paper by Meuwissen et al. in 2001 [1] introduced the concept of genomic selection. Its first routine implementation ensued before the end of the decade in dairy cattle [2]. The methodology quickly gained traction following the publication of various livestock genomes.

Genome publications for chickens in 2004 [3] and for turkeys in 2010 [4] marked early milestones, but the implementation of genomic selection faced various challenges.

Poultry breeding programs typically involve many selection candidates, often in the tens of thousands per year. This high volume enables intense selection and greater genetic gains, but the cost of genotyping per individual becomes a key consideration.

Falling genotyping costs made large-scale poultry genotyping economically viable but posed substantial challenges in sample management, data processing, and storage.

The benefits of cattle genotyping, such as shortened generation intervals and cost reductions in progeny testing for dairy and enhanced pedigrees for beef, do not apply to poultry, which have short generation intervals, no progeny testing, and dense pedigrees.

Amid these challenges, the key benefit of genomic selection in poultry is improved prediction accuracy. Practical implementation depends on decreasing genotyping costs and the availability of tools for large-scale genotype and phenotype datasets.

Genomics selection started at Aviagen in 2013 for chickens [5] and 2016 for turkeys [6].

Resources

Following the genome releases, genomic selection's progression was closely tied to two notable advancements in resources: increasing SNP array density and rapid decline in genotyping cost. The initial chicken SNP array in 2005 had 3,000 markers; subsequently, marker counts increased progressively, reaching a peak with the 600,000-marker array in 2013 [7].

Higher marker density aligned with improved genome revisions, including chromosomal re-arrangements and micro-chromosome additions, capturing genetic variation in gene-rich regions. Simultaneously, annotation enhancements enriched the genome, with current research focused on linking genotype and phenotype through diverse 'omics' data integration.

Ongoing international collaboration projects such as FAANG [8] pave the way for continuous integration of novel data in genome assemblies, while advancements in 'omics' data are transferred in a new improved assembly of the turkeys genome [9].

Methodology

Across species, initial applications of genomic technology in selection primarily focused on candidate gene approaches. This involved using SNP tests to identify major genes to use in MAS (marker-assisted selection) and later conducting GWAS (genome-wide association studies) to locate QTL (quantitative trait loci) with substantial effects, or employing case-control studies to detect genes associated with traits like disease resistance.

Given the quantitative nature of most economically significant livestock traits, it is not surprising that candidate gene approaches had limited practical impact on genetic evaluations.

Chicken and turkey breeders benefited from lessons from the other species, implementing GEBVs (genomic estimated breeding values) by simultaneously considering all SNP markers in the genome to predict genetic merit via GBLUP (Genomic Best Linear Unbiased Prediction) [10].

Imputing missing genotypes in recent generations served as a cost-saving approach, but with substantial reductions in SNP array costs, its utility in genomic evaluation programs is diminished; however, with whole genome sequences available, imputation may regain importance in creating extensive datasets.

Accumulated experience has shown that there is no one-size-fits-all methodology with a clear advantage in terms of accuracy. Instead, results seem to vary by trait and strongly depend on the distinct characteristics of each dataset.



Impact

Genomics selection is a routine component of Aviagen Turkeys breeding programs. This new source of information has allowed substantial increases in selection accuracy in key traits in the breeding goal (Figure 1). Genomics information contributes directly to ensuring long-term genetic progress in turkeys, which ultimately leads to sustainable production.

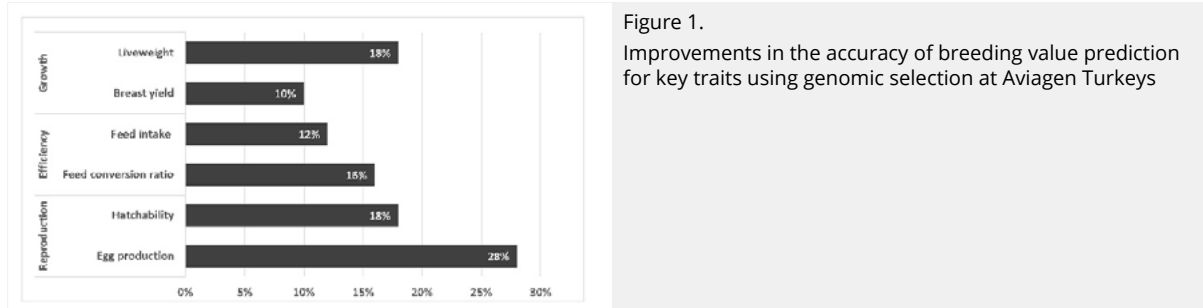


Figure 1.

Improvements in the accuracy of breeding value prediction for key traits using genomic selection at Aviagen Turkeys

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LATEST GENOMIC DEVELOPMENT IN DUCK BREEDING

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Keywords: Genomic, Duck, GWAS

The theory of genomic selection has been presented more than 2 decades ago (Meuwissen et al., 2001). Few years after, genomic has been implemented in many different breeding companies of Livestock. First dairy cattle, then major poultry species (layers, broiler) (Visscher, 2015). If the benefit of genomic selection in terms of accuracy doesn't need to be proved anymore, no tools were available for duck breeding companies until 2019 when a high-density SNP array (700k) has been developed for duck's species (*Anas Platyrhynchos* and *Cairina moschata*) (Tessier et al., 2019).

Based on this array, Orvia genotyped key ancestors from which bio samples have been stored the past years. The best SNPs (classified as PolyHighResolution by AxAS) for its populations have been picked up to build a private middle density array (60k). This array has now been used in a routine breeding program since 2020. By using a single step approach GBLUP on an animal linear mixed model, breeding values are estimated with a better accuracy especially for traits of interest like growth, yield traits measured in slaughterhouse on sibs and/or progenies and reproductive traits (egg number, fertility, hatchability) which are late traits. The use of GBLUP improves the selection process. Accuracy is better and animal ranking changes. For example, for a trait with moderate h^2 , ranking correlation is around 0.8 between GEBV and EBV (both with phenotype). Overlapping between 100 top animals selected on GEBV vs EBV on single trait will be between 62 animals for trait without own performance and 78 animals for traits with performance. Genomic selection implementation gives the opportunity to shorter the generation interval. Genomic brings additional data but doesn't replace data collected in our previous non genomic breeding program. It comes in addition. All in all genetic response of Orvia's balance breeding program per generation increases.

Genome wide association studies are also performed (Liu et al, 2021). As an example, 1116 Orvia Pekin ducks have been genotyped using this 60k SNP array (60281) with the help of gentyane INRAE platform. Those ducks have been grown under commercial circumstances. Individual feed intake, growth and slaughter performances have been measured. AxAS, Plink and R software have been used to perform the entire process of GWAS. The following filtering criteria have been applied: individual with more than 10% missing data, SNP with more than 10% of missing data and with MAF below 0.05 have been removed. Then the missing SNP have been imputed and the GWAS analyse using mixed linear model based on 2 traits: FCR and meat yield have been performed.

Based on the Bonferroni threshold 9 significant SNP have a major effect on breast meat yield. They are located on chromosome 1, 2, 4, 8 and 10 with respectively 3, 3, 1, 1 and 1 SNPs. GWAS peaks on a Manhattan plot for FCR where on chromosomes 1, 2 and 10 with respectively 3, 2 and 1 SNPs. All genes that have significant impact on FCR had also impact on breast meat yield.

FCR is a complex trait. It is influenced by feed intake behaviour, digestion and absorption capacity and meat production. Breast meat yield is also complex. Indeed, it is linked to FCR but also on the metabolic path that promotes breast meat production instead of fat deposition for example. From NCBI (<https://www.ncbi.nlm.nih.gov>) data base several genes have been found like FGF13 and PHACTR1 that have a significant impact based on the outcome of the GWAS. FGF13 is part of the fibroblast growth factor family and is involved in a variety of biological processes like cell growth and tissue repair. PHACTR1 is known as playing a role in cells motility and vascular genesis, which are logical regarding the traits studied. Duck breeding companies have tools to catch up the major avian species in terms of used of genomic, both for regular balance breeding program and for genomic regions identification that affect traits under selection as implemented in Orvia.

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SESSION 6

— Genomic developments in other poultry species

MAIN CHALLENGES FOR RED PARTRIDGE MATING IN CAGE-FREE SYSTEM: REPRODUCTION EFFICIENCY, PEDIGREE RECORDING, AND MANAGEMENT OF GENETIC DIVERSITY

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Keywords: red partridge, cage-free system, parentage assignment

In red partridge French breeding program, pedigree recording relies on cages, where breeders mate as couples. To comply with societal demands regarding animal welfare, breeding partridges in aviaries could be done. But this alternative raises issues that need to be addressed beforehand: reproduction efficiency, pedigree recording, and family structures observed in progeny. Thirty trios (sire-dam-progeny) were genotyped on a KASPar panel of 254 SNP, in order to design a 95 SNP panel for parentage assignment purposes. Four groups of red partridges were bred in four aviaries equipped with nests: two groups gathered 50 males and 50 females, and two others gathered 35 males and 50 females. Eggs were collected for 10 days following the egg laying peak. The percentage of fertile eggs was measured. All breeders and 95 chicks from each aviary were genotyped on the 95 SNP selected for parentage assignment. The laying rate in aviary was 50% inferior to the laying rate observed in cages. Depending on aviaries, the rate of eggs laid in nests varied between 76 and 90%. The assignment rate reached 90%, possibly because some breeders had a too low call rate. Free mating led to an unbalanced contribution of breeders to the progeny.



GENOMIC DIVERSITY AND POPULATION STRUCTURE OF TWELVE ITALIAN LOCAL TURKEY (MELEAGRIS GALLOPAVO) POPULATIONS

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Keywords: Genomic wide analysis, genetic diversity, local turkey breeds

Introduction

The conservation of local turkey breeds and regulating animal biodiversity are crucial. In this study, we assessed genomic diversity and genome-wide comparative analysis among 12 local Italian turkey populations: Basilicata Comune It. (BasBr), Brianzolo (BR), Bronzato Comune It. (BrCI), Bronzato Comune It. × Ibrido Commerciale (BrCI_IBRIDO), Colli Euganei (COEU), Ermellinato di Rovigo (ErRo), Ermellinato di Rovigo × Ibrido Commerciale (ErRo_IBRIDO), Ibrido Commerciale (IBRC), Narragansett (NARR), Nero Italiano (NI), Parma E Piacenza (PrPc) and Romagnolo (ROM)

Methods

Six samples were collected from Basilicata turkey population (BasBr) and were sequenced (12X). We performed MDS analysis to investigate pairwise genetic relationships within and between the breeds by combining our 6 samples with 107 samples of 11 populations.

Results

H_e ranged from 0.091 to 0.413, H_o ranged from 0.094 to 0.354, and MAF ranged from 0.072 to 0.272. The admixture results showed that BasBr and ErRo populations formed nonoverlapping clusters and were separate populations. The F_{ROH} ranged from 0.030 to 0.269.

Conclusion

These results provide insight into genetic diversity and population structure, which could be used to plan and implement conservation strategies.

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POSTER



P-01 | COMPARISON OF PREDICTION ACCURACY OF ESTIMATED BREEDING VALUES WITH PEDIGREE-BASED BLUP AND SINGLE-STEP GBLUP FOR EGG PRODUCTION TRAITS OF NCHU-G101 CHICKEN POPULATION IN TAIWAN

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Keywords: Taiwan country chicken, Single-step GBLUP, Prediction accuracy

Introduction

Compared with the pedigree-based BLUP (PBLUP), ssGBLUP has been proposed to improve the prediction accuracy of EBV. In chicken breeding, ssGBLUP is more beneficial due to larger candidate populations. The NCHU-G101 is one of the breeds of Taiwan country chicken that has been selected for total egg number (EN) as a dam line over 15 generations. The aim of this study was to compare the prediction accuracy of egg production traits with PBLUP and ssGBLUP model, and to determine the optimal model for NCHU-G101 breeding program.

Methods

Phenotypic records on 1069 chickens, 635 genotyped chickens, and 26659 SNPs across 3 generations were used. A single-trait animal model with PBLUP and ssGBLUP using BLUPF90 software was applied to compare prediction accuracy.

Results

The heritability ranged from 0.105 to 0.308 with PBLUP and 0.121 to 0.327 with ssGBLUP. The genetic gain of EN was 0.57 eggs per year with PBLUP and 0.37 with ssGBLUP. The accuracy was increased by an average of 3.3% for all traits with ssGBLUP compared to PBLUP.

Conclusion

The accuracies of most traits were higher with ssGBLUP than with PBLUP in this study. We suggested that ssGBLUP is the optimal model and could be implemented for NCHU-G101 chicken selection.

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P-02 | HEPATIC TRANSCRIPTOME PROFILING REVEALS MODULATION OF GENES INVOLVED IN N-3 PUFAS SYNTHESIS IN RESPONSE TO ORGANIC RAISING SYSTEM IN THAI SLOW-GROWING CHICKENS.

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Keywords: n-3 polyunsaturated fatty acids, organic raising system, RNA-sequencing

Introduction

[Korat chicken (KRC) is a crossbred chicken in Thailand. Rearing KRC under the organic system can improve the proportion of n-3 polyunsaturated fatty acid (n-3 PUFA) and docosahexaenoic acid in the meat. However, their accumulations are variable among chickens. The objective of this study was to investigate the global gene expression and molecular pathways related to n-3 PUFAs synthesis in the liver of organic KRC.]

Methods

[The liver transcriptomic profiles of conventional and organic chickens were compared using RNA sequencing and the function of differentially expressed genes (DEGs) was performed.]

Results

[We identified genes and pathways associated with n-3 PUFAs synthesis in the liver via the fatty acid elongation pathway, and others related to lipid metabolism including steroid biosynthesis, calcium signaling pathway, fatty acid metabolism, carbon metabolism, and steroid hormone biosynthesis. In addition, the protein-protein interaction network analysis of DEGs in the liver revealed that organic KRC might have a better response to pathogens.]

Conclusion

[This study provides a genetic basis for future breeding strategies to improve the genetics of slow-growing chickens to fit with an organic raising system.]

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P-03 | GENOME-WIDE DETECTION AND ANALYSIS OF COPY NUMBER VARIATION IN ITALIAN INDIGENOUS CHICKEN BREEDS

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Keywords: CNV, local population, poultry

Advancements in high-density integrated chips have enabled the identification of copy number variations (CNVs) with increased resolution. This study aimed to identify CNVs within the genome of 530 animals of 23 Italian indigenous chicken breeds and explore their distribution. Animals were genotyped using the 600K Affymetrix Axiom HD chip and CNVs were identified using the Hidden Markov Model using the PennCNV software. A significant number of CNVs were observed in the genomes, with higher distribution in the first six chromosomes and predominantly associated with loss types. A total of 1172 CNV regions (CNVRs) composed of 1084 losses, 36 gains, and 54 mixed CNVRs were defined: 711 CNVRs (60.7%) did not involve any genes and 461 CNVRs (39.3%) encompassed one or more genes, including protein-coding genes (87.8%; *DACH1*, *DMD*, *NTS*), miRNAs (9.8%; *MIR7445-1*), and genes of uncertain function (2.4%). Results provided an insight into the genetic variability and diversity of Italian local chicken population using CNVs, highlighted potential functional associations with crucial genes, and suggested that the breeds are indeed reservoirs of genetic diversity.



P-04 | RE-ESTABLISHMENT OF A BREEDING POPULATION OF A NATIVE PUREBRED DUAL-PURPOSE CHICKEN, THE SUNDHEIMER HUHN: FEASIBILITY ANALYSIS USING FATTENING AND SLAUGHTER PERFORMANCE OF THE ROOSTERS

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Keywords: dual-purpose chicken, native purebred chicken, fattening performance

Introduction

The Sundheimer Huhn is an old, native, dual-purpose and yet, endangered breed. The aim is to analyse its phenotypic and genetic attributes and hence contribute to re-establishment and development of a viable population for use in organic farming.

Methods

About 1000 fertilised eggs were collected from 22 hobby breeders in Germany in 2021 und 2022. After hatching and subsequently, sexing at 8 weeks roosters were kept in 10 compartments of 10 animals each and fed organic pullet feed. Different performance characteristics were measured and observed. At day 119, a sample of one hundred and thirty roosters were slaughtered while the other roosters underwent further tests.

Results

Body weight on day 118 of all roosters averaged 2.1 ± 0.3 kg in the year 2021 and 2.3 ± 0.2 kg in 2022. Group feed consumption, until day 63 was 8.3 kg and 8.9 kg for years 2021 and 2022, respectively for each rooster. For both years, mean carcass weight was 1.2 ± 0.1 kg, and proportion of cuts was: breast = 17.6 ± 1.2 %, leg = 36 ± 1.2 % and wing = 13.5 ± 0.9 %. Temperament was positive.

Conclusion

Performances showed high within and between breeder heterogeneity demonstrating valuable variation needed for breed re-establishment.



P-05 | COMBINED EFFECT OF GENETICS, GUT MICROBIOTA AND HUSBANDRY ON VARIATIONS IN VACCINE RESPONSE AND THEIR IMPACT ON WELFARE IN LAYING HENS

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Keywords: Gut Microbiota, Vaccine Response, Genetics

Vaccination is an effective way of preventing infectious diseases, but its efficiency is variable. We led three experiments to assess the effect of genetics, microbiota, outdoor rearing and their interactions on individual vaccine responses and behavior in four laying hen lines: two commercial lines (Novogen) and two experimental lines. With the first experiment we developed a microbiota perturbation model induced by the ingestion of antibiotics on 200 birds from the four lines raised in collective cages. The second experiment used the same model of microbiota perturbation, but on a total of 400 birds from the two commercial lines raised this time on the floor, with a higher number of phenotypic assessments: several vaccine responses, immune parameters, individual and collective behavior. We also studied the impact of outdoor rearing on these parameters. With the third experiment, we will perform genome wide association analyses to determine the genetic architecture of vaccine responses, microbiota composition and behavior in a progeny of 400 hens from one of the commercial lines, with access to outdoor for all. Results confirm the triple impact of genetics, microbiota and rearing environment on vaccine responses and hen behavior.



P-06 | TWO STEPS OF GENETIC EVALUATION IN LAYING HENS

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Keywords: BLUP, egg production, selection

The objective of the research project is to extend the number of performance traits and to modify the methodology of genetic evaluation in laying hens. In the approach used so far, five traits recorded in the first laying period were used in the genetic improvement program: body weight [BW], average egg weight [AEW], initial egg production [IEP], rate of initial egg production [RIEP] and sexual maturity [SM]. Recently, the trait panel has been extended to include the average eggshell thickness [AET]. Moreover, a second level of genetic evaluation was introduced (mainly for selection of sires), covering “re-productive success” (defined as ratio of number of healthy chicks and number of set eggs) and additionally the shell thickness and hatching egg weight and egg production during this performance period. Three chicken strains from pedigree farm were included into the study. Estimated heritability of AET was moderate. As expected, the genetic correlations between AET and egg production (IEP and RIEP) were negative.

The study is financed by the GENDROB research project of „DZIAŁANIE 16 WSPÓŁPRACA”, contract nr 00038.DDD.6509.00101.2019.06.



P-07 | A DYSFUNCTIONAL MUTATION IN SERUM AMYLOID A IS ASSOCIATED WITH RESISTANCE TO CHICKEN AMYLOID ARTHROPATHY

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Keywords: Amyloid Arthropathy SAA

Introduction

Amyloid arthropathy (AA) in chickens is characterized by serum amyloid A (SAA) depositions in the knee joints and is triggered by bacterial infection. Brown egg-laying chickens are susceptible, whereas white egg-laying chickens (WL) are resistant to AA. We functionally characterized a missense mutation in the SAA gene which is associated with disease resistance.

Methods

Candidate variant identification, production of stable chicken hepatocellular carcinoma (LMH) cell lines, Western blots, qPCR, IF microscopy, and RNAseq analysis were performed with established protocols [1].

Results

The discovered variant is predicted to result in an Arg to Ser substitution at position 90 (SAA.R90S) and was fixed in WL (FST = 0.935). When overexpressed in LMH cells, SAA.R90S was expressed at a higher rate and secreted to a greater degree than wild-type SAA. Differential expression analysis showed that the R90S mutant exerted a differential effect on the expression of core transcription factors linked to immune cell differentiation.

Conclusion

We conclude that the SAA.R90S mutation is causative for AA resistance by modulating the immune response to bacterial infection, which is currently under investigation in a suitable cell model.

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P-08 | BIAS IN ESTIMATED VARIANCE COMPONENTS AND BREEDING VALUES DUE TO PRE-CORRECTION OF SYSTEMATIC EFFECTS

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Keywords: big data, modelling, genetic parameters

Introduction

Analyses of large datasets are often simplified with a two-step procedure. In the first step, the data is pre-corrected by subtracting estimated systematic effects (e.g. sex, herd or litter) from the raw phenotypes. In the second step, heritabilities or breeding values are estimated from the pre-corrected phenotypes. Even though pre-correction is widely used, the consequences for estimated variance components have received little attention.

Results

Our results show that pre-correction creates bias in the estimated variance components. For balanced data, we provide simple mathematical expressions for the bias, showing that variance components are underestimated unless the reliability of the pre-correction is one. Bias occurs both with fixed and random effect pre-correction, albeit the use of fixed effects results in stronger bias (16% versus 10%). These theoretical results were consistent with the results from simulations.

Conclusion

We will present further results from simulations of data sets with family structure and genotypes, and from an empirical data set. Our research will help to better understand the effects of data pre-correction, and will ideally provide a solution for the resulting bias.



P-09 | EXPRESSION ANALYSIS OF GROWTH REGULATORY GENES AND OPTIMIZATION OF CRISPR/CAS9 MEDIATED GENE EDITING OF ACTIVIN RECEPTOR TYPE IIB (ACVR2B) IN BROILER CHICKEN

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Keywords: Myogenesis, Long-non coding RNAs, ACVR2B genome editing

Growth in broilers is a complex phenomenon with interplay of physiological, metabolic and hormonal events.

The expression of myogenic regulatory genes and long non-coding RNAs in pectoralis major of coloured broilers at different post hatch time points were studied. Bulk wave electroporation conditions were optimized for primary CEFs and DF-1 cell lines. CRISPR/Cas 9 mediated gene editing of ACVR2B gene was also optimized with guides targeting exon 2.

Current study witnessed lower expression of negative growth factors (MSTN, ACVR2A, ACVR2B) with advancing age. IGF-1 showed a stable expression pattern in liver. A total of 156 novel lncRNAs were observed. DF-1 cells when double pulsed at 300 and 350 v/10 ms resulted in increase of efficiency scores with 53.84 & 25.53% viability respectively. Co-transfection of guides at different PAM seq reveals better editing efficiencies (40.72%) than single (0%) resulting in double stranded breaks and deletion.

Pulsing conditions and buffer composition are essential to maximize cell viability and electro-transfection efficiency. This study revealed the role of lncRNAs at different physiological stages of both rapid and slow growing meat chickens and provides theoretical basis for future research.

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P-10 | SIMULATION OF GENE EDITING IN DUAL-PURPOSE CHICKEN BREEDING PROGRAMS

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Keywords: Gene editing, Dual-purpose chicken, Simulation

In response to the growing interest in dual-purpose chickens that are good in both meat and egg production, this study explores the potential of gene editing in dual-purpose chicken breeding programmes by stochastic simulation. This simulation considers two negatively correlated traits (-0.4) to represent the performance in egg production and meat production. An additional trait was also simulated as a health indicator and is negatively correlated with both production traits (-0.2). The simulation demonstrates that gene editing could accelerate the genetic improvement in two different breeding schemes: the first breeding scheme improves the performance of offspring from a layer-broiler cross (sped up by 36%), while the second breeding scheme improves the offspring of the laying hen line (sped up by 57%). Editing more SNPs yields faster improvement in the first generations, but the extra genetic gain slows down after 20 generations. This is because the QTNs with large effects were fixed, and gene editing on small QTNs had a higher risk than benefits. In conclusion, gene editing could speed up the improvement of dual-purpose chicken when large QTNs could be improved and the risk of gene editing was low.



P-11 | INVESTIGATION OF RELATIONSHIP BETWEEN RESIDUAL FEED INTAKE (RFI) AND MEAT QUALITY IN SLOW-GROWING KORAT CHICKEN

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Keywords: Slow-growing chicken, Meat quality, Residual feed intake

Introduction

This study aimed to investigate the relationship between Residual feed intake (RFI) and meat quality in Korat chicken (KRC), a crossbreed chicken in Thailand. Based on KRC has low feed efficiency (FE) and adverse effects on high production costs. Also, in the past, genetic selection to improve FE has shown obvious negative consequences for meat quality, thus, RFI that is used for estimating FE was considered.

Methods

In this study, High (HRFI) and low (LRFI) KRC were collected.

Results

The results found that there were no differences in drip loss, ultimate pH, and water holding capacity (WHC) between two groups ($P > 0.05$). Pearson's correlation revealed that meat characteristics had negative correlation with growth parameters i.e. feed conversion ratio (FCR), body weight gain (BWG), etc. Based on Principal Component Analysis results, HRFI was significantly associated with all growth performance. While, LRFI has no significantly associated with any parameters.

Conclusion

Interestingly, all meat characteristics did not either correlate with any groups. These findings indicated that RFI had a high potential to be used as a tool for breeding selection, without any effect on its meat quality.



P-12 | FINE-MAPPING OF GENETIC VARIANTS FOR BODY MASS IN LAYING HENS

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Keywords: body size, gwas, fine-mapping

Introduction

Body mass is a model quantitative trait that shows substantial heritability, a largely polygenic architecture, as well as major associations in many species including the chicken. In particular, orthologous loci are associated with body size traits in several species. In this case, we aim to fine-map such major loci in laying hens, to narrow down the underlying genomic regions.

Methods

We performed genome-wide associations studies of body mass in commercial laying hens of two crossbreds (with sample sizes of 415 and 428 animals) using 50k SNP chip genotypes and linear mixed models. Further, we whole-genome sequenced 48 hens to detect and impute SNPs and indels for statistical fine-mapping.

Results

Our genome-wide association studies detect three significant loci for body mass on chromosomes 4, 6, and 27. They overlap regions previously associated with body mass, including the *LCORL/NCAPG* and *CCKAR* genes. The genome-wide association studies are published [1], and the fine-mapping work is ongoing.

Conclusion

While it is unlikely that fine-mapping will identify individual causative variants, it will show what level of resolution can be achieved in crossbred hens, and whether any candidate genes can be ruled out.

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P-13 | ACCESS TO OPEN-AIR INFLUENCES MEAT QUALITY AND ANIMAL GROWTH BUT NOT YIELDS IN A STRAIN OF SLOW-GROWING NAKED NECK CHICKEN.

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Keywords: broiler selection, outdoor access, genotype by environment interaction

To limit sanitary risk and maximize the expression of genetic potential, selection of Label Rouge chickens takes place indoors while animals in production are reared with access to outdoors. The effectiveness of selection may be compromised by the presence of genotype by environment interactions. Our study aims at estimating the intensity of these interactions on traits with high impact on profitability and satisfaction of consumer demand such as growth, carcass composition and meat quality. By rearing 600 related broilers of a naked neck strain indoors or outdoors (300 birds/group), we showed that outdoor access affects meat quality with a more acidic (5.69 vs 5.77), paler (L^* : 46.4 vs 45.4), more exudative (13.8% vs 12.8%) and less tender fillet (16.3 N vs 14.2 N). Carcass fattening (2.8% vs 3.4%) and male weight at 9 weeks (2.08 kg vs 2.23 kg) were lower outdoors. Genetic correlations between indoors and outdoors showed that genotype by environment interactions exist for acidity, exudation and tenderness ($r_g = 0.69, 0.61$ and 0.30 , respectively) of breast meat. Genetic analyses will be consolidated by doubling the number of animals measured and by enlarging recorded traits to feed efficiency and rearing quality of birds.



P-14 | TOWARDS THE PROBLEM OF IDENTIFYING AND INDIVIDUALLY ASSIGNING LAID EGG AND LAYING HEN: A COMPUTER VISION BASED AUTOMATIC DETECTION METHOD OF FLOOR EGGS

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Keywords: computer vision, laying hen, floor eggs

Individual assignment of egg and corresponding laying hen is crucial when conducting genetic or physiological studies of correlations between laying characteristics and functional regulation. With the ban on keeping individual laying hens in cages in Germany, this has become difficult. Electronical systems using RFID technology individually record eggs being laid in nests, but eggs laid on the floor remain unassigned. We present first steps towards developing an automatic system to detect floor eggs using deep learning-based computer vision. Videos were recorded in one pen housing 20 laying hens. In 328 images a total of 6443 hens and 479 eggs were labeled[1]. Labeled images were used to train a deep learning-based object detector[2,3]. Egg detections were tracked, clustered and compared to manual video observations. First results for one day show that in all areas (dividing pen into a 2x4 grid) eggs were laid, automatic detections were made. Still existing problems are false positive detections and discrepancy between observed and automatically detected time of egg laying. This is likely to be improved by extending the data set to include more eggs. Future steps also include automatic identification of the egg laying hen.

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P-15 | EXPRESSION DYNAMICS OF GRP78 AND HSP70 GENES IN SLOW GROWING POULTRY WITH RESPONSE TO THERMAL CONDITIONING DURING EMBRYONIC AND EARLY POST-HATCH PERIOD

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Keywords: Thermotolerance, Slow-growing backyard poultry, Embryonic thermal conditioning

Study was undertaken to elucidate effect of embryonic thermal-conditioning in combination with early post-hatch thermal-stress in CARI-Debendra chicken. Embryonic thermal-manipulation (ETM) was done during 11-16th day at 39.5°C with RH 60-70% for 5hrs/day and standard incubational conditions was maintained for rest period. After hatching, chicks were randomly distributed in three sub-groups (No manipulation, embryonic and early post-hatch thermal-manipulation). Early post-hatch thermal-manipulation was done for first five weeks in chicks with brooding at 37±1°C. Hatchability didn't differ between ETM (63.23%) and control (63.53%) but, hatch weight and pipping time showed significant variation. In ETM group, rectal and surface temperature were +1.04 and +4.76% higher than control at hatch but, later waned into insignificant. Heat-conditioning showed 1% increase in body weight than control at 6wk. Serum concentration of thyroxin, potassium and creatinine-kinase was higher in ETM than control, while low H/L and tri-iodothyronine indicated acquisition of thermotolerance. In ETM group, *HSP70* expression was significant in liver (P=0.06) and intestine (P=0.09) tissues, whereas upregulation of *GRP78* revealed intestinal tissue specificity.

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P-16 | COMPARISON OF STRUCTURAL VARIANTS IN CHICKEN TRIOS CALLED FROM ILLUMINA, PACBIO AND NANOPORE SEQUENCING DATA

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Keywords: structural variation, long-read-sequencing

Introduction

Structural variants (SV) are known to strongly influence heritable traits of livestock. Discovery of SV was usually based on sequencing with Illumina's short-read approach during the last decade, which produces reads shorter than most SV and thus required the use of auxiliary information to call SVs. Further, mapping issues are observed in low complexity regions, which are known to trigger SV formation. The recent development of long-read (LR) technologies from Pacific Biosciences (PacBio) and Oxford Nanopore Technologies (ONT) promises to overcome those issues.

Methods

We sequenced four chicken trios using Illumina, PacBio and ONT sequencing. SV were called independently from the different data types using state-of-the-art tools and then compared with regard to number, overlap, length and mendelian errors.

Results

While the technologies resulted in comparable numbers of SV called, LR technologies showed a lower number of mendelian errors than Illumina sequencing (4 vs 8%), indicating more accurate SV calls from LR sequences.

Conclusion

LR sequencing allows more accurate discovery of SV than Illumina sequencing and might help uncovering a yet underestimated source of genomic variation.

P-17 | JOINING OF GENOTYPING DATA OF DIVERSE SETS OF CHICKEN SAMPLES ORIGINATING FROM DIFFERENT ARRAYS CAN BE IMPROVED BY IMPUTATION WITH SEQUENCE DATA

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Keywords: Genomic Data, Diversity, Imputation

Background

The last two decades of diversity analyses have been dominated by the use of high-throughput genotyping with genotyping arrays. In Chicken, different versions of arrays became popular over time, which are only moderately comparable in terms of positional overlap. Extensive studies have been conducted to gather and characterize large datasets of various breeds and ecotypes using different arrays. Consequently, such datasets cannot easily be subjected to a joint analysis.

Material & Methods

About 380 roosters from various breeds were genotyped with the IMAGE-multi-species array to be combined with the Synbreed Chicken Diversity Panel (SCDP, 600k Axiom array). SNP positions of the first set were systematically pruned and reimputed in a 3-fold cross validation, using (1) SCDP array data and (2) SCDP array data and sequences of 68 IMAGE cocks, extended by 90 commercial layers and broilers as reference.

Results

Genotype concordance between masked and imputed loci could be elevated from 70 to 80 % on average when the reference set was pre-imputed with sequence data. Imputation accuracy was clearly dependent on minor allele frequency. Concordance was on average 11% higher for frequent loci (MAF > 0.25). The maximum concordance reached was 88% using sequence dense reference on frequent loci.

Conclusion

Using sequence data to pre-impute array reference data increases imputation accuracy in IMAGE array data, but larger and more diverse reference sets are needed for further improvements.



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