



LOHMANN INFORMATION

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BREEDING FOR SUCCESS ... TOGETHER



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TIERZUCHT

Contents

Introduction	3
150 years Gregor Mendel – from counting peas to gene editing	4
Applied research to support cooperative breeding programs	10
Uncovering causal relationships between feather pecking and related behaviours using structural equation models (SEM)	16
Selection on beak shape to reduce feather pecking in laying hens	22
Double-yolk eggs in commercial laying hens and parent flocks	28
Making worst case scenarios real: The introduction of highly pathogenic avian influenza of subtype H5N8 led to the largest fowl plague outbreak ever recorded in Germany	36



Dear colleagues and friends,

Avian Influenza remains the most important threat of poultry production worldwide. After disastrous outbreaks in the US in 2014/15, Europe was hit in 2016 by a modified type of HPAI virus. **Franz J. Conraths** describes the dynamics of the recent problem in Europe and measures in Germany to minimize the spread of the disease. We will continue to provide up-to-date information on the development of HPAI.

While focusing on serious problems of the present time, we should not forget historical aspects of natural sciences without which the development of efficient plant and animal production and food security for a growing world population would have been impossible.

The introductory article of **Gottfried Brem** recalls the publication of "Mendel's laws" 150 years ago. The history of Mendel's research reflects the problems of scientists who trespass frontiers of established theories. Resistance against new developments in genetics and the application in animal and plant breeding persists especially in Western societies, and some creationists even deny the principles of evolution.

As reported in the article of **Kay-Uwe Götz** and Malena Erbe, genomic information from large volumes of data in different countries is being used to assure that regional producers can keep up with global progress in the efficiency of meat and milk production, taking into account regional production conditions and preferences.

Feather pecking and cannibalism are a major issue in poultry production, especially in countries where beak treatment is no longer permitted. **Jörn Bennewitz** and co-authors review motivations of feather pecking and relationships with other behaviors. This information is important for genomic studies on feather pecking.

Wiebke Icken and co-authors studied the question to what extent beak shape varies and may be changed by selection. Beak shape can be measured in large flocks of pedigreed hens, and genetic changes may help in the long run to lower the risk of feather pecking. The control of feather pecking in commercial poultry flocks will remain a challenge.

Double-yolk eggs in chickens are rare and only of interest for niche markets. They are commonly observed at the beginning of the laying period, and the frequency depends on lighting programs during sexual maturation. **Dietmar Flock** and co-authors review earlier work and add new information from commercial white-egg and brown-egg layers.



Prof. Dr. Dietmar K. Flock



Prof. Dr. Werner Bessei



Gottfried Brem

Professor Dr. Dr. h.c. Gottfried Brem studied veterinary medicine, agricultural sciences and business economics in Munich and Weihenstephan, Germany. He was first Professor at the Ludwig Maximilian University of Munich, Germany, and is at present Full Professor at the Institute of Animal Breeding and Genetics, section Biology of Reproduction at the Veterinary University of Vienna, Austria. His special field of research is cloning of mammalian genes and transgene models in experimental and farm animals. His present work is focused on automatic phenotyping of diverse Y-chromosomal markers for the establishment of male genealogy in horses.

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150 years Gregor Mendel – from counting peas to gene editing

Preface

The present article is based on the introductory lecture of the author “150 Jahre Mendel’sche Regeln: vom Erbsenzählen zum Gen-Editieren” at the joint Symposium of the Austrian Academy of Sciences and the Gregor-Mendel-Society, held in Vienna March 2016. The article focuses on the scientific work of Mendel, with special reference to the social and scientific conditions of his time.

Abstract

Genetics and genomics have shown unexpected progress in the last decades. Their impact has spread from the traditional fields of interest, breeding of plants and animals, towards all fields of life sciences, medical diagnostics and remedies, food and feed technology, fuel production, etc. The 150th anniversary of the publication of “Mendel’s laws” is an opportunity to recall the historic framework of the epoch-making findings on the nature of inheritance. The career of Mendel started under highly unfavorable conditions. Born as son of a poor farmer, he had to enroll as cleric to receive higher education. His famous crossbreeding experiments were carried out during his spare time after all routine obligations as teacher, monk and finally as abbot of his monastery. Based on observations and statistical analysis of large scale breeding experiments, Mendel developed the theory of inheritance including dominant and recessive effects. His theory was not unanimously accepted by contemporary scientists, but Mendel showed no ambition to defend it in public. He was convinced that his work would be recognized in due time when he said “meine Zeit wird kommen - my time will come”. The validity of Mendel’s theory was indeed confirmed by experiments of de Vries, Correns and the brothers Tschermak-Seysenegg about 30 years later. Again one generation later, geneticists discovered that Mendel’s laws not only explained qualitative genetic (i.e. single gene) effects but represent the basis for quantitative genetics. To appreciate Mendel’s scientific merits, we should remember the unfavorable working conditions of those times. Not only was he lacking technical support, even more problems arose from the adverse attitude of the church towards new insight in science. It is interesting to note that important scientific inventions have been made by other scientists with a clerical background (Galilei, Copernicus, Kepler, Lamarck and Darwin) despite the conservative spirit of the Christian church. Mendel owed his successful work not only to his excellent education, but also his particular character, which combined hard and meticulous scientific work with virtues of the Christian and the antique philosophy.

Keywords

Genetics, Mendel’s law, history



Figure 1: Gregor Mendel |

Source: © Unknown / wikimedia.org / CC0

Education and first experience

Johann Mendel was born on 20 July 1822 as son of a poor farmer in the small village Heinzendorf, Silesia (today: Vracne, Poland). He was noticed as an intelligent school boy with outstanding results. Since his family was not in a position to pay for higher education, his school mentor proposed that Johann should study theology and become a monk in an Augustinian monastery. In this case the convent of the monastery would pay for his study. Following this recommendation, Mendel enrolled in the Augustinian St Thomas Abbey in Brunn, Austria-Hungary (today: Brno in the Czech Republic) in 1840 and adopted the clerical name Gregor.

In this monastery Gregor came into contact with animal and plant breeding activities. The monastery belonged to the "Hermits of St. Augustinus", a mendicant order. Abdication of property was a fundamental principle of this order. This life style differed from the capitulars, which reside in the monastery Kloster Neuburg near Vienna, and are considered the wealthy part of the order St. Augustinus. The founder of the order was St. Augustin of Hippo, an an-

tique city in Northern Africa. He lived from 354 until 430 and was one of the "fathers of the church" in the late antique and the early mediaeval time. He was an important philosopher with substantial influence on the thinking of the Christian world in those days. The long list of well-known members of the Augustinian hermits includes two with outstanding influence: Martin Luther, the reformer of the Christian church in the 16th century and translator of the Bible into German, and Gregor Mendel as the mentor of modern genetics.

Four years after his enrolment in the monastery St. Thomas, Gregor Mendel was ordained priest and acted as teacher. His abbot, Franz Cyril Napp, recognized the scientific quality of Gregor Mendel and sent him to Vienna for further studies. Here he met famous professors, like Doppler (mathematics and physics), Redtenbach (chemistry), Eduard Fenzl and Franz Unger (botany), von Ettinghausen and von Baumgartner (physics). All of them were members of the Imperial Academy of Sciences of Vienna (Figure 2). Gregor Mendel was particularly influenced by the professors in physics, Doppler and Ettinghausen, and the professor in botany, Franz Unger. From Franz Unger he learned methods of

crossbreeding and combination, which allowed recognizing relationships between groups. For this reason, Mendel meticulously recorded and analysed the results of his crossbreeding experiments.

In 1856 Gregor Mendel failed in an exam which was required for the nomination as teacher. It has been speculated that his failure was caused by a dispute within the Academy of Science: The botanist Eduard Fenzl denied the theory of his colleague Franz Unger, that fertilization is the result of melting of male and female cells. Mendel, in contrast, supported the theory of the combination of male and female cells. Unfortunately Mendel was not examined by Franz Unger, but by his adverse colleague Fenzl, and this was probably the reason of his failure to obtain the intended academic title. Possibly it was this failure which stimulated his scientific ambition. Back to Brunn, he started the famous experiments with peas in the garden of the monastery.

Experimental work and deduction of genetic rules

Using specialized techniques of artificial fertilization, Mendel produced about 15,000 controlled crosses of different varieties of peas. He carefully followed the



Figure 2: Members of the Academy of Sciences of Vienna and teachers of Gregor Mendel

development of the hybrids and their offspring using statistical methods. On the basis of the phenotypic distribution of the crosses he developed the so-called rules and pattern of inheritance, though the expression “inherited” appeared only once in his publications. Mendel found that criteria or properties of parents are transferred unchanged and in constant frequencies to offspring as inherited characteristics. He further concluded that each individual has two complete sets of heritable factors, one from each parent, and that the contribution of each set was independent from the sex of the parent. The heritable factors may not be expressed in all cases. Criteria which appeared in the hybrids he called “dominant” characteristics and those which became latent, he called “recessive” characteristics. He chose the expression “recessive”, e.g. not appearing, because these criteria reappeared again in unchanged pattern in offspring of the hybrids. On 8 February and 8 March 1865, Mendel reported his crossbreeding experiments in two sessions of the Association of Natural Sciences in Brünn. One year later, in 1866, his reports were published in Volume 4 of the “Verhandlungen des Naturforschenden Vereins”, a leading journal of natural sciences at the time. The so-called laws of Mendel led to a revolution not only in biology and agricultural sciences, but also in various other disciplines.

Mendel's rules explained qualitative gene effects which are based on single (major) genes. Methods to identify Mendelian effects cannot be applied in quantitative genetic traits. The next important milestone in genetics was the transition from Mendel's qualitative genetics towards quantitative genetics around the year 1920. Fisher (1918) and Wright (1921) showed that quantitative genetic effects are the result of simultaneous segregati-

on of genes on multiple locations. Hence quantitative genetics represents an extension of Mendel's rules, not a contrast.

Today, 150 years after Gregor Mendel, molecular genetics plays a dominant role in natural sciences. These new methods influence not only biology and agricultural sciences. High precision diagnostic tools are indispensable for treatment of human and animal diseases and the development of new medical drugs. The development and use of gene editing to replace single base nucleotides in live organisms needs to be carefully observed and evaluated by scientists and the society to ensure that they will be used to improve the health of the human population.

Gregor Mendel was elected abbot of his monastery in 1868. This means he was charged with all obligations of the Augustinian order. Nevertheless, he devoted his spare time to scientific activities, mainly to scientific questions in agriculture, meteorology and forestry. He died on 6 January 1884. His scientific work was not recognized and honored at that time. The quote “my time will come” goes back to the time when he acted as abbot of his monastery. Continuing progress of genetics confirms the importance of his findings and shows that his prediction became true.

The role of clerical scientists in advancement of science

It took about 30 years until Mendel's laws were recognized. Nobody knows for sure whether this delay was due to the fact that Mendel was a monk and not member of a renowned scientific institute. In contrast to the present time, when religion and natural sciences appear to be incompatible and far from each other, many important scientists in the past were clerics or had

an education in a religious institution. Important universities in Europe were founded by churches. The universities in central Europe emerged from the mediaeval Christian education system which has been established by monasteries and domes in the 6th century. Priests, monks and nuns have discovered and invented a large number of important innovations. The official Christian church, however, was not always pleased with some of these innovators, who had to suffer for their creativity. The best known story of a monk's discovery is that the Franciscan monk Berthold Schwarz from Freiburg, Germany, invented the “black” gun powder, which was named “Schwarzpulver” after him. Priests and clergymen worked on many topics outside their proper destination: electricity, magnetism, perception of colors, optics, telecommunication, production of paper from wood. Even more recently, clerics developed slow motion movies and the first serial production of washing machines. Health care was a prominent activity of monasteries, and medicine during the mediaeval time in central Europe was mainly in the hands of monks and nuns. Hospitals were operated by monasteries in the early medieval time, and there were virtually no physicians outside the monasteries. Nurses and barber-surgeons were the only “authorities” to transfer accumulated experience from one generation to the next. The monastery's medicine was predominantly based on naturopathy, hydrotherapy and phytotherapy, using water and medicinal herbs as remedies. The naturopathy has a long tradition in the western medicine, from the abbess Hildegard von Bingen in the 12th century (1098 – 1179) to the priest Sebastian Kneipp (1821 – 1897), who brought hydrotherapy to new blooming.

Modern natural science based on mathematics started with Galileo Galilei (1564



Figure 3: The shape of chicken combs are based on qualitative genetics (Darwin, 1868)

– 1642). He was the first who used mathematics to study natural events. Galilei was educated in a monastery and intended to join the Benedictine order. His father, however, sent him for medical studies to Pisa, where his main interest changed from medicine to mathematics. With his telescope he confirmed the heliocentric picture of the universe of Nicolaus Copernicus (1473 - 1543). This was against the prevailing theory of the Christian church, which still believed in the Ptolemaic theory of the universe. Galilei was prosecuted by the inquisition, the powerful institution of the Christian church to fight heresy. Galilei's tragedy was that he was still a strong believer in Christianity, but attempted to correct the erroneous perception of the universe. He was convinced that god's creation could be explained completely through experiments and logical thinking. This was also against the church's official point of view of that time that god's work in nature could never be understood and explained by the limited mental capacity of human beings. Until the time of Galilei, the church was under the influence of the scholastic theology, which had been developed some centuries before by Albertus Magnus (1200 – 1280) and Thomas Aquinas (1225 – 1274). This theology was

still based on natural philosophy and the logics of Aristotle. As founder of the "pre-critical sciences", Galilei was still under the influence of the traditional theology when he began his studies, and he searched for the truth. The fundamental difference between science and religion is that science-based insight is subject to the principle of falsification. In his "Logic of Research", the philosopher Karl Popper postulated that theories have to be subjected to experimental examination, e.g. the theory must be refutable. The principle of falsification of theories is the basis of progress in and success of natural sciences. This is in contrast to the religion-based approach, which tries to confirm an anticipated theory.

The above-mentioned Nicolaus Copernicus, who studied theology in Krakov (Poland), had no problems to believe in god and, at the same time, accepting that the sun and not the earth is the center of our universe. Similarly, Johannes Kepler (1571 - 1630), who studied theology, mathematics and astronomy at the University of Tübingen, Germany, could combine his faith in god with the fact that the planets move around the sun in elliptic orbits. In this context we should also remember Isaac Newton (1642 – 1726), one of the most

prominent scientists of his time and founder of modern understanding of natural sciences, who was educated in a Christian institute, the Trinity College of Cambridge. Although Newton did not become a cleric as intended, he remained a strong believer in Christian theology. A few areas remained, where scientists could not overcome the gap between science and religion. This was also true for genetics and the theory of evolution.

Jean Baptiste Lamarck (1744 – 1829) attended the Jesuit College in Amiens, France, with the intention to become a cleric. Three decades before Charles Darwin he developed a theory of evolution. According to Lamarck's theory, life had begun through spontaneous creation and then developed, under the influence of natural forces, goal-oriented from simple to more complex forms. He also thought that the use of organs would lead to functional modifications, which are transferred from one generation to the next. Organs which are not used would shrink. Lamarck's assumption that life was not the result of god's creation but develops under natural driving forces was appalling to the prevailing theology. His theory of inheritance of environmentally acquired characteristics was also criticized by contemporary scientists. Interestingly, Lamarck's theory has been adopted by Lyssenko (1898 – 1976), the most influential geneticist in the Soviet Union and other socialist countries in the first half of the 20th century. Lyssenko was of the opinion that environmentally acquired traits of plants would be transferred to the next generation. Lyssenko's theory was in contrast to the basic findings of Mendel, Darwin and contemporary geneticists. Hence, teaching their theories was prohibited in the Soviet Union up to 1960. Charles Darwin (1809 – 1882) began to study medicine. But he was not satisfied

with this discipline and changed to theology - on the recommendation of his father, with the aim to become a cleric of the Church of England. He passed the exams with distinction and received the Baccalaureate in 1831. He did not continue the clerical career and focused his work on entomology, botany and geology. It is often overlooked that Darwin studied not only feral animal species, like the well-known finches of the Galapagos Islands, but also domestic animals. Darwin described in detail the variation and extreme phenotypes of domestic birds and mammals. The inheritance of some anatomic characteristics, such as the form of the chicken comb (figure 3), follows the Mendelian rules and is still being used to demonstrate the effect of qualitative genetics. Darwin had no understanding of genetics and could not explain how the variation of criteria could be passed from one generation to the next. The insight in these mechanisms became evident only when Mendel's findings were considered.

Rediscovery of Mendel's laws

Discoveries sometimes turn into tragedies when researchers realize that their findings have already been recognized and published by others before. Discoveries may also be made too early and ignored or even combated by the contemporaries. This was the case with Mendel's rules of inheritance. Mendel's findings were one generation ahead of the general development of natural sciences, and the society of his decades was not prepared to recognize his epochal discovery. One generation later, de Vries, Correns and the brothers Tschermak-Seysenegg found the same results as Gregor Mendel. Their publication in 1902 confirmed Mendel's rules. Unfortunately the authors had to realize that they were not the first to uncover the principles of inheritance.

Mendel and his ethical mind

Gregor Mendel was a solid, conscientious and ingenious natural scientist and not a visionary or a speculator. A visionary would have speculated on the potential results of his findings. All he wanted was to demonstrate existing natural rules as the result of creation. In this sense we understand his saying: "my time is still to come", when he realized that the scientific community was not willing to recognize his findings. Mendel's thinking was based on the Christian anthropology. He was deeply influenced by the Christian virtues: belief, love and hope. He was also aware of the virtues of the antique philosophy: wisdom, justness, courage, and modesty. He practiced this attitude in his function as monk and later as abbot. As reported by his colleagues, he was always kind and benevolent to everybody: "affabilis unicuique". Mendel's deep compassion with suffering humans is also expressed in a letter to his mother: "There is little new in our monastery, except the enrolment of two novices, one from Brunn and one foreigner from Bavaria. ... But not everywhere in the world it is as quiet and peaceful. There is an outbreak of war in Italy, bloodier than ever before. Thousands have lost their lives and any hour we expect news about a main battle, which is foreseen in the last days of this month. May God give a fortunate end for our side. The screams sent to heaven by thousands of mothers whose sons are sacrificed, should be heard by god". Mendel's letter is dated Brunn, 25th June 1859. The main battle he mentioned had already happened the day before. It was the battle of Solferino, Italy, where Austria lost the Kingdom of Sardinia-Piemont in the "Sardinian War" against Napoleon. The small city of Castiglione had to accommodate 8000 victims of the war. They were looked after by Henry Dunant and the women of

the city under the motto "Tutti fratelli" (we are all brothers). The impression of these events was the driving force of the foundation of the International Committee of the Red Cross in Geneva, Switzerland, in the following years.

Further reading

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NOTES



Kay-Uwe Götz

Co-author: Malena Erbe

Kay-Uwe Götz studied animal breeding at the University of Göttingen, Germany. In 1989 he obtained a doctoral degree in animal breeding for a research project on the estimation of cross-breeding parameters in a selection experiment with mice. In 1990 and 1991 he worked as a post-doc at the Institut National de la Recherche Agronomique in Jouy-en-Josas (F), working on the detection of marker-QTL linkage in animal breeding. In 1993 he became senior researcher at the Bavarian State Research Institute for Animal Breeding (BLT) in Grub. His research focus in the following years was on the development of BLUP breeding value estimations in pigs and cattle and the design of breeding programs. In 1999 he became head of the department for animal genetics and IT at BLT. Since 2003 he is director of the Institute of Animal Breeding at the Bavarian State Research Institute for Agriculture (LfL) and chairman of the Alpine Genetic Evaluation Team. Since 2010 he teaches animal genetics at the Technical University of Munich. From 2012 to 2016 he was coordinator of the LfL focus group on animal welfare and since 2010 he is member of the board of the German Agricultural Research Alliance (DAFA).

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Applied research to support cooperative breeding programs

Abstract

Cooperative forms of animal breeding are still dominating in Southern Germany, Austria and Switzerland. The breeding organizations are supported by public research facilities which allow Bavarian farmers to compete in genetic progress and to apply state of the art methods although the majority of herd book and AI-organizations are way too small to run meaningful research units. The competence centre in Grub comprises a research station, an AI-organization, a veterinary service and research unit, a laboratory for routine genotyping and the head offices of several breeding organizations. The Institute of Animal Breeding runs genetic and genomic evaluations for cattle, pigs, sheep and horses in Bavaria and beyond and has developed cooperation with many other countries in the alpine region. These collaborations are continuing to grow, but have recently been challenged by new developments in international cattle breeding that might also affect the collaboration in genetic evaluation.

Keywords

Animal breeding research, cattle breeding, pig breeding, genomic selection, genetic evaluation, polled cattle

Introduction

Animal breeding is an important global contribution to meet the growing demand for animal protein. Across the world, different forms of organization have developed, ranging from community based breeding systems in developing countries

and cooperative breeding programs across several countries, to global enterprise breeding programs that supply different varieties of their breeding stock to customers in many countries. Which type of breeding program dominates in a certain region depends on the state of develop-

ment as well as on species, tradition, competition and public support.

The state of Bavaria in Germany has pursued a policy known as "the Bavarian Way" ever since 1969 that regards rural politics as closely related to social policy and

tried to slow down the growth of farming structures while maintaining competitive income and avoiding fallow areas in unfavorable regions [1]. One element of the Bavarian Way was the provision of public capacities for applied research in conjunction with a publicly funded extension service. Other elements were the improvement of agricultural education systems and the promotion of cooperative formats like groups of producers.

The Bavarian State Research Center for Agriculture (LfL) was founded in 2003 in the course of a reorganization of 17 different units dedicated to research, education and other public tasks. The research station in Grub is today a hotspot of Bavarian animal breeding and includes the research station, an AI-organization, a veterinary service and research unit, a laboratory for routine genotyping and the head offices of several breeding organizations. Overall, there are more than 300 employees working for Bavarian animal breeders. The research in Grub covers all areas of animal production although only animal breeding will be described here.

The Institute of Animal Breeding of LfL

The Institute of Animal Breeding (ITZ) was founded as the department for animal genetics and IT in the 1970s under the lead of Gottfried Averdunk. Since 1990 the institute became responsible for genetic evaluations in Bavaria, and Averdunk was one of the initiators of Interbull in the 1980s. Animal breeding research at ITZ covers cattle, pigs, sheep/goats and horses, but no longer poultry. Grub used to host one of the last independent layer breeding programs in Europe, the so-called "Meisterhybriden", until the selection unit was shut down in 1997 for "post-factual" reasons: the Bavarian government pushed the exit from conventional cages before the EU, and

breed improvement without testing pedigree stock in cages seemed impossible. Lohmann acquired two of the brown layer lines in 1997 which became an ingredient of Lohmann "Tradition" [2].

Apart from applied animal breeding research, the Institute of Animal Breeding of LfL (ITZ) runs a performance testing station for 4000 pigs/yr. and is responsible for conformation scoring in progeny of AI-bulls (50.000 cows/yr.). It is also responsible for routine genetic evaluations in cattle, pigs, sheep and horses. Research cooperation exists with all German agricultural faculties and many others. A staff of ten senior researchers together with several PhD-students conducts applied research in quantitative genetics and the design of breeding programs.

Genetic evaluation in dual-purpose cattle

Cattle breeding has been a very open process for the last 70 years. Semen of bulls with high breeding values is traded worldwide and many AI-organizations in Europe and the US make a living from exporting semen to less developed countries. From the international exchange of semen and competition between AI-organizations in different countries, a need for comparable breeding values arose, which led to the foundation of "Interbull" and the development of the international genetic evaluation system for bulls known as MACE [3]. MACE supports the international trade of semen, but it does not offer the importing countries an opportunity to participate in international breeding programs, because it only provides "international" breeding values for AI-bulls, which may not adequately reflect the definition of traits in different countries. For the Fleckvieh breed we chose a different approach and developed an international genetic evaluation for bulls and

cows giving comparable breeding values for all breeding animals and allowing for an across-country selection of sires as well as of (bull-)dams. Starting with a small national cooperation in 1995 that estimated breeding values for fat and protein yield, the genetic evaluation system for Fleckvieh (Simmental) and Braunvieh (Brown Swiss) was stepwise extended to more than 50 traits in breeding stock from Austria, Czech Republic, Italy, Hungary and Germany.

The unique approach of the "Alpine Genetic Evaluation Team" (AGET) spreads the work load between three computing centers in Grub, Stuttgart and Vienna. Each team specializes on a specific group of traits and accumulates specific knowledge in this field. ITZ is focused on milk production and conformation traits, Stuttgart deals with the beef traits and Vienna specializes in reproduction, longevity and health traits. The scientists are coordinated by a technical committee and report to a transnational board of herd book organizations, AI-organizations and public servants. The basic idea behind the AGET approach is that it is relatively easy to extend a genetic evaluation to accommodate more data, while it is relatively complex to establish a genetic evaluation for a new trait.

Since 2009 a genomic evaluation system was developed and put into practice in 2011. AGET applies the same division of labor as for the conventional evaluations, thus allowing specific knowledge about the data in different trait groups to be used. The collaboration between the participating countries comprises not only the genomic evaluation, but logistics of DNA-samples, genotyping and genomic databases are shared in the same way. Conventional breeding values are published three times a year, while genomic breeding values are estimated monthly.

International trends in cattle breeding

The idea of sharing all the knowledge and breeding values across several countries and competing organizations may appear strange to pig and poultry breeders. However, this approach in cattle breeding has proven to be very successful in the past decades. The main reason for this was that performance testing of AI-bulls could only be organized using data from many (if not all) farms. Even non-herd book farms have contributed to performance testing and paid considerable shares of the recording system. Due to the necessity of progeny testing and the free distribution of semen, different breeding programs showed very similar rates of genetic progress. With the advent of genomic selection, the generation interval of a dairy cattle population began to depend heavily on the proportion of genomic young bulls used for AI. Varying proportions of young bulls lead to very different generation intervals and genetic progress becomes more heterogeneous across populations. In the long run the more progressive organizations will take the lead and will keep the best bull-dams of the breed for themselves, instead of sharing their genetic potential with all competitors. First tendencies can already be seen in the worldwide Holstein population [4]. Sexing Technologies, a relatively new player in the field of AI, uses promising new bulls exclusively for their own breeding program and later on some bulls are only available as sexed semen, not suitable for producing male calves. Other companies like Select Sires or Evolution distribute bulls to the general public only after a grace period of 4-6 months.

This tendency is boosted by another trend in worldwide cattle breeding: For new traits, where phenotypes for historic breeding animals cannot be generated ex

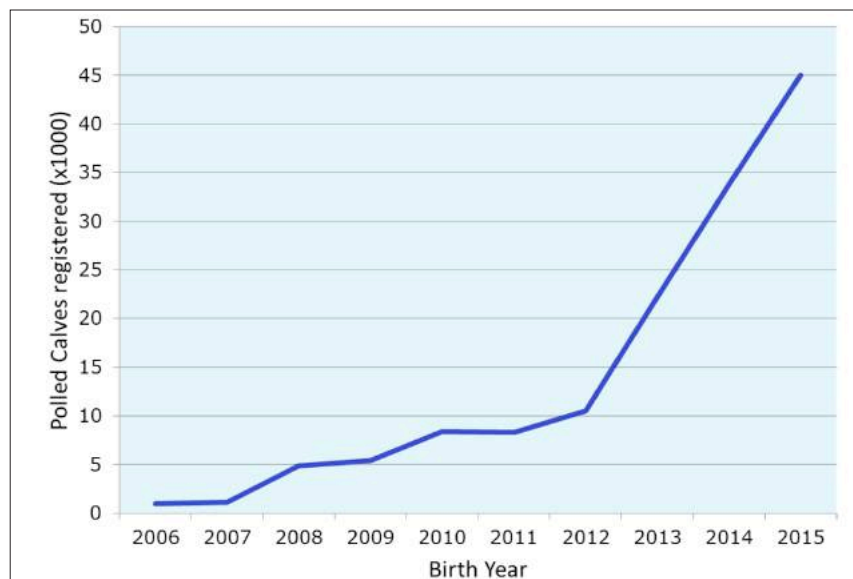


Figure 1 : Number of polled female calves born in herds under milk recording in Bavaria

post, the genotyping of large cow samples is the method of choice. This requires large investments of the breeding organizations for genotyping and large efforts to supervise the participating farms. Breeding companies that have made these investments will not be willing to share the benefits with competitors. It is very likely that we will see a change of paradigm in the breeding of AI-bulls over the next few years. Cooperative breeding programs do not yet have developed a conclusive strategy for this new era.

Breeding of polled Fleckvieh

Most of the cows in present husbandry systems are kept in free stall systems, which require the cows to be hornless. However, the practice of dehorning causes pain for the animals and ethical concerns in the public. Polled breeds of cattle have been used since a long time, but polledness was usually not appreciated in multi-purpose breeds which were used for field work.

Polledness is caused by a single locus which appears to be identical in all polled *bos taurus* breeds [5]. However, in most

breeds used for dairy production polledness occurs only at very low frequencies, which results in a lack of polled AI-bulls and makes selection for polledness difficult.

Systematic breeding of polled Fleckvieh in Bavaria dates back until 1974 when the first polled cow was purchased for breeding purposes. Until 1985 three polled bulls and two more polled dams were purchased and from 1984 on the first homozygous bulls were produced via embryo transfer. Until 1990 the polled allele was only common in beef strains of the Fleckvieh breed. At the beginning of the 1990's the first polled alleles were introduced into the dairy herd of the state research farm in Grub. This research farm acted as the nucleus for the introgression of the polled allele in the dual-purpose lines of the Fleckvieh breed. Since 2003 polled calves in all Bavarian herds under milk recording were systematically recorded and the polled phenotype was regularly examined by employees of ITZ. At the same time a systematic cooperation with Bavarian herd book organizations and AI-stations was established. Between 2000 and

2011 in total 193 polled bulls were raised at LfL, of which 37 entered AI-stations. The success of these bulls is also a nice example to demonstrate that a systematic application of quantitative genetic principles can lead to considerable progress even in species with long generation intervals.

Currently the dual-purpose population of Fleckvieh comprises 60 polled AI-sires and 15.000 polled cows. While the number of cows corresponds to 2% of the population, in 2016 already 18% of all inseminations were made with polled sires, 85% of them being young sires with only a genomic breeding value. The analysis of relationships of polled sires showed that the introgression has caused negligible amounts of additional inbreeding [6]. Fig. 1 shows the number of female polled calves registered by the Bavarian milk recording organization. Due to the incredible dynamics of the use of semen of polled bulls, it is very difficult to predict the share of polled cows in the future. However, from calves born in 2016 it is certain that the proportion will be close to 25% by 2021.

Genomic Selection in Pigs

In Bavaria, herd book breeders are still supplying 79% of the genetic material used in piglet production, which is unique in Germany. Performance testing is very intense with approx. 300 newly recruited AI-boars per year providing 8.000 progeny tested on station. Until recently, genomic selection and routine genomic breeding value estimation has only been established in larger (international) pig breeding companies, while smaller breeding organizations are still working on the implementation, frequently struggling with small calibration sets. In Bavaria, a three-year project called InGeniS ("Integrierte genomische Forschung und Anwendung in der bayerischen Schweinezucht" - integrated geno-

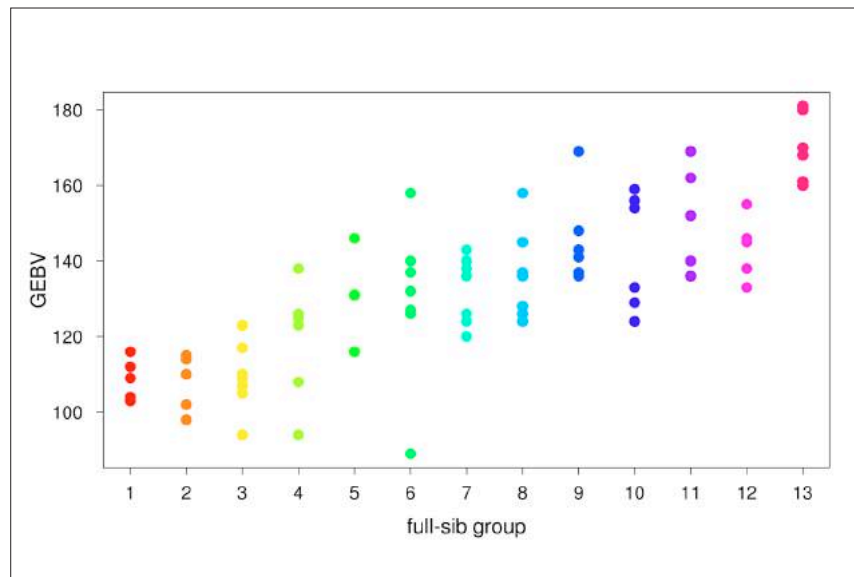


Fig. 2: Genomically enhanced breeding values (GEBV) for members of thirteen different full-sib groups from the Piétrain breed with at least six members. Each dot within a full-sib group represents an individual GEBV of one of the members

mic research and application in Bavarian pig breeding) has been the kick-off for genomic selection in pigs. Within this project it was possible to genotype more or less all AI boars for the breeds Piétrain and German Landrace used as sires in Bavaria during the last decades with the Illumina PorcineSNP60 v2 BeadChip (around 60.000 markers). All these genotyped individuals build the basis for a routine genomic evaluation system which itself is the basis for applied genomic selection.

Genomically enhanced breeding values (GEBV) can only be calculated if there is a sufficiently large set of individuals with both genotypic and phenotypic information that are used to calibrate the system, the so called reference set. These individuals are used to estimate the effects markers have on a specific trait. This will work out the better, the more individuals are available for the reference set, the better they represent the breeding population and the more own or progeny performance records are available. For the Piétrain breed in Bavaria there exist reference

sets for around 2.500 individuals. This is one of the largest reference sets available for this breed and forms an excellent basis for the GEBV estimation.

Routine genomic evaluation in cattle normally involves a multi-step procedure with pedigree-based breeding value estimation, subsequent direct genomic breeding value estimation and finally a combination of both results [7]. A calibration set of many bulls with very reliable progeny-based breeding values is a good basis for such a procedure; however, such data is normally not available in pigs and thus small errors accumulate to a considerable amount across the different steps. Thus, a procedure called 'single step' is preferred for routine genomic evaluation [8]. This modelling is computationally more expensive, but provides the advantage of modelling all available information (phenotypic observations, pedigree data and genomic data for the genotyped individuals) in one single step, GEBV for all individuals directly.

In pig breeding it is very common that a young selection candidate does not have own or progeny performance at the time of selection. Using conventional breeding value estimation the expected breeding value of such a candidate will be the mean of the parents' breeding values. This parent average (with a reliability of around 20-30%, depending on breed) can only be used for selection between, but not within full-sib groups. The situation is similar to the one in layer breeding where early selection of males in the purebred lines is at a point in time where only phenotypes of full-sib sisters are available. In a genomic evaluation system, each selection candidate receives an individual GEBV from the moment its genotypic information is included into the single step model.

In Piétrain pigs in Bavaria reliability of the GEBV is approx. 50% compared to approx. 30% for a conventional parent average. With respect to the level of reliability, an early GEBV is comparable to the breeding value of a boar based on six crossbred progeny tested in the testing station. Apart from this increase in reliability the GEBV allows us to select within full-sib groups based on an individual breeding value for each member of the full-sib group. Figure 2 shows that clear differences between and within full-sib groups can be observed with GEBV. AI stations thus can use GEBV to decide which young boar to purchase and breeders can use GEBV to select the best females for their breeding stock.

Official genomic evaluation for pigs in Bavaria was introduced in May 2016 for the Piétrain breed and in December 2016 for German Landrace. Since then, GEBV are calculated for all individuals of the breeding populations on a weekly basis.

Conclusions

The public research allows Bavarian farmers to compete in genetic progress and to apply state of the art methods although the majority of herd book and AI-organizations are way too small to run meaningful research units. Until now the Bavarian approach has proven to be resilient against purely market driven breeding programs. The availability of publically financed research allows also for the consideration of public goods in the breeding process. For example, meat quality traits in pigs have been considered since 1986, longevity, udder health and calving ease in cattle since 2002 and recently strong efforts have been made to prevent dehorning of cattle and tail-biting in pigs by means of animal breeding.

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NOTES



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Uncovering causal relationships between feather pecking and related behaviours using structural equation models (SEM)

Abstract

Feather pecking is a serious problem in poultry production. It causes high economical losses and suffering in the affected birds, especially in countries where beak trimming is prohibited. In spite of intensive research, the causes of this damaging behaviour are not fully understood. Most approaches to solve the problem were focused on nutrition and management. All measures have shown only attenuating or no effects on feather pecking. Genetic studies revealed sufficient genetic variation for selection against the damaging behaviour. However, feather pecking is a complex behaviour and more detailed information on the genetic background of the motivation is required to successfully implement this trait in breeding programmes. The prevailing hypothesis explains feather pecking as misdirected foraging behaviour, but other motivations, such as feather eating, aggression, fear and general locomotor activity may be involved. The interrelationships between the above mentioned behaviours have been studied using more than 900 birds of a F2-cross of two lines which have been selected for high and low feather pecking. Heritability, genetic and phenotypic correlations between the traits were estimated using standard statistical models. In addition, structural equation models (SEM) were applied to estimate causal relationships between feather pecking and other traits. Genetic correlation and Lambda coefficients as parameter of the causal link, showed a strong causal effect of feather eating and feather pecking. This supports the hypothesis that feather eating represents a primary cause of feather pecking. There was a substantial causal influence of aggression and general locomotor activity on feather pecking. Open-field activity (fear) and foraging in contrast did not show clear effects on feather pecking.

Keywords

Laying hens, feather pecking, aggression, fear, activity, exploration, genetics

Introduction

Welfare has become a major issue in poultry production in industrialised countries. Intensive management systems and selection for production traits are assumed to compromise animal welfare. Examples for the antagonism of performance and welfare-related traits are leg weakness, ascites, sudden death in fast growing broilers (Bessei and Gerken, 2006) and bone breakage in laying hens (Moinard et al., 1998). The discussion has gained momentum through the development of genomic tools which, when applied in commercial breeding, will further increase performance. In the past high performance has been considered as an indirect criterion of welfare. There is however, increasing awareness of the fact that selection for growth or laying rate may lead to welfare problems, and the responsibility of scientists for the wellbeing of farm animals has been expressed in particular by the Precision Animal Breeding framework (Flint and Woolliams, 2007) and the SEFABAR Project ("Sustainable European Farm Animal Breeding and Reproduction", SEFABAR, 2003). Economic and welfare-related criteria are not necessarily in contrast. Damaging feather pecking and cannibalism for instance, represent severe welfare problems in poultry and have important economic implications. The problem will become more important when beak trimming is banned for welfare reason. Recent experiments with intact-beak birds have shown that management procedures may attenuate feather pecking but do not prevent it. Since feather pecking has a strong genetic component, methods are sought to include this behaviour in selection programmes. Understanding the causes of feather pecking is a prerequisite to solve the problem. In the following we present quantitative genetic analyses of feather pecking and causal relationships between feather pecking and selected behavioural criteria

using structural equation models as introduced by Gianola and Soerensen (2004).

State of information and experimental approach

Damaging feather pecking is a complex behaviour. Despite extensive research during the last decades the underlying causes are not known. There exist however various hypotheses in its motivation. The most widespread theory relates feather pecking with feeding and feed searching (foraging) (Blokhuys, 1986; Huber-Eicher and Wechsler, 1998). According to the foraging theory it is assumed that foraging behaviour is redirected towards the feathers of group mates. The foraging theory however, has failed to explain feather pecking in various studies (Hocking et al., 2004; Newberry et al., 2007). Bessei and Kjaer (2015) proposed feather eating as primary underlying motivation. Other motivations for feather pecking which are under discussion are aggression (Bessei et al., 2013), fear (Rodenburg et al., 2010) and spontaneous locomotor activity (Kjaer, 2009). The assumption that feather pecking depends on dust-bathing (Vestergaard and Lisborg, 1993) has not been confirmed in later experiments and is not further considered.

Assumptions on the above mentioned association of feather pecking and other behaviours are mainly based on the comparison of group means and on conventional correlation estimates which do not reveal causal relationships. There exist however, methods such as Structural Equation Models (SEM), to estimate causal effects among traits (Gianola and Soerensen, 2004). Observations of feather pecking (FP), feather eating (FE), aggressive pecking (AP), open-field activity (fear) (OFA), general locomotor activity (GLA) and foraging (FOR) have been observed in a large

F2-cross population (> 900 birds) originating from two lines selected for high and low feather pecking (Figure 1). standard multi-trait models (SMS) and structural equation models (SEM) have been used to estimate genetic parameters and to discover causal relationships between the above mentioned behaviours. The application of SEM requires precise a priori definitions of the hypotheses. Therefore, sets of three behaviours each were used. Only behaviours with assumed causal relationships have been chosen.

Feather pecking, feather eating and aggressive pecking

Previous studies have shown that feather pecking and feather eating are closely related. Birds with a high propensity of severe feather pecking of pen mates were found to eat more feathers from plastic dishes when kept individually (McKeegan and Savory, 2001). Laying hens of a high feather pecking line preferred feathers vs. wood shavings in choice experiments and ate more feathers from a plastic dish than hens from a low feather pecking line (Harlander-Matauschek and Häusler, 2009). On the basis of these results we hypothesized that feather eating is the primary motivation which influence feather pecking. It is generally acknowledged that aggressive pecking and feather pecking clearly differ in their phenotypic pattern and are driven by different motivations (Savory, 1995; Bilezik and Keeling, 1999). Aggressive behaviour has proved to be heritable in quantitative genetic studies and selection experiments (Craig et al., 1965).

Results have been reported in detail by Bennewitz et al. (2014) and Grams et al. (2014). The heritability estimates for FP varied between 0.11 and 0.20, depending



Figure 1: Feather pecking as recorded in direct observation (a), feather eating measured in a feather eating test (b), aggression recorded by direct observation (c), general locomotor activity measured by RFID techniques and exploration (d) open-field activity in the open-field test (e) recorded by video observations

on the statistical model. FE showed considerably higher heritabilities (0.36 – 0.57). There were very high genetic correlations of FP with FE and AP. All phenotypic correlations were positive but on a lower level than the genetic correlations. On the basis of previous information we hypothesised that FE influences FP and AP, and that AP influences FP. The estimated causal effect of FE on FP, $\lambda_{FP,FE}$, was 5.94 (figure 2). For the influence of feather eating on aggressive pecking $\lambda_{AP,FE}$ and of aggressive pecking on feather pecking $\lambda_{FP,AP}$, the λ -values were positive (0.11 and 0.23) but considerably lower than for the effect of feather eating on feather pecking. The results confirmed our hypothesis that FE is the primary motivation for FP. The effect of FE on AP was also low. The high genetic correlation between feather pecking and aggressive pecking and the positive $\lambda_{FP,AP}$ show that the influence of aggression has been underestimated in the past. Though aggression and feather pecking are different in their motoric pattern and the underlying motivation, aggression may reinforce the expression of FP.

Feather pecking, feather eating and general locomotor activity

Locomotor activity is usually activated through numerous different motivations, such as exploration, aggression, egg production and flight. As mentioned below, locomotor activity can be inhibited by fear. Under practical conditions it is not possible to determine the underlying motivation.

There exist individual differences in the level of locomotor activity of animals which are independent of particular motivations. This “General Locomotor Activity” (GLA) is considered the result of a genetically determined spontaneous activity. Heritability estimates for GLA have been reported by McClearn (1961) in mice and Jezierski and Bessei (1978). Kjaer (2009) selected chicken lines divergently for high and low GLA

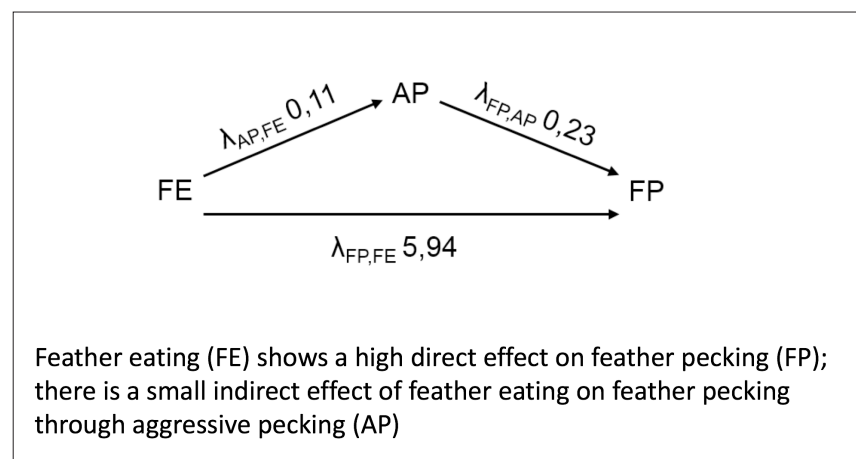


Figure 2 Causal relationships between feather eating (FE), feather pecking (FP) and aggressive pecking (AP) in a F2-cross of HFP and LFP lines (Grams et al., 2014) The effects were estimated using Structured Equation Models

using the same recording system. He estimated heritability coefficients between 0.20 and 0.30 (pers. communication). The author hypothesised that feather pecking may be the result of a hyperactivity disorder. This hypothesis was supported by physiological studies, which showed that both, feather pecking and hyperactivity are influenced by the dopamine system (Kjaer et al., 2004; van Hierden et al., 2005). Long-time recording of locomotor activity in pullets of the above mentioned F2-cross were used to test this hypothesis (Lutz et al., 2016). At 18 weeks of age locomotor activity was recorded during 9 consecutive days using RFID techniques. The heritability of GLA was 0.29 and both, phenotypic and genetic correlations with FP were positive (0.16 and 0.47). Regarding the causal associations between the traits we hypothesized that GLA influences FP and that FE influences FP and GLA. The results from SEM are shown in figure 3. Structure coefficients revealed a causal influence of GLA on FP ($\lambda_{FP, GLA} = 1.20$) and of FE on FP ($\lambda = 5.71$). The effect of FE on GLA was low. The results confirm the hypothesis that GLA influences FP. However, the effect of FE as primary motivation on FP is not mediated through the GLA. The effect of GLA on FP is obviously smaller than the effect of FE on FP. Both effects seem to be independent from each other.

Feather pecking, foraging and fear

Foraging (FOR) and fear play an important role in the surviving strategies of feral animals. High fear levels inhibit exploration and, thus, exploitation of unknown feed resources. However, fear is essential for survival under high pressure of predators. Hence the foraging activity observed under natural conditions is considered a compromise of exploration and fear. Recent observations have shown that this

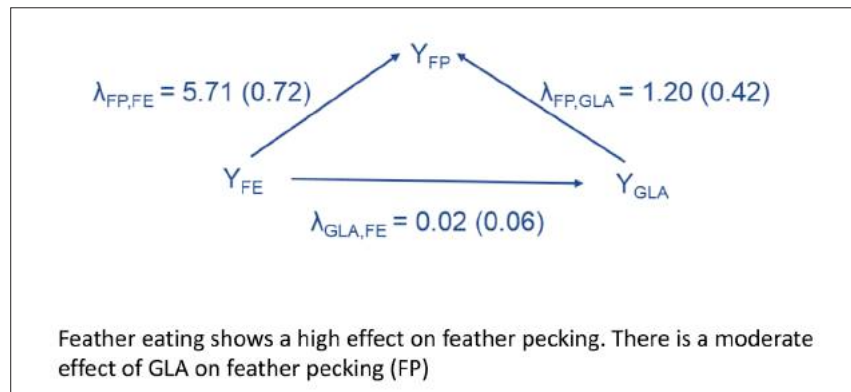


Figure 3 Causal relationships between Feather Eating (FE), Feather Pecking (FP), General Locomotor Activity (GLA) (Lutz et al., 2015)

mechanism still exists in the domestic fowl kept in free range. Hens showing high fear in the Tonic Immobility test spent less time in the free range (Hartcher et al., 2016). There exist numerous tests to assess fear in animals. The open-field test has frequently been used in laboratory animals and in the domestic fowl. Exposing the animals to the open-field situation induces immobility as initial fear response, which is followed by locomotor activity. High open-field activity (OFA) indicates low level of fear and is considered as exploratory activity (Jones, 1989). The open-field test was considered suitable for the present study because of its implication on exploratory behaviour. Moreover, high OFA in young chicks was reported to be predictive for low incidence of feather pecking (Rodenburg et

al., 2004). Data of FP, OFA and FOR of the F2-cross of the lines selected for high and low feather pecking have therefore been analysed using SEM. OFA was recorded as number of steps in a 3 minutes test at 7 and 8 days of age. FOR was defined as time spent walking and litter pecking in the home pen during a 20 minutes observation period at 3 and 4 weeks of age. It was hypothesised that OFA and FOR influence FP, and FOR influence OFA. The heritability of FOR was zero. Therefore no genetic correlations could be estimated for this trait. The heritability of OFA was 0.21. The phenotypic correlation between FP, OFA and FOR as well as the genetic correlation between FP and OFA were close to zero (Grams et al., 2014). All λ -coefficients were close to zero (figure 4).

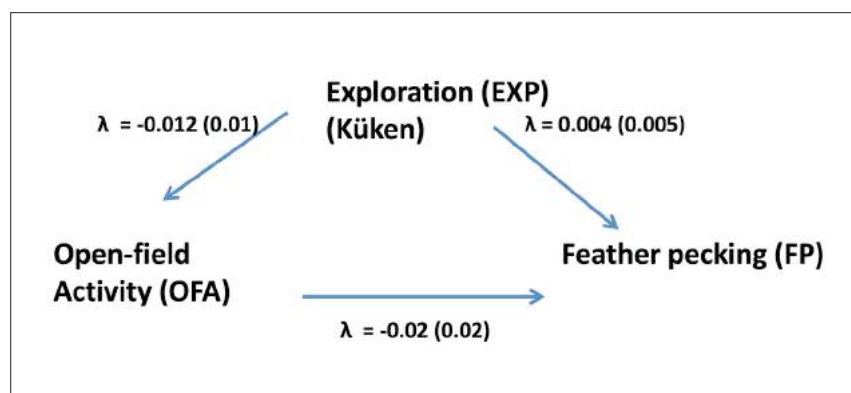


Figure 4 Causal relationships between feather pecking (FP), exploration (EXP) and open-field activity (OFA) in a F2-cross of HFP and LFP lines (Bessei et al., in prep.)

The results show that the assumed role of foraging and fear on feather pecking as influencing factors for feather pecking have been over-estimated. In contrast to the prevailing hypotheses neither FOR nor OFA showed a relevant causal effect on FP.

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Selection on beak shape to reduce feather pecking in laying hens

Abstract

Feather pecking and mortality due to cannibalism in laying hens are of economic and welfare concern in all housing systems. Traditional family selection on the basis of liveability and feather condition in group cages has reduced, but not eliminated this misbehaviour. Actually, the change to non-cage housing systems and the ban on beak treatment in several countries have increased the risk of feather pecking for egg producers. Primary breeders have tried to find novel selection criteria which can be determined under commercial conditions on a large number of birds. Lohmann Tierzucht started several years ago to measure beak shape in pedigreed hens of different lines and estimated genetic correlations with feather cover. In group cages, birds with shorter and blunt beaks tended to have better feather quality and lower mortality. However, the correlations vary considerably between lines ($r_g = -0.30$ to $+0.05$ for feather quality and -0.08 to $+0.19$ for mortality). We conclude from our results that individual selection for blunt beaks in addition to family selection for intact feather cover and liveability may accelerate the reduction of feather pecking and cannibalism, while efforts continue to optimize management practices.

Keywords

laying hens, animal welfare, feather pecking, beak shape

Introduction

During recent decades, the social behaviour and welfare of laying hens have received more attention in laying hen breeding programs. However, the great

variability of behaviours makes it difficult to define what is disturbed behaviour and negatively affecting the wellbeing of other birds in a group. Only damaging behaviours such as feather pecking and

cannibalism are unanimously recognized as disturbed behaviour (Bessei, 2016). Feather pecking is of special concern, and this welfare issue is getting more attention since beak treatment will be ban-

ned in many countries in the near future (Rodenburg et al., 2013).

Different theories have been proposed to explain the behavioural cause of feather pecking. The most commonly accepted one is based on the two main components of foraging behaviour in birds: food searching (the appetitive phase) and food consumption (the consummatory phase). Keeling (2002) argued, that feeding *ad libitum* may satisfy the motivation for the consummatory phase, however the birds will still exhibit food searching behaviours, such as ground pecking and scratching, which might be redirected in severe feather pecking (Blockhuis, 1986). De Haas et al. (2014) found that the provision of pecking blocks, good quality litter and an enriched environment on commercial farms resulted in less feather damage.

Regardless what the real origin of this behaviour might be, the fact is, that feather pecking is affected by many different factors and although many of them have been identified and intensively studied, it remains largely unpredictable and difficult to control (Hartcher et al., 2016). Therefore a multifactorial approach attending to different parameters should be used to minimize its negative impact. This misbehaviour can occur in every housing system; however it is especially relevant and more variable in cage-free housing systems, due to larger groups of birds and a more complex environment. Feather pecking and cannibalism was recently reviewed in an invited lecture during the World Poultry Congress in Beijing by Bessei (2016), which is highly recommended as background for the present paper.

Daigle et al. (2015) described three different pecking behaviour levels: (i) gentle feather pecking (which does not result in

the removal of a feather); (ii) severe feather pecking (which leads to feather losses at the back, rump or tail of the victim); and (iii) aggressive pecking (the most serious type of feather pecking, usually directed at the head and often associated with a chase, standoff, or leap from the aggressor). The last two types are the most relevant from a bird welfare point of view. One of the approaches to reduce this problem is to select directly or indirectly against this antagonistic behaviour.

Feather eating has been associated with feather pecking (Haarlander-Matauscheck and Häusler, 2008; Bennewitz et al., 2014). It has also been suggested that there may be “primary feather-peckers” in a flock with a genetic predisposition to feather pecking due to an increased appetite for feathers and fibre as a consequence of their intestinal microbiota (Meyer et al., 2013; Bessei and Kjaer, 2015). Therefore, it could be used as an indirect trait to select against feather pecking. Hen specific data on “feather eating” was recorded by McKeegan and Savory (2001) and related to feather pecking. In their study, peckers ate, picked up and manipulated feathers significantly more often than non-peckers. However, in commercial group housing, Riber and Hinrichsen (2016) found no correlation between the prevalence of poor plumage condition and the prevalence of droppings with feather content. These authors leave the question unanswered from where feathers are selected for ingestion. Remaining feathers in droppings could be the result of eaten feathers which were picked from the floor litter, plucked directly from other hens or dislodged during preening of own feathers. Thus, data on feathers found in droppings is not a reliable indicator for feather pecking and therefore does not seem useful as a selection trait to improve plumage condition and reduce feather pecking.

Traits related to the incidence of feather pecking such as plumage condition or mortality due to cannibalism recorded from relatives (full and half sibs) in group cages, not only housed in the breeding farms, but also under field conditions in commercial farms has been already included into the selection index of Lohmann layers since decades. The heritability of plumage conditions has a moderate heritability ($h^2 = 0.15-0.30$). This selection has allowed reducing the incidence of feather pecking to a low level. However, due to the multifactorial nature of this misbehaviour, it will be difficult to eliminate it completely.

Bennewitz et al. (2014) analyzed individual behaviour data from 910 hens in pens with 36 to 42 birds, collected by seven experienced observers and estimated a heritability of $h^2 = 0.10$ for feather pecking and aggressive pecking, which were positively correlated ($+0.81$). Unfortunately, the results indicated a negative genetic correlation between feather pecking and egg production ($r_g = -0.5$). The automatic direct observation and evaluation of individual bird behaviour in a small group is a technical challenge, and it is extremely time consuming to do it manually.

Kjaer (2009) observed a positive relationship between the locomotor activity of layers and their pecking behaviour from which activity characteristics such as the free range behaviour and nest acceptance were derived. Hen specific data recording on these traits supports a balanced breeding for layers with calm social behaviour and sufficient locomotor activity to move adequately in group housing.

Different management strategies can be applied to reduce the incidence of feather pecking and cannibalism. A widely



Figure 1: Measurement of beak shape in terms of the difference between upper and lower beak

used method that results in fewer non-agonistic pecks is beak treatment of chicks in the hatchery or before 10 days of age. Various publications describe the pros and cons of different beak trimming methods, however all variations are beneficial in reducing beak-inflicted feather loss and mortality from cannibalistic pecking in commercial flocks. According to Damme and Urselmans (2013) the goal of any beak treatment must be to destroy as little nerve tissue as possible and to induce quick recovery, while reducing the frequency of aggressive pecking. The treatment should minimize pain and interfere as little as possible with feed selection, feed intake and preening, but prevent the misuse of the

Table 1: Averages \pm standard deviations for beak length (mm) at 23 and 48 weeks of age and their phenotypic correlation for Lohmann Brown pure lines

Line	23 weeks	48 weeks	Correlation
A	3.5 \pm 0.7	3.5 \pm 0.6	+0.41
B	3.4 \pm 0.6	3.5 \pm 0.6	+0.42
C	3.5 \pm 0.7	3.7 \pm 0.8	+0.30
D	3.1 \pm 0.6	3.0 \pm 0.6	+0.30

beak as a pair of tweezers to pull feathers of other hens. The goal is a round beak without sharp or pointed ends, which can be obtained by adequate infrared treatment. Although this beak treatment has been proven to be very effective in preventing feather pecking, ethicists consider this practice as an amputation. Some countries have banned completely this practice and others will join this initiative soon. Banning of beak treatment is a new driving force to find solutions to reduce the incidence of feather pecking.

Pecking stones are being used in commercial farms for layers and turkeys. Abrasive materials for beak blunting may be an effective alternative to beak treatment. In a recent review, van der Linde (2016) noticed that the continuous use of pecking stones in turkeys blunt the beak, so that after 3 to 6 months around 80% of the birds showed no difference between birds with and without beak treatment. Morrissey et al. (2017) also reported that the use of cuttlebones (but not blunting boards) showed encouraging results for shortening upper mandibles in laying hens. Parallel to these

management practices, primary breeding companies could select pedigree birds on beak shape under commercial management conditions, as a long-term program with cumulative effects. To what extent genetic selection on beak shape can contribute to this goal will be discussed on the basis of experimental results from pedigree brown-egg and white-egg layers.

Measurement of beak shape

A special device was developed to measure beak shape in terms of the extension of the upper beak beyond the lower beak in pedigree hens and to evaluate the usefulness of this criterion as an additional selection criterion to reduce feather pecking. The working hypothesis was that birds with blunt beaks should be less inclined or less successful in pulling feathers from group mates.

With this equipment, the excess length of the upper beak compared to the lower beak is measured and automatically saved in a database (Fig. 1). Three generations of objective and reliable data on beak shape from Lohmann pure line layers were available for this study. Since the beaks not only continue to grow, but at the same time respond to abrasion in the given environment, the age at the time of measurement has to be standardized to compare individual hens in a group.

Table 2: Comparison of beak shape in brown-egg vs. white-egg lines at 30 weeks of age: means with coefficient of variation (CV)

Line	Lohmann Brown		Lohmann LSL	
	Mean (mm)	CV (%)	Mean (mm)	CV (%)
A	3.4	19	2.7	25
B	3.2	20	2.5	23
C	3.3	23	2.6	26
D	2.9	24	2.5	26

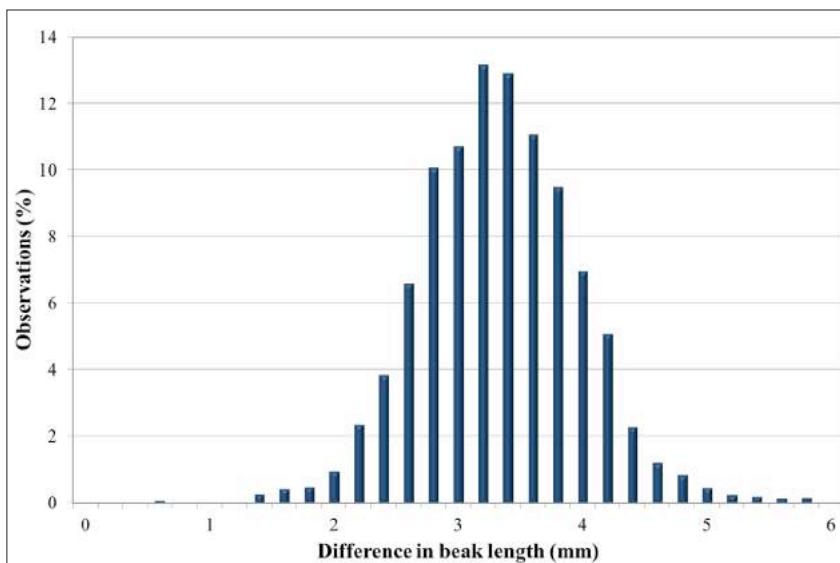


Figure 2: Distribution of the difference of beak length for Lohmann Brown - Line A.

As shown in Table 1, average excess of the upper beak was similar at different ages and for different brown egg lines. Apparently the growth of the beak tissue compensated or even exceeded the abrasion in single hen cages. The tested hens were housed in modified single bird cages that include perches, a scratching area as well as a special claw abrasion board. Drinking nipples and a commercial feed chain ensure the ad libitum supply of nutrients for the layers. Line D has a shorter beak, whereas differences between the other lines are marginal. The phenotypic correlations between measurements at 23 and

48 weeks of age within line vary between $r_p = +0.30$ and $r_p = +0.42$, indicating satisfactory repeatability of measurements at different ages. Thus, beak shape appears to be a promising trait for selection against feather pecking, as long as it is recorded at the same age.

Brown-egg and white-egg pure-lines are compared in table 2. These averages are based on about 3,000 individual hens per line, measured at about 30 weeks of age. As in table 1, the beaks in the brown-egg line D are slightly shorter. However, differences between brown and white layers

are larger. At the same age, the differences between upper and lower beak of LSL layers are 0.4 to 0.8 mm shorter compared to the lines of Lohmann Brown. Individual variation within line in difference of beak is substantial; the variation coefficient is more than 20.

The trait follows a normal distribution, as shown in Fig. 2 for one line. The results in tables 3 and 4 show to what extent this variation is genetically determined.

The heritability estimates for beak shape range from 0.13 to 0.25 and from 0.09 to 0.26 for four lines each of the Lohmann Brown and LSL breeding programs, respectively. These genetic parameters and the high variability of the trait suggest that a reduction of beak length through genetic selection should be feasible. The heritabilities are at a similar level as for traits like plumage condition or persistency of egg production. Before including a new trait in a selection index for commercial lines, genetic correlations with other important traits must be analysed and taken into account.

Genetic correlations between beak shape and the egg number at different production levels are shown in tables 3 and 4 for Lohmann Brown and LSL respectively. In three out of four brown-egg lines, hens with shorter upper beak tended to lay more eggs, whereas a longer upper beak was positively correlated with egg production in the white-egg lines (table 4). However, most of the correlations are non-significant and the differences between lines are difficult to explain.

Pedigreed hens of the commercial Lohmann Brown and LSL lines have been scored for their plumage condition since more than twenty years, based full-sibs and half-sibs housed in group cages, both

Table 3: Heritability of beak shape and genetic correlations with other traits – Lohmann Brown

Line	A	B	C	D
Length of beak (h^2)	0.21	0.25	0.13	0.16
Start of Lay	- 0.10	- 0.12	- 0.28	- 0.23
Laying peak	- 0.13	+ 0.22	- 0.22	- 0.08
Persistency	- 0.13	+ 0.44	- 0.12	- 0.08
Egg weight	+ 0.10	- 0.04	+ 0.11	+ 0.14
Shell strength	+ 0.03	+ 0.12	+ 0.19	+ 0.11
Body weight	+ 0.21	+ 0.11	+ 0.03	+ 0.23
Feed Intake	+ 0.16	- 0.02	+ 0.15	+ 0.18

Table 4: Heritability of beak shape and genetic correlations with other traits – Lohmann LSL

Line	A	B	C	D
Length of beak (h^2)	0.21	0.26	0.09	0.12
Start of Lay	-0.19	-0.19	-0.09	+0.11
Laying peak	-0.08	+0.09	+0.45	+0.10
Persistency	+0.12	+0.03	+0.22	-0.01
Egg weight	+0.06	+0.24	+0.15	+0.16
Shell strength	+0.18	+0.04	-0.12	-0.25
Body weight	+0.21	+0.46	+0.20	+0.08
Feed Intake	+0.13	+0.34	+0.26	+0.03

in the breeding farms as well as in commercial farms under field conditions.

In the field tests, the layers are scored for their plumage condition at about 40 and 75 weeks of age. Families with intact plumage are getting the top score 9, families with damaged feathering are downgraded according to the amount of feather loss. Based on this information, genetic correlations were estimated between beak length and plumage condition and mortality. As shown in table 5, the correlation between mortality and beak length is mostly a positive, the correlation between beak length and plumage condition mostly negative. Although the standard errors of the estimates are high, birds with shorter beaks tend to have lower mortality and better plumage condition, which supports our initial working hypothesis, especially in the white egg layers (Lohmann LSL).

Discussion

For students of evolution, the different beak shapes of finches on the Galapagos Islands are a familiar model to support Darwin's theory of evolution in response to a changing environment. Weiner (1984) quoted more recent observations on these finches as evidence that evolution continues and is measurable in terms of changing bird populations in response to "dry" and "wet" seasons. The beak of chickens evolved as a useful tool to search for food in a free range environment, and this tool is misused by some birds as a "weapon" for aggressive pecking and/or cannibalism. Beak treatment of chicks at an early age had been used successfully in the poultry industry as a very effective means to minimize the risk of feather pecking and related mortality. The ban on beak treatment forces primary breeders to take a closer look at genetic variation between and within lines in measurable traits which are directly or indirectly

related to feather pecking and cannibalism. Measuring the shape of beaks as described in this study has the advantage that it can be applied in large numbers of birds in a non-cage environment.

Summary and Conclusions

The reduction of feather pecking of laying hens is recognized as a major challenge for egg producers and primary breeders of laying hens due to its negative association with the wellbeing of the birds. To reduce the incidence, geneticists in the past have relied mainly on family selection for liveability and feather condition in group cages. Beak shape, defined as the excess in length of the upper beak over the lower beak, was measured in pedigreed hens over a period of three generation to estimate genetic parameters, which are reported here. The results suggest that including beak shape as an indicator of feather pecking behaviour in a multi-trait index may accelerate the desired reduction of feather pecking and cannibalism in flocks of laying hens which are not beak treated. However, family selection for intact feather cover and liveability should continue and management practices have to be further improved.

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Table 5: Genetic correlations between beak shape and plumage condition and mortality in group cages in commercial farms under field conditions

	Lohmann LSL		Lohmann Brown	
	Male line	Female line	Male line	Female line
Mortality	+ 0.05	+ 0.19	- 0.08	+ 0.11
Plumage condition	- 0.30	- 0.20	+ 0.05	- 0.05

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Double-yolk eggs in commercial laying hens and parent flocks

Abstract

Mature hens typically ovulate in precise daily cycles, adding albumen and shell within 24 hours and depositing the egg before the next ovulation. As deviation from this precision production of eggs, two or more ovulations may happen within a few hours, and then two or more yolks are found in one shell. Double-yolk (DY) eggs are mainly found and are easy to recognize in young flocks, because they are much larger than single-yolk eggs of the same flock. Researchers have studied genetic differences and environmental effects on the frequency of DY eggs. Genetic parameters indicate that an increase by selection would be possible, but may be at the expense of persistency. Rapid increase in day length at the time of sexual maturity and transfer from rearing to laying facilities contributes to more DY eggs in young flocks. Recent data suggest that the frequency of DY eggs in egg-type chickens has not increased since the early 1980s. In broiler and layer breeder flocks, appropriate lighting programs and controlled feeding are used to optimize the age at sexual maturity and to maximize the number of hatching eggs. To develop a niche market for DY eggs as a premium price specialty would require a pool of different age flocks and promotion throughout the year.

Keywords

chickens, egg production, double-yolk eggs, age, lighting

Introduction

During evolutionary times, the size and composition of eggs in avian species must have been optimized for efficient reproduction, and the eggs within a clutch are similar in size. When humans started to breed chickens for egg production, they

discovered that egg weight varies with season, age of the hens and nutrition. After Mendel's laws had been re-discovered, geneticists took a closer look at the variation among laying hens in egg weight. Egg weight seemed of special interest, because it varies a lot, consumers are willing

to pay a higher price for larger eggs, and the egg weight distribution during a full laying year can be changed by selection.

Pullet growers apply controlled lighting and nutrition to optimize the age at sexual maturity and early egg size; egg producers

use phase feeding to maximize egg income over feed cost. Good management practice should assure that the hens eat enough, continue to gain weight and increase early egg weight quickly, because small eggs are difficult to sell at production cost. Physiologists have analyzed and described the process of egg formation from maturing ova to ovulation and oviposition. As explained by Kaspers (2016), follicles with different size develop with the onset of sexual maturity, resulting in a hierarchy of follicles. Normally only one pre-ovulatory follicle develops per day, but occasionally two ova are released almost at the same time, within a few hours, resulting in eggs with two yolks. These double-yolk (DY) eggs have attracted the interest of researchers and egg producers.

Our interest in the subject goes back to the late 1960s, when the senior author reviewed the literature and estimated genetic parameters in commercial White Leghorn lines (Flock, 1984). Renewed interest was triggered by the recent publication "EggSignals" (Simons, 2017), which contains a lot of information on DY eggs, but little about genetic aspects. With courtesy of Roodbont Agricultural Publishers, two pictures of DY eggs are shown in Fig. 1 and Fig. 2:

With reference to earlier publications, we will discuss the following questions:

- (1) Are today's commercial laying hens with close to 100% peak production laying more DY eggs than reported in previous studies?
- (2) To what extent is the incidence of DY eggs influenced by genetic disposition and/or management?
- (3) Is the incidence of DY eggs a useful indicator of good management practice?
- (4) How are commercial broiler breeders and hatching egg producers controlling erratic oviposition in parent flocks today?

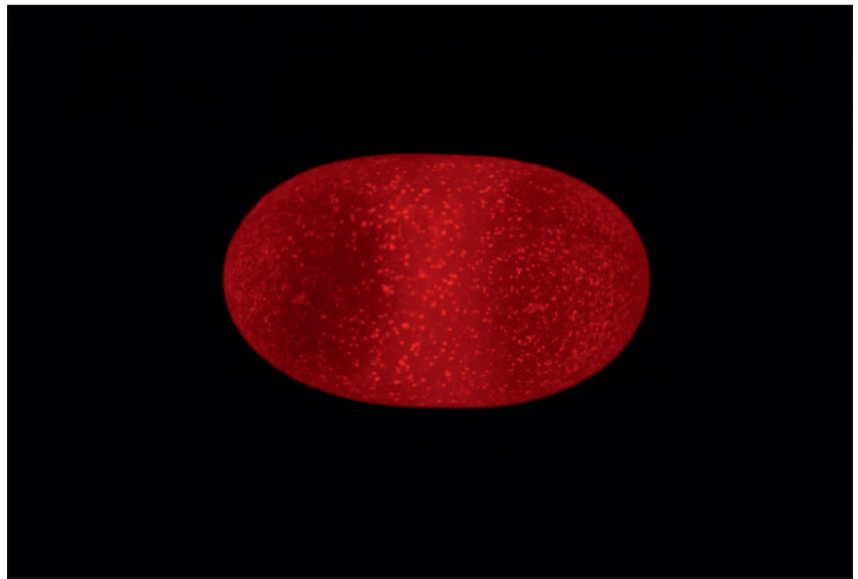


Figure 1: Double-yolk egg under a candling light (Source: Roodbont publishers B.V., Egg Signals)

Results of a selection experiment with White Leghorns in California

Poultry researchers at the University of California started selection experiments with chickens about a century ago to demonstrate that Mendel's laws of inheritance also applied to quantitative traits like egg production. These experiments were a useful tool to teach students the principles of ge-

netics. In 1964, a selection experiment was started to find out whether the incidence of DY eggs in White Leghorns would also respond to selection. Abplanalp et al. (1977) reviewed the literature on DY egg production and documented the progress in 10 generations, from about 2% to 30% in two closed lines, while a control line remained essentially unchanged (Fig. 3).



Figure 2: Boiled double-yolk eggs (Source: Roodbont publishers B.V., Egg Signals)

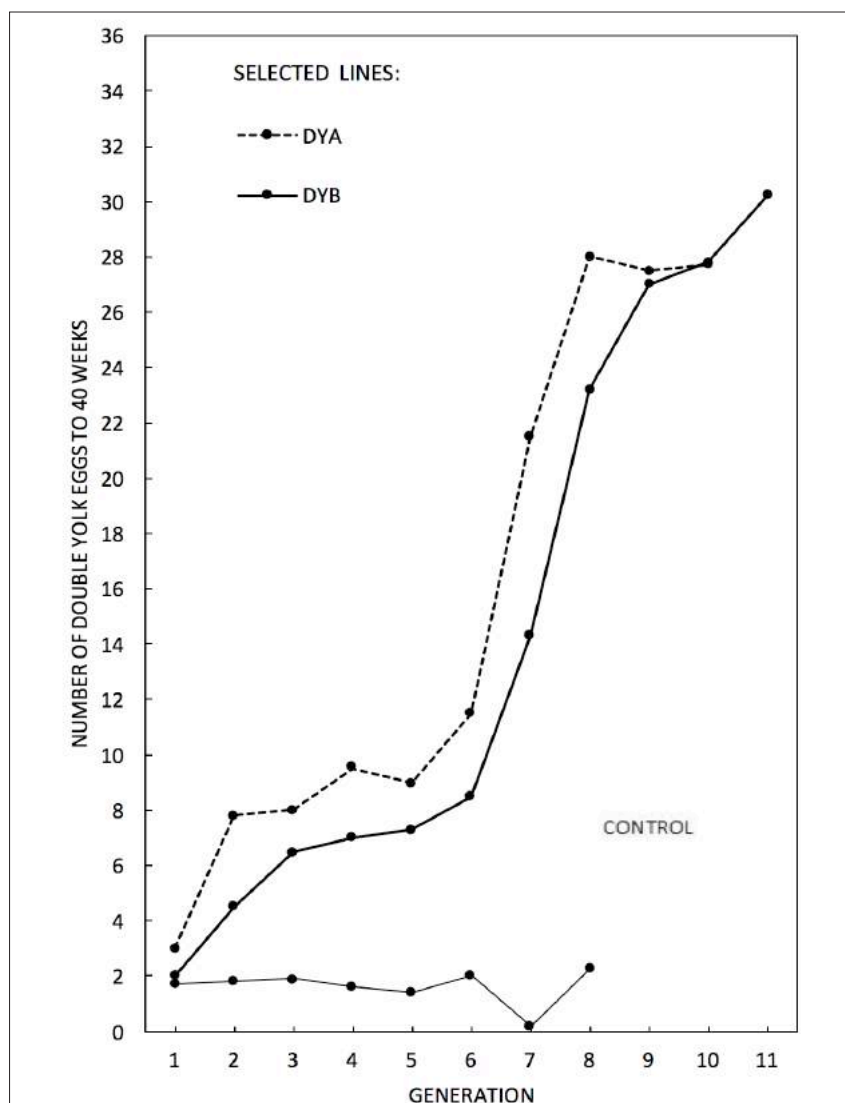


Figure 3: Incidence of double-yolk eggs in two lines of White Leghorn hens selected for incidence of double-yolk eggs (Abplanalp et al., 1977)

In the final generation, the yolk production in single and double eggs were recorded for a cross of the two selected lines from to 32 weeks of age, and a replicate of this cross was tested at the Spelderholt Institute in The Netherlands to 55 weeks. Results of this test are shown in Fig. 4:

Variation among laying strains and seasonal effects in Florida tests

Christmas and Harms (1982) analyzed differences between 16 commercial White Leghorn strains, based on repeated entries

in seven trials at the Florida Strain Evaluation Center. All birds were grown under natural daylight conditions and subjected to 14-15 hours light in the laying house after transfer at 150 days of age. The period from 150 days of age to age at 50% production varied from 27 to 35 days. Each test lasted 400 days, and therefore the age at 50% in subsequent trials also changed. The data were analyzed as an incomplete block design, to estimate the differences due to strain and season as independent effects.

On average, these 16 strains reached 50%

production at an age of 163 ± 3.6 days and laid 1.77 ± 0.62 % DY eggs. The differences between strains were statistically significant (range from 1.12 to 3.52 %). Seasonal effects showed a consistently higher frequency of DY eggs when test flocks reached sexual maturity between March and July (2.0-3.0 %), compared to flocks reaching sexual maturity between September and January (0.7-1.8 %), in eggs and age at 50 % production during the first 27 to 35 days in the laying house.

Genetic parameters in commercial White Leghorn lines

After Abplanalp et al. (1977) had shown in a long-term selection experiment that the incidence of DY eggs responded to single-trait selection in two experimental White Leghorn lines, we verified these findings by estimating genetic parameters in a large population of commercial White Leghorns (LSL). A total of about 12,000 pedigree chicks of three pure lines and two single crosses were hatched over a period of 5 weeks (late March to early May 1982), reared under floor conditions in windowless houses and transferred to the test farm within two weeks, when the oldest birds were about 20 weeks of age and began to lay. At transfer, the daily light hours were increased from 8 to 12 hours, and the feed was changed from developer to layer mash.

Under these conditions, we could estimate the combined effects of light stimulation and change to layer feed as "age at transfer" and estimate genetic parameters with a mixed model. Across all genetic groups, the percentage DY eggs increased from 1.64 to 2.49% during the first 8 weeks and from 0.77 to 1.10% during the first 24 weeks of production in response to 10 days earlier transfer to the laying house. Pullets from the first hatch were housed in

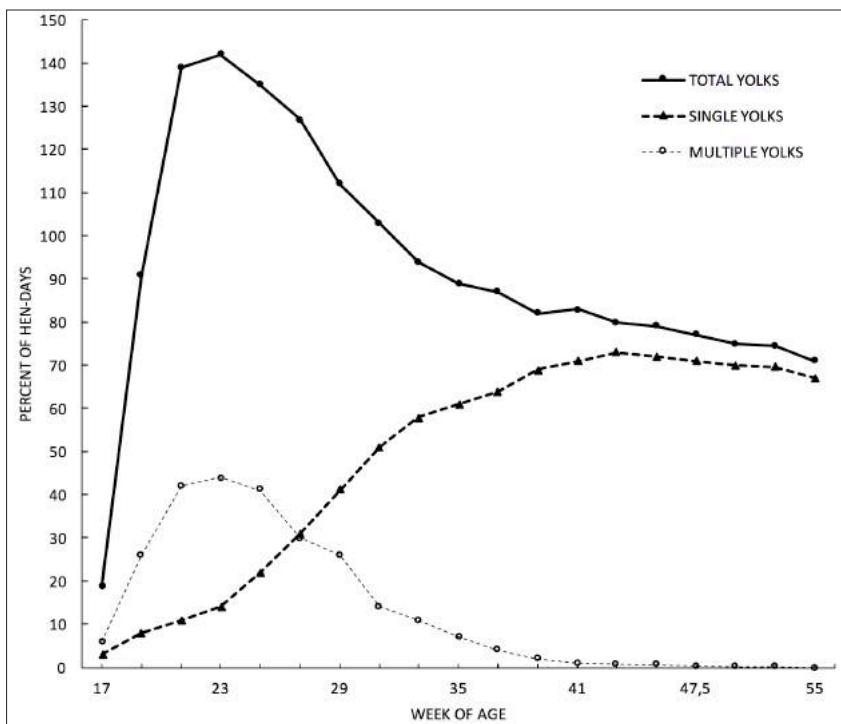


Figure 4: Hen-day yolk production in an F1 cross of two White Leghorn lines selected for DY egg production over 10 generations (Abplanalp et al., 1977).

group cages and not used to estimate genetic parameters (Flock, 1984).

From the weekly results per line and cross we calculated the "heterosis" for the incidence and weight of DY eggs, defined as excess of the cross over the parent average, as shown in Table 1:

The percentage of DY eggs (relative to the number of eggs laid per week) reached its maximum before peak production, while the number of DY eggs continued to increase with increasing rate of lay. The incidence of DY eggs was significantly higher in the crosses than in the pure-lines, and the difference between reciprocal crosses suggests that maternal effects may be important. The average weight of DY eggs increased with the age of the hens, from about 35 to 45% above single yolk eggs.

Heritabilities and genetic correlations were estimated only for hens in single cages

which had survived to 44 weeks of age. Mortality was negligible under these management conditions on the breeding farm.

Table 1: Frequency and average weight of DY eggs by age (modified from Flock, 1984)

Hen age weeks	AA		BB		AB		BA		Heterosis* (%)	
	%DY	EW	%DY	EW	%DY	EW	%DY	EW	%DY	EW
21	1.8	64.3	1.6	67.1	3.2	72.9	2.4	72.9	64.7	11.0
22	1.5	68.4	1.8	69.7	3.4	75.6	2.2	75.9	69.7	9.7
23	1.4	70.4	2.0	72.1	3.0	78.3	2.0	77.7	47.1	9.5
24	1.6	73.5	2.0	74.1	3.0	79.5	1.7	79.4	30.6	7.7
25	1.7	75.6	1.8	76.2	3.0	80.8	1.5	80.9	28.6	6.5
26	1.5	77.8	1.3	77.5	2.3	82.6	1.6	82.3	39.3	6.2
27	1.4	79.8	1.2	79.0	2.2	84.3	1.4	83.6	38.5	5.7
28	0.9	80.9	1.0	81.3	1.4	80.6	0.8	86.0	15.8	2.7
29	1.1	84.0	0.7	83.6	1.3	88.2	0.9	88.6	22.2	5.5
30	0.7	87.0	0.7	85.8	1.4	87.7	0.8	87.2	57.1	1.2
31	0.6	87.4	0.9	85.8	1.3	90.1	0.8	89.4	40.0	3.6
32	0.4	88.9	0.6	86.7	1.4	90.0	0.9	91.0	30.0	3.1
21-32	1.1	78.2	1.3	78.2	2.2	82.6	1.4	82.9	40.9	5.9

* excess of reciprocal crosses over parent lines

The number of DY eggs to 44 weeks of age had a higher heritability (0.26 ± 0.04) than the total number of eggs (0.12 ± 0.02), and the pooled genetic correlation was negative ($r_g = -0.18$). Correlations between pure-line and cross-line sire progeny groups were higher for the number of DY eggs (0.54 to 0.61) than for the total number of eggs to 44 weeks of age (0.32 to 0.44).

The negative correlation between DY egg production to 44 weeks of age and full year production is confirmed by the results in table 2:

The top 10 DY layers had a significantly higher ovulation rate during the first 16 weeks of production, but then lost in terms of cumulative egg mass due to inferior persistency. These results suggested that selection should focus on persistency of egg production and shell quality rather than more DY eggs during early production.

Table 2: Full-year egg mass production per hen housed (HH) of the top 10 hens with the highest number of DY eggs, compared to the average (modified from Flock, 1984)

4-week period	Average cross AB		Top 10 DY laying hens		Egg mass kg/HH
	Production %/HH	Egg mass kg/HH	Production %/HH	Ovulation %/HH	
1	81.4	1.118	72.9	91.8	1.132
2	92.1	1.396	82.9	109.6	1.459
3	92.5	1.494	81.8	97.1	1.445
4	93.2	1.558	83.6	96.8	1.507
5	92.1	1.573	86.8	91.8	1.525
6	91.4	1.589	87.8	89.6	1.487
7	90.0	1.592	81.7	81.7	1.446
8	86.8	1.561	76.9	76.9	1.380
9	86.1	1.565	75.0	75.0	1.363
10	81.8	1.503	70.0	70.0	1.286
11	79.6	1.475	67.1	67.1	1.243
12	75.0	1.400	63.2	63.2	1.180
Average	86.8	–	77.5	84.2	–
Total	–	17.824	–	–	16.454

Recent results from commercial White Leghorns in Japan

Since our studies in the 1970s and 1980s, genetic improvement of commercial lines continued, while the structure of primary breeding companies changed and farms with pedigreed stock were located on different continents to assure continuous supply of parent stock to customers around the world. With progressing globalization of the egg industry, the H&N breeding program was relocated from the USA to Germany, and brown-egg breeding has become as important as white-egg breeding. For the present study, we can therefore refer to recent data from white-egg layers in Japan and brown-egg layers in Spain.

Japan is perhaps the best country in the world to study egg marketing and to discuss breeding goals with business partners focused on efficient production of top quality eggs. As a common management tool, daily results are recorded and com-

Table 3: Production and egg quality of a recent “Julia Lite” parent flock in Japan

Week of age	Female Livability	% H.D. Prod.	Settable eggs %	Undergrade eggs %	Cracked eggs %	Misshapen eggs %	XL eggs %	DY eggs per 10.000 hens
20	99.9	40.4	0.0	93.9	0.2	0.4	0.9	35
21	99.8	70.8	0.0	95.2	0.2	0.2	1.5	103
22	99.8	87.8	0.0	95.0	0.2	0.2	1.8	157
23	99.8	93.5	0.0	95.0	0.2	0.3	2.1	200
24	99.7	95.0	0.0	95.6	0.1	0.3	1.7	166
25	99.6	95.8	56.8	38.9	0.2	1.5	1.5	142
26	99.6	96.1	80.7	16.4	0.1	0.6	1.2	119
27	99.5	97.2	84.6	12.8	0.1	0.5	1.0	101
28	99.4	97.1	90.8	6.7	0.2	0.7	0.8	75
29	99.4	96.9	93.1	4.3	0.1	1.0	0.6	62
30	99.3	97.4	94.5	3.2	0.3	0.8	0.5	45
31	99.2	97.3	95.4	2.3	0.1	1.0	0.5	45
32	99.2	96.5	95.1	1.4	0.1	2.2	0.5	46
20-32								1,295

pared with previous flocks or standards in management guides. Table 3 shows an extract from a much larger document with detailed results of a recent parent flock (courtesy of Ghen Corporation):

No eggs were recorded before 20 weeks of age, and all eggs were graded on size. Only normal eggs between 52 to 72 g are used as hatching eggs to assure uniform chick quality. DY eggs are sorted out mechanically by weight and/or by subjective inspection of eggs from young flocks. Since few DY eggs will be laid after 34 weeks of age, this parent flock will average less than 0.14 DY eggs per hen housed.

The incidence of DY eggs varies more on commercial farms than on parent farms with top management. As an example, the following Table 4 shows an unusually high percentage of DY eggs in a flock of 6,326 "Julia Lite" which hatched in February 2016 and was reared under conditions of open housing during a period of increasing natural daylight. The flock lacked uniformity when it reached sexual maturity at an average age of 145 days of age, started with low average egg weight and reached peak daily egg mass production close to 50 weeks of age.

Recent results from commercial brown-egg lines in Spain

We are not aware of any published data on the frequency of DY eggs in brown-egg lines and the composition of DY eggs compared to single yolk (SY) eggs. We have therefore summarized daily records from two pedigreed commercial brown-egg lines currently on test in single and group cages in Spain. The weekly incidence is shown in table 5:

The pullets had been reared under environment-controlled conditions and were 27

Table 4: High incidence of DY eggs in a flock of "Julia Lite" White Leghorns, hatched 20-02-2016 and reared with increasing natural day-light (Source of data: Farm Kitagawa yohkei; courtesy of Ghen Corporation, Japan)

Age weeks	Livability %	Hen-day Prod. %	Egg Wt. g	Egg Mass g/hen-day	DY eggs %	DY eggs No.
19	99.9	4.3	42.0	1.8	4.09	78
20	99.8	23.8	43.7	10.4	3.61	381
21	99.6	51.7	46.6	24.1	3.41	778
22	99.6	71.4	48.6	34.7	3.41	1072
23	99.5	80.4	50.8	40.9	2.75	974
24	99.3	86.8	52.1	45.2	2.45	935
25	98.9	87.2	52.5	45.8	1.62	619
26	98.8	92.4	53.6	49.5	1.27	517
27	98.6	88.3	54.3	48.0	1.14	441
28	98.5	92.2	55.3	51.0	1.14	458
29	98.4	89.7	56.2	50.4	0.92	360
30	98.3	92.3	56.9	52.5	0.89	357
31-35	97.8	93.7	58.9	54.9	0.57	1160
36-40	97.4	94.0	61.7	58.0	0.27	545
41-49	96.7	93.1	63.5	59.1	0.15	542

and 30 weeks of age when a sample of 120 DY and 120 SY eggs per line were broken out to determine the composition of DY eggs. The results are summarized in table 6:

Table 5: Incidence of double-yolk eggs in two commercial brown-egg lines

Age weeks	Total Egg No.	DY Eggs No.	%
21	51,610	607	1.18
22	68,448	697	1.02
23	83,100	678	0.82
24	88,490	580	0.66
25	89,297	676	0.76
26	89,658	573	0.64
27	89,552	529	0.59
28	89,391	437	0.49
29	90,069	400	0.44
Total	739,615	5,177	0.70

Weight and percentage of albumen can be calculated as whole egg minus shell and yolk. On average, DY eggs were 37.3 % heavier than single-yolk eggs from the same group of hens, mainly due to almost double yolk weight (+92.8%). DY eggs had 12.8% higher shell weight and 19.9% higher albumen weight, but lower percentage shell and percentage albumen than single-yolk eggs.

Depending on the time laps between the first and second ovulation, the egg white in DY eggs may enclose each yolk or both yolks, and more egg white may be seen around the first yolk. Additional shell weight suggests more than 24 hours from first ovulation to oviposition, and a DY egg is often followed by a day without oviposition.

Table 6: Weight and composition of double-yolk vs. single-yolk eggs

Line (Age)		Double-yolk eggs					Single-yolk eggs				
		Egg Wt. g	Shell g	Yolk g	Shell %	Yolk %	Egg Wt. g	Shell g	Yolk g	Shell %	Yolk %
B (27 wks)	Mean	77.1	9.0	28.0	11.6	36.3	53.9	7.8	13.7	14.4	25.5
	STD	3.7	1.0	2.4	1.0	2.5	3.5	0.7	0.9	0.9	1.5
	CV (%)	4.7	11.0	8.4	9.0	7.0	6.4	8.6	6.7	6.2	5.7
	Min	69.8	7.1	23.4	9.2	30.4	47.8	6.6	12.1	12.5	22.2
	Max	84.3	11.4	33.9	14.2	44.8	62.5	9.8	15.8	16.9	31.0
A (30 wks)	Mean	82.3	9.5	28.5	11.5	34.6	62.2	8.6	15.6	13.8	25.1
	STD	7.4	1.0	2.6	0.9	2.0	4.9	0.9	1.4	1.4	2.4
	CV (%)	9.0	10.6	9.0	8.1	5.8	7.8	10.7	8.9	9.9	9.4
	Min	69.2	7.1	24.1	9.1	30.0	50.9	6.5	12.1	10.9	19.0
	Max	99.8	12.3	35.0	14.1	39.9	78.5	11.8	19.3	17.1	32.0

Controlling the incidence of DY eggs in meat-type breeders

Jaap and Muir (1968) studied non-rhythmic laying patterns in large-bodied broiler lines during the first three month of lay and found that between 9 to 20% of their ovulations resulted in defective eggs: 5.0 to 12.5 % were double-yolked, 3.7 to 7.0% soft-shelled or shell-less, and 1.6% to 6.4% were laid as two eggs within 24 hours.

Early work on DY eggs of meat-type chickens is based on data with ad libitum

feeding to 8 weeks, which used to be the standard age at which broiler lines were selected for rapid juvenile growth rate. When the intensive selection with focus on early growth rate started to show undesirable correlated effects on reproductive performance, specialized sire and dam lines were selected on an index which emphasized broiler growth rate and meat quality, while index selection of female lines was based on a combination of broiler traits and reproductive performance.

Van Middelkoop (1973) reviewed the literature on effects of the dw gene on ovulation rate and oviposition in egg-type and meat-type chickens. At the Spelderholt Institute for Poultry Research, he mated females of three heavy White Rock lines to heterozygous Dw/dw males and compared the reproductive performance of normal sized (Dw) and dwarf (dw) daughters of the same sires over a laying period of 32 weeks. As shown in the following table 5, the heavy Dw- hens produced on average 10 more yolks than their smaller dw- sisters, whereas the dwarf hens produced 10 more normal eggs.

Table 7: Influence of the dwarf gene dw on ovulation rate and production of normal eggs in three experimental White Rock lines (Source: van Middelkoop 1973)

Genotype	Number of ovulations		Number of normal eggs	
	Dw-	dw-	Dw-	dw-
Line DY	155.6	141.2	123.9	138.4
Line TE/D*	139.0	134.1	116.6	131.4
Line N	150.5	139.1	135.7	135.1
Average	148.4	138.1	125.4	135.0

* Two eggs on one day

In this experiment, the advantage of the dw- gene was most pronounced in the lines previously selected for DY eggs or two eggs a day and during the first 8 weeks of production. Differences in early egg weight were not discussed, but would be of interest for commercial hatcheries which set eggs only above a minimum weight for good chick quality and early growth rate.

Several broiler breeding companies have introduced the dw- gene in a line used as maternal grandsire of dwarf parents to reduce the feed cost per hatching egg. Mated to heavy males, they produce normal sized broilers. With sex-separate feeding and spiking the flock with young males, adequate fertility can be achieved. The argument of fewer DY eggs has never been a commercial issue in promoting dwarf breeders. Light control and modern technology to control feed intake in breeder flocks helps to reduce erratic ovulation and the incidence of non-settable eggs. Broiler breeder field data indicate that double yolk incidence is normally between 1-2%. This is within the range observed in pedigree flocks which has stayed unchanged for the last 20 years. In commercial PS flocks, incidence above 2% are related to flock management issues, typically poor uniformity through rear resulting in variable response to light stimulation and a consequent increase in incidence of double yolks (Avendano. 2017).

Discussion and Conclusions

Most of today's commercial layers will produce one normal shell egg per day and follow the diurnal pattern recently described by Kaspers (2016), provided they are properly managed during rearing and especially during the critical time of transfer to the laying house. Personal information from colleagues who are in close contact

with the egg market suggests that DY eggs are found in every young white-egg and brown-egg flock, with variable frequency, and that egg producers never complain about too many. About 2% DY eggs are common at the time when pullets reach sexual maturity, and there is no evidence that the rate has changed significantly in recent years. A higher incidence is most likely due to overstimulation, when the pullets are exposed to an abrupt increase of day length and/or light intensity at the time of transfer to the laying house, which should be avoided with good management practice.

In our earlier data (Flock, 1984), we could not test whether laying of DY eggs involves an increased risk of mortality due to prolapse or vent picking, because the hens were kept in single cages, with essentially no mortality. In case of significant mortality due to vent picking, it is recommended to take a closer look at the conditions during rearing and laying: the pullets should not be fat when they lay their first egg, and their cloaca should be out of sight for other hens while laying an egg. Mucous membranes of the uterus are everted when an egg is laid, and it may take longer to retract them after laying a DY egg. These membranes attract the attention of other hens, and this may initiate vent picking and cannibalism in the flock, especially if the light intensity is too high.

While egg producers are unlikely to complain about "too many" DY eggs, the negative correlation between DY egg production at onset of lay and persistency of production should discourage producers from overstimulating pullets at the time of transfer from rearing to laying facilities. Systematic feed formulation to support high early egg weight is a better solution to meet the demand for larger eggs in young flocks.

The incidence of DY eggs in chickens is much lower than twinning in humans, and it is to some extent heritable. The incidence in today's commercial white-egg and brown-egg layers is apparently not higher and perhaps even lower than a few decades ago, when peak production was much lower.

If primary breeders wanted to develop a line with higher incidence of DY eggs for a special niche market, they could focus on early production instead of persistency and credit these eggs with their true sales value instead of counting them only as "saleable" eggs at an age when eggs are small. Selection intensity would be high as long as the frequency of DY eggs is low.

Unfortunately, DY eggs often end up in processing plants, where their added value is not recognized; or they are sold on open markets as a seasonal specialty but are no longer available if customers ask for them again. Developing an attractive niche market for DY eggs would require coordination between enough egg producers to assure continuous supply from young flocks, to collect these eggs carefully on special flats to minimize shell damage, and - last not least - a smart marketing approach with quality assurance for consumers who are prepared to pay for the added value of these eggs.

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Making worst case scenarios real: The introduction of highly pathogenic avian influenza of subtype H5N8 led to the largest fowl plague outbreak ever recorded in Germany

Abstract

Between 8th November 2016 and 31st May 2017, more than 1,150 cases of HPAI H5N8 in wild birds and 107 out-breaks in birds kept in captivity (92 poultry holdings and 15 zoos/animal parks) were reported in Germany. This HPAI epidemic is the most severe recorded in Germany so far. In total, 29 European states have been affected. In addition to H5N8, two other HPAI subtypes have been detected in Europe, i.e. HPAI H5N5 (in seven states) and H5N6 (in Greece). The viruses were probably introduced by migratory birds, sparking an epidemic among wild birds with occasional incursions into poultry holdings, zoos and animal parks, which were usually rapidly controlled. In many cases, substantial gaps in farm biosecurity may have eased the path for virus entry. Only in the late phase of the epidemic, there was also epidemiological and molecular evidence for transmission of the infections between commercial holdings, which caused approximately 20-25 percent of the total number of outbreaks. Biosecurity measures should be checked and maintained at a high level. This includes, inter alia, wearing of stable specific protective clothing and footwear, cleaning and disinfection of equipment that has been in contact with birds and the best possible prevention of contacts between poultry and wild birds.

Keywords

Avian influenza, H5N8, fowl plague, epidemiology, Germany, biosecurity

Background

Avian Influenza, colloquially called 'bird flu', is an infectious disease in poultry caused by influenza A viruses, which are enveloped viruses with a segmented single-

stranded RNA genome. They belong to the family Orthomyxoviridae. These viruses occur in two variants (low/highly pathogenic) and different subtypes. Wild water birds (Anseriformes) as well as gulls, terns

and wader birds (Charadriiformes) are regarded as the natural reservoir for all low pathogenic avian influenza viruses (LPAIV), i.e. viruses of the subtypes H1-H16 and N1-N9. LPAIV of the subtypes H5 and H7 may

cause almost no or only mild disease in domestic poultry. Particularly in ducks and geese, the infection may be asymptomatic or lead to very mild symptoms. However, these subtypes have the capacity to evolve spontaneously into a highly pathogenic form (highly pathogenic avian influenza viruses, HPAIV), which usually happens in domestic poultry after transmission of the low pathogenic progenitors from wild birds (Jones et al., 2012). The highly pathogenic form clinically manifests in poultry as fowl plague, which causes drastic losses especially in turkeys and chickens. In ducks and geese, however, the clinical signs of an HPAIV infection may be mild and mortality can be lower than in turkey and chickens. Therefore, HPAI viruses may circulate in waterfowl undetected, whereas mortality is always very high in Galliformes (75-100%; Swayne and Suarez, 2000).

Upon exposure to a high infectious dose, usually after direct contact to infected birds, some avian influenza viruses (e.g. HPAIV H5N1 and H5N6, LPAIV H7N9 in China, of which a HPAI variant has recently been detected), can be transmitted to humans and have the potential to cause fatal human disease. Due to the segmented genome of influenza A viruses, new viruses can evolve when simultaneous infections of a single host with different influenza A viruses allow mixing (reassortment) of the genome segments. Therefore, there is a permanent risk for the generation of novel influenza viruses with pandemic potential if different influenza A virus strains co-circulate (Lam et al., 2010).

In 1996, an HPAIV of subtype H5N1 originating from geese (goose/Guangdong/96) caused outbreaks in chickens and disease in 18 humans with six fatalities. This virus subsequently evolved steadily and extremely successfully during the following

Table 1: HPAI outbreaks in poultry and number of locations of HPAI infections in wild birds in European states in 2016 and 2017

State	Poultry holdings 2016/2017	Wild birds 2016/2017	zoo 2016/2017	total 2016/2017
Austria	1/1	7/47	0/1	8/49
Belgium	0/0	0/3	0/2	0/5
Bosnia und Herzegowina	0/1	0/1	0/1	0/3
Bulgaria	8/63	1/12	0/2	9/77
Croatia	0/11	2/10	0/0	2/21
Czech Republic	0/38	0/39	0/1	0/78
Denmark	1/0	35/14	0/1	36/15
Finnland	0/0	9/6	0/0	9/6
France	84/401	3/47	1/2	88/450
Greece	0/6	1/8	0/0	1/14
Hungary	221/21	4/59	0/5	225/85
Ireland	0/0	1/9	0/0	1/9
Italy	0/15	1/4	0/0	1/19
Lithuania	0/0	0/5	0/0	0/5
FYROM	0/1	0/1	0/0	0/2
Montenegro	0/0	1/0	0/0	1/0
Netherlands	9/0	37/9	3/7	49/16
Poland	22/43	5/63	0/0	27/106
Portugal	0/0	0/1	0/0	0/1
Romania	1/44	6/87	0/2	7/133
Serbia	3/1	4/16	0/0	7/17
Slowakia	1/8	0/58	0/2	1/68
Slowenia	0/0	0/44	0/0	0/44
Spain	0/10	0/2	0/0	0/12
Sweden	2/2	10/20	0/2	12/24
Switzerland	0/0	3/84	0/0	84/3
Ukraine	1/3	0/1	0/1	1/5
United Kingdom	1/11	8/14	0/0	9/24
TOTAL	355/680	221/583	5/29	578/1.292

two decades into various phylogenetic clades, subtypes and numerous genotypes. A combination of vaccination of poultry against HPAI H5, live bird markets and the traditional way of keeping waterfowl, for example in rice fields, in contact to wild birds is a perfect source for the genesis, emergence and evolution of new HPAIV in large parts of Asia, especially in South

East Asia. Wild birds mixing with poultry may contribute to the development of new viruses by reassortment and to the intra- and intercontinental spread of newly reassorted viruses. Many of the H5-descendants caused serious outbreaks of fowl plague in poultry in South East Asia and some managed to find their way to Europe: in 2005/2006 (H5N1 clade 2.2), in

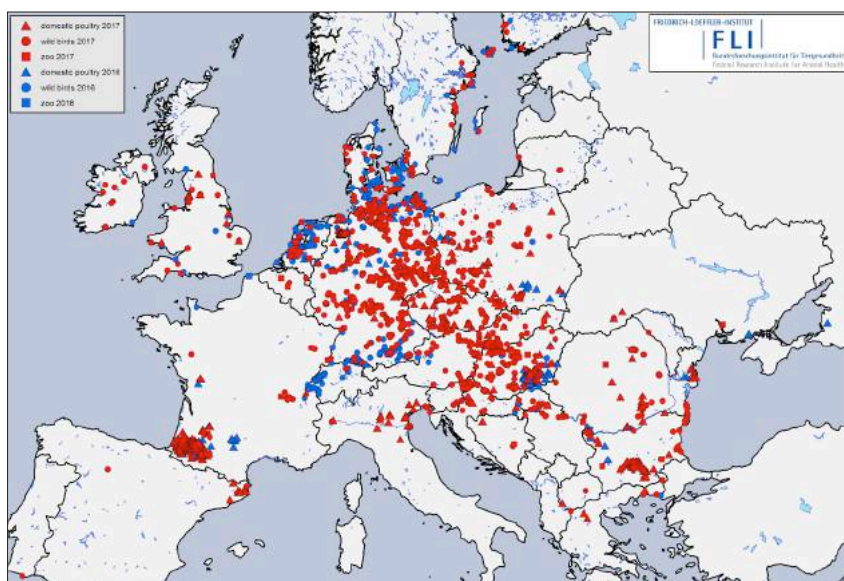


Figure 1: Confirmed cases of HPAI in Europe in 2016 (blue symbols) and 2017 (red symbols) in wild birds (circles), poultry (triangles) and in animal parks/zoological gardens (squares) registered in ADNS (Animal Disease Notification System of the European Union), Empres-i (FAO) or TSN (German Animal Disease Notification System).

2010 (H5N1 clade 2.3.2.1c) and 2014 (H5N8 clade 2.3.4.4a). This development has led to a massive increase of HPAI outbreaks worldwide since 1996 (Guan and Smith 2013; Lee et al., 2016). Some, but not all, of these HPAI H5 strains can cause clinical infections also in humans. The generation of a potentially pandemic virus that is able

to spread among humans is of worldwide concern and under careful observation.

HPAI H5N8 CLADE 2.3.4.4 B outbreaks in Europe and Germany

In September 2016, the FAO released a risk alert about the potential westward spread

of a novel HPAI H5N8 virus of clade 2.3.4.4 B, which was detected through surveillance of wild migratory birds in the Tyva Republic, Russian Federation (Sims et al., 2016). Only one month later Hungary and then also Poland notified the first cases of HPAI H5N8 clade 2.3.4.4 b in dead wild birds (a swan in Hungary and ducks as well as gulls in Poland).

A few days later, on 7 November 2016, an increased mortality of uncertain cause was first reported in tufted ducks (*Aythya fuligula*) at Lake Constance. One day later, on 8 November, HPAI H5N8 was confirmed in dead wild birds (mostly tufted ducks) at Lake Constance in Baden-Wuerttemberg, Southern Germany, as well as in tufted ducks found dead at Lake Plön in Schleswig-Holstein, Northern Germany. Simultaneously, an increased number of water birds and sea gulls were found dead at the Eastern coast of Schleswig-Holstein, around Lake Constance in Switzerland, Austria and Germany (Bavaria and Baden-Wuerttemberg) as well as in Mecklenburg-Western Pomerania, North-Eastern Germany. Genetic analysis and animal experiments showed later that there was no indication of a zoonotic potential of the H5N8 virus (EFSA, 2017) and no human infections with this virus have indeed been reported so far.

Soon, the epidemic nature of the HPAI H5N8 strain for birds became obvious as it spread across Germany affecting mainly wild waterbirds overwintering at lakes and rivers or along the coast, and scavenging birds (white-tailed sea eagles, buzzards, crows, gulls, owls, etc.) that had apparently fed on infected carcasses. The virus was isolated from at least 53 wild bird species. Besides Germany, 28 other European states have been hit by the epidemic (Table 1, Figure 1). A severe outbreak series occurred

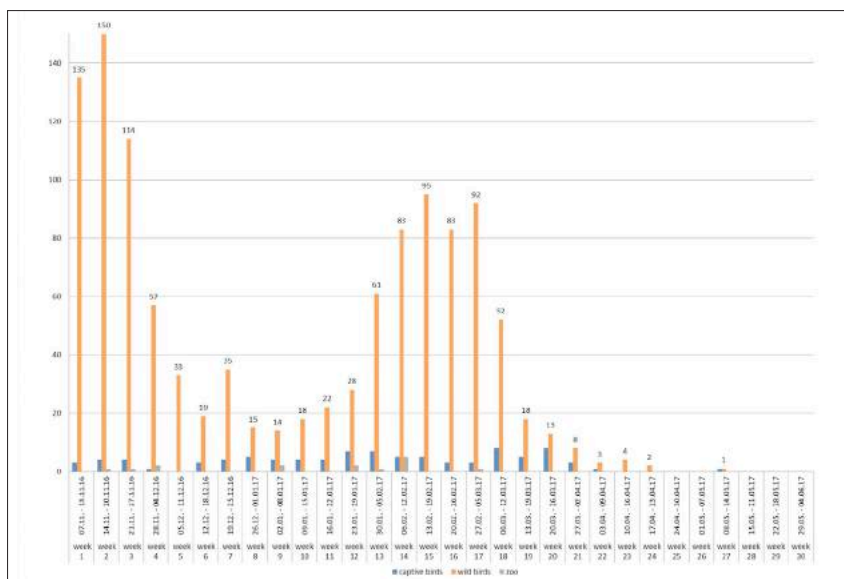


Figure 2: Weekly numbers of outbreaks of HPAI in poultry (red columns), zoos (blue columns) and cases in wild birds (green columns) in Germany (November 2016– May 2017).

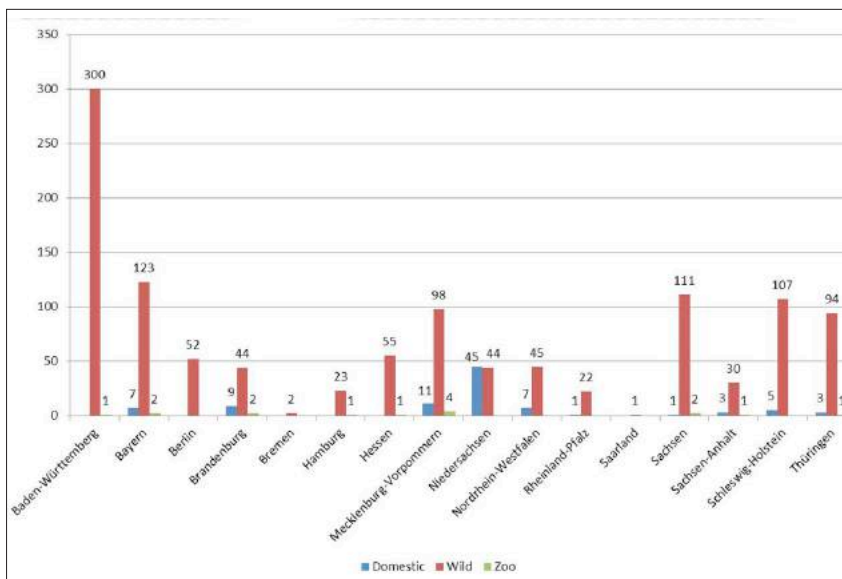


Figure 3: Numbers of reported cases in wild birds and outbreaks in poultry in the German Federal States.

particularly in France, but also in Bulgaria, Romania, Poland, Czech Republic and Hungary, where many domestic duck and geese flocks were infected. To control the circulation of various HPAI and LPAI H5 viruses including HPAIV H5N8 in domestic waterfowl in South-Western France, the government took drastic measures including the depopulation of all kept waterfowl and prohibition of keeping ducks and geese for some time in regions, where infections were mainly detected by surveillance.

Between 8th November 2016 and 15th May 2017, more than 1,150 cases of HPAI H5N8 in wild birds and 107 outbreaks in birds kept in captivity (92 poultry holdings and 15 zoos or animal parks) were reported in Germany. The last outbreak in poultry was reported on 9th May 2017. Thus, the HPAI epidemic seemed to be waning in Germany since April 2017 most likely due to a rise in temperature and increasing UV radiation as well as due to lower densities of overwintering waterfowl on lakes and rivers. This is the most severe epidemic of HPAI recorded in Germany so far.

Generally, the temporal course of the epi-

demic in wild birds was characterized by at least two waves, with maxima in mid-November 2016 and mid-February 2017, respectively (Figure 2). A few days after the detection of HPAIV H5N8 in wild birds, the first outbreaks were reported in non-commercial poultry (backyard) and subsequently also in large commercial poultry farms. By the end of February 2017, all federal states of Germany had reported HPAIV H5N8 infections in wild birds or poultry (Figures 1, 3). During the second wave of the epidemic, further HPAI H5-reassortants were found in wild birds and domestic poultry (turkey) in Schleswig-Holstein. These strains could be clearly distinguished from the first reported strains as they belonged to different genotypes involving several gene segments including another NA subtype (N5). Phylogenetic analyses indicated that multiple independent entries of viruses into Germany had occurred more or less at the same time (Pohlmann et al., 2017).

Epidemiological investigations of outbreaks in poultry holdings

A total of 68 commercial poultry holdings were affected by the epidemic; thereof 52 turkey, 5 laying hen, 9 duck and 2 geese holdings (Figure 4). Moreover, 24 small scale, non-commercial poultry holdings were affected by HPAIV H5N8. They were distributed almost all over Germany and were, like the outbreaks in captive birds in zoos, most likely caused by primary virus introduction into the holdings/zoos via direct contact to infected wild birds (where captive birds were kept outdoors and with access to ponds also visited by wild birds) or via indirect contact (feces or carcass contaminated material). No links regarding trade of live animals, feed or products of animal origin were detected between the outbreaks in Germany. The majority of outbreaks in large commercial poultry holdings were apparently caused by single incursion events, often affecting only one stable. In many cases, substantial gaps in farm biosecurity may have eased the path for virus entry. Only in the late phase of the epidemic, there was also epidemiological and molecular evidence for transmission of the infections between commercial holdings, which caused approximately 20-25 percent of the total number of outbreaks.

Conclusions and Outlook

The simultaneous endemic circulation of various HPAI and LPAI viruses in parts of Asia and Africa in wild birds and a substantial number of outbreaks in poultry do not give an optimistic view on the chances to eradicate these viruses. On the contrary, the poultry industry, politics and poultry associations must prepare for further outbreaks in future. Fortunately, the current HPAIV H5N8 clade 2.3.4.4b had no zoonotic potential, but this can change as new viruses have evolved in Asia, which

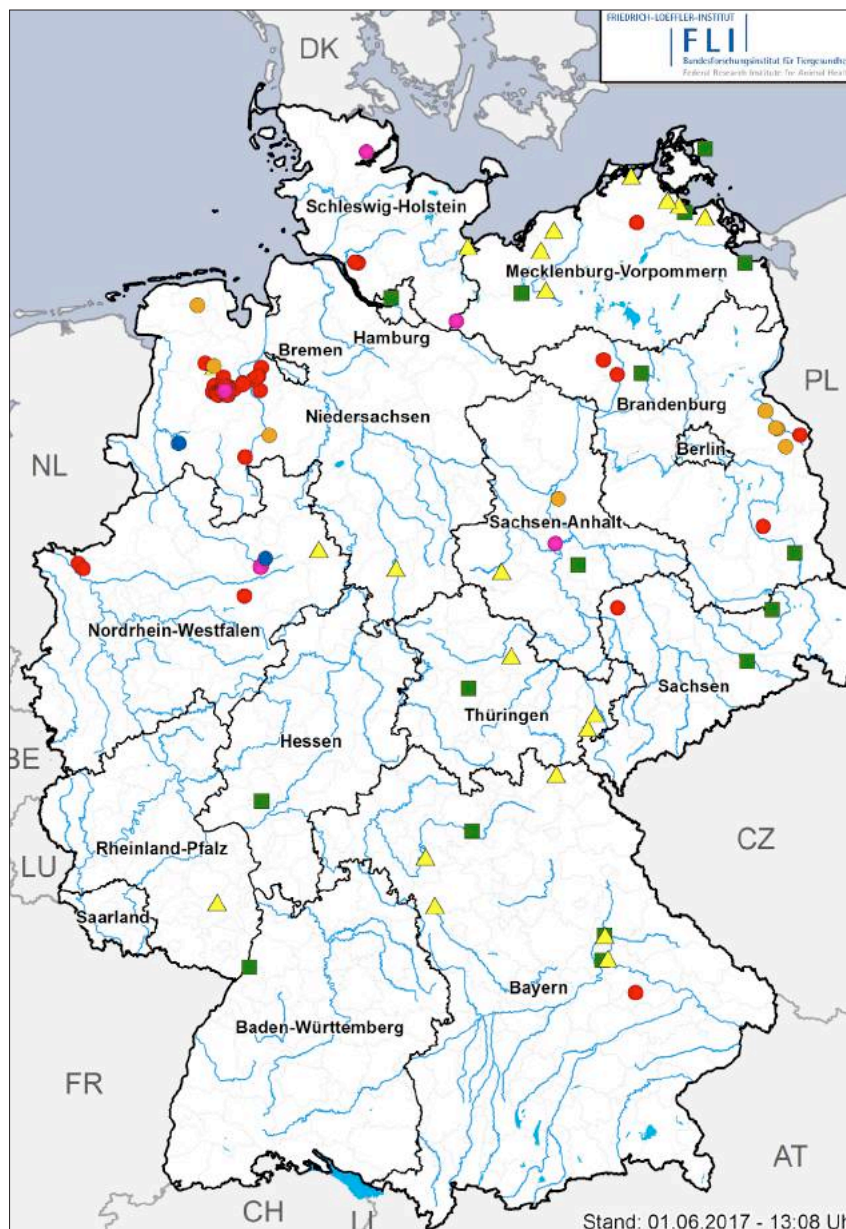


Figure 4: HPAI in captive bird holdings in Germany (08. November - 18 May 2017). Red circles: turkeys (52), orange circles: ducks (9), blue circles: geese (2), pink circles: laying hens (5), yellow triangles: small scale, mixed holdings (24), green squares: zoos (15).

can lead to fatal infections in mammals. Vaccination has mostly complicated the situation. The only efficient measures to prevent the spread of avian influenza viruses are prompt detection of infection, closure of the holding, immediate depopulation and cleansing/disinfection of the affected holding, as well as a temporary ban on restocking (EFSA, 2017). Moreover, potential contact to wild birds, mode and

frequency of farm visits, biosecurity practices, and the density of poultry holdings in a specific region are relevant risk factors for the introduction and the spread of HPAI (Ssematimba et al., 2013). Strictly-enforced farm biosecurity rules are essential to prevent the introduction and spread in poultry farms in high density areas, particularly in times of epidemics. Particularly in high risk periods and locations, losses should be

fully compensated only if biosecurity rules had been established and enforced.

Currently, the risk of introduction into domestic poultry holdings and captive bird holdings in zoological gardens through direct and indirect contacts between wild birds and domestic poultry is estimated to be moderate for Germany and Europe (FLI, 2017; DEFRA, 2017). By the end of summer, this risk will probably rise again, particularly for holdings in the vicinity of resting and gathering areas of wild waterfowl, including agricultural crop land.

Protection of domestic poultry holdings from infection with HPAIV H5N8 has the highest priority. Emphasis is put on the creation of a physical and functional barrier between wild bird habitats and domestic poultry holdings. Among other biosafety measures, mandatory indoor housing of poultry or the use of protected shelters (fenced and covered with fabric) minimize the risk of direct and indirect contact with infected wild birds. In particular, indirect introduction routes, e.g. through feed contaminated by wild birds, contaminated water, litter and objects (shoes, wheelbarrows, vehicles etc.) must be cut off and adequate disinfection measures must be taken. Revision, optimization and strict implementation of biosafety measures are of utmost importance. Poultry farmers are obliged by law to observe basic biosafety rules. The FLI has published a biosafety checklist together with the German Poultry Association (Zentralverband der Deutschen Geflügelwirtschaft e.V.), which is based on an EFSA opinion (EFSA, 2017). The document can be downloaded from https://www.openagrar.de/servlets/MCRFileNodeServlet/openagrar_derivate_00002067/Checkliste-Gefluegelpest-2017-03-17.pdf (only in German). Some interesting farm

biosafety guidelines and toolkits can be found under <http://www.farmbiosecurity.com.au/industry/chickens/>.

Further information on the HPAIV H5 distribution (maps) and current risk assessments on the introduction of HPAI viruses to Germany is available from <https://www.fli.de/en/news/animal-disease-situation/avian-influenza-ai-fowl-plague/> for Germany. FAO releases on a regular basis information about the spread of relevant avian influenza viruses, especially HPAI H5N1 and LPAI/HPAI H7N9 (<http://www.fao.org/avianflu/En/index.html>, <http://www.fao.org/ag/againfo/programmes/en/empres/H7N9/>).

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