Lohmann Information

Cuxhaven, April 2010

Prof. Dietmar Flock,
Editor

Editorial

The editorial board of Lohmann Information meets every six months close to the time when the current issue is being published. Typically, several topics are suggested from Lohmann Tierzucht and Lohmann Animal Health colleagues for the next issue, based on recent symposia or internal developments. It is then the pleasure of the editor to persuade authors to translate excellent power point slide presentations into publishable text versions. In 2009, Lohmann Tierzucht celebrated “50 years layer breeding in Cuxhaven” and the Solutions Department of Lohmann Animal Health organized a workshop „Water supply in poultry farming – a key factor for health and performance“. We decided to include one paper from each meeting, supplemented with a related general paper from external authors.

It so happened that National Geographic just published an excellent special issue (April 2010) **“Water – our thirsty world”**, which we recommend to our readers for more general information on world water resources.

This issue of Lohmann Information includes 9 contributions as „food for thought“:

1. **Harm Specht**, former board member of Lohmann & Co. AG and acting president of the Heinz Lohmann Foundation, presented a portrait of Heinz Lohmann in the first online edition of Lohmann Information (2006).
   In this issue, he presents **“The Heinz Lohmann Foundation – honoring a pioneer in modern agriculture”**, based on an address given on the occasion of a reception 14th April 2010 before unveiling a memorial on the Lohmann Campus in Cuxhaven. The non-profit Foundation was established in 1997 as a subsidiary of Lohmann & Co. AG.

2. **Prof. Dr. Heiner Niemann**, Director of the Friedrich Löffler Institute for Farm Animal Genetics in Mariensee (formerly Max-Planck and later FAL Institute) recently published a short perspective on **“Animal Production and Research in Germany”** in the Journal Züchtungskunde, which may also be of interest for our international readers and was therefore translated from the original publication.

3. **Prof. Dr. Dorian Garrick**, Jay Lush Chair of Animal Breeding at Iowa State University in Ames, Iowa, USA, presents the theoretical basis for modern methods of breed improvement in his article **“The Revolution in Genetic Improvement”**, which combines traditional quantitative genetics with DNA information. This is an excellent general introduction to the next paper.

4. **Prof. Dr. Rudolf Preisinger**, Lohmann Tierzucht GmbH, Cuxhaven, looks at the same topic from a more practical angle of current application and future potential for breed improvement in his paper **“Genome-wide Selection in Poultry”**. Laboratory techniques to identify individual genes are being refined to analyze genetic variation in each line in order to select more accurately for complex traits.

LOHMANN TIERZUCHT

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Managing Directors: Dr. Bruno Kaesler, Markus Wesjohann

Lohmann Animal Health

5. **Dr. Wiebke Icken** and co-workers, Lohmann Tierzucht GmbH, Cuxhaven, present a new technique developed in cooperation between Lohmann Tierzucht and the Institute for Agricultural Engineering and Animal Husbandry of the Bavarian State Research Centre for Agriculture of the Technical University Munich-Weihenstephan. In the article **"New selection traits from group housing systems"** the authors describe how pedigreed hens are being tested under floor conditions as a basis to improve adaptability to non-cage management systems:

6. **Dr. Barbara Tzschentke**, Institute of Biology, Perinatal Adaptation, Humboldt-University Berlin, and **Dr. Ingrid Halle**, Friedrich-Loeffler-Institute, Federal Research Institute for Animal Health, Institute of Animal Nutrition, Braunschweig, Germany present results of a series of trials with heat treatment of broiler hatching eggs on hatchability, chick quality and broiler performance in their paper **"Temperature training" during the last days of incubation: a new method to improve poultry performance"**.

7. **Dr. A.M. Manschadi** and co-workers, Center for Development Research (ZEF), University of Bonn, Germany, describe a project focused on efficient use of water resources. In the article **"White Gold" and Aral Sea disaster – Towards more efficient use of water resources in the Khorezm region, Uzbekistan"**, the authors analyze the effects of irrigating cotton fields and related factors contributing to one of the world's most dramatic losses of water resources. Possible actions to minimize further deterioration are identified. Political support and incentives to use resources more sustainably are required to benefit fully from the recommendations.

8. **Prof. Dr. G. Schlenker**, emeritus of the department of animal and environmental hygiene of the Veterinary Faculty of the Free University in Berlin, **Dr. Ina Bräunig** and **D. Windhorst**, Lohmann Animal Health GmbH & Co. KG, Cuxhaven, review water as the most important nutrient in poultry nutrition in their paper **"Technological and health aspects of drinking water for broiler chickens"**. The review is based on papers presented at a workshop of the Lohmann Animal Health division "Solutions" held in Cuxhaven, 20-21 October 2009 „Water supply in poultry farming – a key factor for health and performance".

9. Attila von Hankó contributed an introduction to "Autogenous Vaccines" in the October 2009 issue of Lohmann Information.
   In this issue, **Dr. Wiebke Icken** and co-workers present first encouraging field results in their paper **"Use of different autogenous vaccines in commercial layers to reduce *E. coli* infection"**.

Please feel free to pass Lohmann Information to colleagues or send their name and address to the editor for future direct mailing: Dietmar.Flock@lohmann-information.com

With kind regards,

Prof. Dietmar Flock
Editor

The Heinz Lohmann Foundation - honoring a pioneer in modern animal agriculture

Harm Specht, Cuxhaven

The Heinz-Lohmann Foundation was initiated by the brothers Paul-Heinz and Erich Wesjohann and registered on 4th August 1997 as a non-profit subsidiary of Lohmann & Co. AG, which belongs to the PHW Group and is wholly owned by the Paul-Heinz Wesjohann family. The PHW Group is one of the leading German companies with major activities in the areas of nutrition, health and agriculture and best known by the poultry brand name WIESENHOF.

After the reception, a memorial was unveiled, shown in the picture with curators of the Foundation (from left: Hans-Werner Hannemann, Peter Bleser MdB, Prof. Dr. Werner Zwingmann, Renate Grothkopf (CEO), Paul-Heinz Wesjohann, Freifrau Dagmar von Cramm, Dr. Reinhard Grandke, Peter Wesjohann, Alfons Frenk, Peter Engel and Harm Specht).

„Heinz Lohmann Memorial on Lohmann Campus

Photo: PHW-Group

The Wesjohann brothers chose the name of the foundation in memory of the founder Heinz Lohmann (1901-1975), who laid the foundation for today's Lohmann & Co AG in 1932 with a fishmeal factory in Cuxhaven. His approach to business was to utilize scientific knowledge for the benefit of agriculture and his company. For decades he trained his staff and hired qualified people to spread the latest knowledge on modern animal and poultry breeding, nutrition, health and management. The Wesjohann brothers established the foundation to keep this tradition alive and to express their responsibility as industry leaders for food quality and related concerns of society.

Since the establishment of the foundation, 7 Nutrition Symposia were organized as a platform for 39 invited speakers from universities and research institutes, trade organizations and society to review recent scientific results and developments related to the future of human nutrition:

1997: **Nutrition in 2010** – what will we eat the day after tomorrow?

1998: **Meat** – food with controversial perceptions of society

2000: **Food Quality** – joint responsibility of producers, industry and trade

2002: **Changing Agriculture or Consumers** – who determines the market?

2004: **Globalization of Nutrition** – can we uphold German quality standards?

2006: **Wholesome or Cheap?** – the future of nutrition

2008: **Energy vs. Food** – will food prices increase?

At a reception on April 14th 2010, members of the Lohmann and Wesjohann families, administration and regional politicians, former employees and business associates who remember Heinz Lohmann from his active years, current managers of the PHW and EW Groups and board members of the Heinz Lohmann Foundation met in Cuxhaven to reflect on the lasting impact of Heinz Lohmann and to review the dynamic development of the PHW and WE Groups since the take-over of Lohmann & Co. AG by the Wesjohann family 23 years ago.

Next time you are in Cuxhaven and visit the Lohmann Campus, make sure you take a close look at this beautiful 4 t Diabas Tuff rock, which originates from the Marmara region of Turkey and dates back to Palaeozoic times.

A portrait of Heinz Lohmann, founder of the Lohmann Group, was published in the first online edition of Lohmann Information (Specht, 2006) and is still top of the list according to the number of visits for individual papers on the website, which suggests that readers of our time are still trying to learn from Heinz Lohmann 35 years after his death. What was so special about Heinz Lohmann and his approach to business?

He started his first business during the depression period in 1932: a fishmeal factory in Cuxhaven as a joint project with 20 partners in the local fish industry. Fishmeal was considered as a valuable protein source for animal nutrition, especially for pigs and poultry, and it was good business to turn offal of the fish industry (with distinctive odour!) into a valuable product for the feed industry.
His training as apprentice in business included experience gained during his visit of the USA during the 1930s. After World War II, Heinz Lohmann was keen to learn from developments in the USA and imported valuable know-how for the German feed industry. In 1952 he signed a licence agreement with the US company Lederle to produce the antibiotic substance CTC, which was marketed under the trade name **Lohmacin**. The fermentation unit started to operate already three months after signing the agreement – today it would probably take three years to pass all legal hurdles for a new process and product. The scientific information provided by the US partner had to be substantiated to meet German legal requirements, the sales and service staff had to be trained to explain the benefits of feed additives to the feed industry, and feed analysis was offered free of charge to help in designing optimal rations including Lohmacin for specific groups of animals.

The use of antibiotics has been gradually eliminated by EU directives since the early 1970s. **Lohmann Animal Health** introduced alternative feed additives in time and today supplies the poultry and animal industry with a complete range of products for efficient production of animal protein from healthy animals.

After Word War II, the growing demand for poultry meat and eggs could not be met with traditional breeds and production systems. In 1956 Lohmann signed a licence agreement with the US breeding company Nichols to breed and distribute **Lohmann Broilers**, and two years later a similar agreement with the US breeding company Heisdorf & Nelson to breed and distribute **HNL Nick Chick** layers. These contracts enabled Lohmann to import not only specialized pure lines, but also the complete knowledge of modern breeding, nutrition, disease prevention and management.

After the expiration of the licence agreements, Lohmann Tierzucht was able to continue the genetic improvement and is today a key player in the world market for egg-type chickens and SPF eggs for vaccine production. Broiler breeding has been discontinued in Cuxhaven in the 1990s, when the EW Group acquired Aviagen and concentrated broiler and turkey breeding in Scotland.

The pioneer years were not without major problems. Disease prophylaxis as we know it today was still in its infancy. Specialized poultry pathologists had to be trained and new vaccines had to be developed against a range of common diseases. Lohmann established a **Veterinary laboratory** with the capacity to monitor the disease status of the own breeding flocks and offer diagnostic services to customers. Today Lohmann Animal Health produces a range of poultry vaccines in its own plant, based on **SPF eggs** supplied by Lohmann Tierzucht.

Lohmann started in the mid 1950s to sell day-old broilers to farmers interested in growing broilers, but soon recognized that it was necessary to provide processing capacity for the growers and to get involved in marketing. The brand name “Goldhähnchen” was introduced and later changed to “WIESENHOF”, which became a well-known brand in Europe and is recognized as market leader and quality brand in Germany.

Human Nutrition and Pharmaceuticals

Heinz Lohmann also ventured early into human nutrition with his purchase of **Pomps** in 1938 to produce baby food in Cuxhaven. Production capacities were expanded before this company was sold to Maizena in 1968. His son Bernd Lohmann started the company **TAD** in the late 1960s to produce pharmaceuticals for animals and humans. In the 1990s, the animal health activities were combined with animal nutrition and TAD focused on a range of human pharmaceuticals. TAD was sold in 2007 and continues to operate in Cuxhaven within a larger group.

The company **Nutrilo** was founded in 1984 and is successfully expanding with food supplements and special products for human health.

The entrepreneur Heinz Lohmann

As businessman in Cuxhaven, Heinz Lohmann became known for his energy and integrative approach to pursue his goals. To appreciate his impact in modernizing poultry and animal production in Germany, we should remember the opposition he faced not only from established trade organizations, but also from politics and administration. He imported knowhow from the USA and continued the developments in different areas with a qualified staff, based on scientific methods. In retrospect, we can say that one of his major contributions was to recognize the profound changes in consumer demand and methods of efficient food production earlier than most people in Europe and acted accordingly. Professionalism and an open mind for innovations, combined with positive risk assessment and deep personal involvement, were typical for Heinz Lohmann.

Lohmann & Co sold to Wesjohann

During the 1980s the Lohmann Group was faced with serious financial problems in connection with large turn-key projects. As a result, Lohmann & Co was sold in 1987 to the Wesjohann family, which had been a long-term partner and knew the Lohmann business well enough to recognize potential synergism and possibilities to participate in the growing global market for products and services required for efficient poultry and animal production.

The Wesjohann business was restructured in 1998 and divided among the two brothers: Paul-Heinz Wesjohann and his family own the **PHW Group**, Erich Wesjohann and his family own the **EW Group**. Each of the two groups employs more than 5,000 people worldwide today, including three companies on the Lohmann Campus in Cuxhaven with currently about 530 employees: Lohmann Animal Health (400), Nutrilo (110) and Vibalogic (20).

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Perspectives of Animal Production and Farm Animal Research in Germany

Heiner Niemann, ING-FLI Mariensee

Introduction

In Germany, animal production represents more than 60% of the total value of agricultural output. As in other Western countries, the focus is no longer on volume (or mass production?), but increasingly on product quality and variety. According to recent estimates of the FAO, the world population will increase to 8.3 billion by 2030. With the predicted increase in affluence, the demand for high quality animal protein will increase significantly. In Asia, an increase by 40% is expected within the next decade.

In order to meet the growing demands, it will be mandatory to further increase the efficiency of production, while greenhouse gases from animal production have to be reduced in parallel. Under European conditions farm animals will also play an increasing role in maintaining the landscape, for leisure and sports as well as production for niche markets.

Animal breeders have to maintain genetic diversity as a reservoir for future adaptation of livestock populations to changing goals and production needs. Modern breeding methods applied since the second half of the 20th century, have changed populations of farm animals considerably. A small number of highly productive breeds have become dominant at the expense of local breeds and survival of some of these is now endangered. The distribution of high yielding and efficient breeding stock will become even more global in the future. More and more traits are being included in selection programs to develop farm animal populations for specific future needs.

Man's association with farm animals dates back to pre-historic times. The characteristics of today's farm animals are the result of centuries of deliberate selection. More recently, sequencing and annotating the genomes of farm animal species has contributed considerably to our understanding of the genetics of farm animals, including cattle, pigs, poultry, horses, dogs and honey bees. New genetic tools not only allow us to analyze genomic variation, but also to modify genotypes. Combined with advances in reproductive technologies, limitations of traditional breeding can now be overcome and new horizons will be opened.

The German Society of Animal Breeding (DGfZ) has developed the following guidelines for future animal breeding and farm animal research in Germany.

Application of molecular genetics in practical breeding:

The latest results from research on molecular genetics biotechnology should be combined with traditional methods of breeding and reproduction. Systematic analysis and detailed description of relevant traits using molecular genetics information is required to combine quantitative genetics with modern tools for genomic selection. This is the only way to assure continued genetic progress of farm animal populations. In cattle breeding genomic selection is already being practiced. Similar developments expected in other species will benefit from interdisciplinary research and development of new methods.

Preservation of genetic diversity and avoidance of inbreeding:

Accumulating knowledge from genome analysis will add novel possibilities to describe genetic diversity and to increase diversity in farm animal populations. Improved biotechno-logical tools and computer software as well as simplification of international exchange of genetic material (primordial germ cells, embryos, semen, somatic cells) can contribute to maintenance and increase of genetic diversity. The potential of these developments should be exploited in internationally competitive research and for developing novel strategies in domestic animal breeding and reproduction.

Molecular processes will become more important in applied breeding:

Animal farming is following the same path with regard to applied genomics as we have already seen with humans and laboratory animals. With suitable array techniques it is already possible to describe the expression of mRNA and/or proteins of organs or whole organ systems. On this basis it is possible to gain a better understanding of interactions between genes and gene clusters as well as processes within animals in response to given environmental conditions. Intensive inter-disciplinary and cooperative research will be required, and the farm animal industry should be open and receptive for the application of results.

Understanding epigenetic effects will contribute to improved reproduction:

Genomic information and the high degree of epigenetic plasticity offer new possibilities to reduce early embryonic mortality, to improve fertility and to shorten the generation interval by using prepuberal animals. Research with embryonic development of farm animals, predominantly cattle, is increasingly being used as model for human medicine, specifically for assisted reproductive technologies. Epigenetic research with farm animals is still in its infancy, but results from model populations suggest that epigenetics acts as bridge between genotype and environment and will thus become more important in explaining phenotypic observations in farm animal populations. The full exploitation of this potential will require innovative research.

Transgenic animals will become more important:

It is already possible today, to produce farm animals with specific genetic changes by combining molecular genetic tools with somatic cell nuclear transfer. Techniques which are being used in mice to achieve a tightly regulated expression of genetic elements are increasingly being adapted to farm animals. In addition to the use of transgenic animals for biomedical purposes, including gene pharming and xenotransplantation, research will address possible application for agricultural purposes related to efficiency, environmental impact and sustainability. Potential application of research results has to be validated, and potential risks and opportunities have to be communicated early with the public, which is known for a strong bias against genetically modified organisms.

Farm animals as model for human disease:

Transgenic animals are increasingly being used as model to study human diseases and disorders like mucoviscidosis, diabetes, different forms of cancer and diseases of the vasculatory system. This is not limited to basic research, but may also involve pre-clinical testing. Genetically modified farm animals will increasingly be used to test novel stem cell therapies. Research is needed to exploit these new areas of development and to train young scientists for these challenges.

To realize these promising perspectives will require innovative interdisciplinary research with international cooperation. To secure a sufficient supply of high quality animal protein for the growing world population will require continued interdisciplinary research and introduction of science-based innovations in practice. Highly qualified specialists need to be trained to respond to new challenges in animal breeding and reproduction, farm management, nutrition and disease control.

The rapid development of molecular genetic tools requires the protection of intellectual property and technical innovation, e.g. by patenting. However, progress in animal breeding should not be limited by patents. It is up to patent courts, politics and administrations to find practical solutions which protect new developments without hampering practical animal breeding and production.

In view of the global challenge to feeding the growing world population a return to traditional animal farming is no realistic option. Research and animal industry are called upon to continue the road of innovation with due responsibility.

Zusammenfassung:

Stellungnahme der Deutschen Gesellschaft für Züchtungskunde zur Zukunft der Tierzucht und Tierzuchtforschung in Deutschland

Dr. Ernst-Jürgen Lode, Präsident der Deutschen Gesellschaft für Züchtungskunde (DGfZ) und Prof. Dr. Heiner Niemann, Vorsitzender des Fachbeirats der DGfZ, haben Leitlinien für die künftige Tierzucht und Tierzuchtforschung zu folgenden Themen vorgestellt:

- Übertragung molekulargenetischer Erkenntnisse in die praktische Tierzucht
- Genetische Vielfalt zum Erhalt genetischer Ressourcen und zur Vermeidung von Inzucht
- Zunehmende Bedeutung molekularer Prozesse und Einfluss auf die züchterische Arbeit
- Bedeutung epigenetischer Erkenntnisse für eine Steigerung der Fruchtbarkeit und Erklärung von Umwelteinflüsse auf das Tier
- Künftige Bedeutung transgener Tiere
- Nutztiere als neues Modell für menschliche Erkrankungen

Einzelheiten sind dem Originalbeitrag im März/April-Heft 2009 der Züchtungskunde (82: 99-101) zu entnehmen.

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The revolution in genetic improvement

Dorian Garrick, ISU, Ames

Introduction

Livestock improvement through selection has been remarkably successful over the last 50-100 years, despite some industries adopting and implementing modern principles more slowly than others. The foundation for improvement in all livestock industries has been quite simple - the selection of above-average candidates to be the parents of the next generation. Within that context, there have been a number of major changes. The manner and scope for defining overall merit has evolved from visual appraisal against an ideal, to a performance-based approach. In some industries it has gone further to a single index value representing aggregate economic merit, by combining evaluations on a portfolio of traits according to their economic values. There has been continuous evolution of the technical approaches to combine information on correlated traits and on relatives to improve the accuracy of prediction in the selection candidates. However, in most species, the approach using pedigree and performance information has suffered from a major technical drawback that has limited the annual response to selection. Genomics now offers the potential to overcome that limitation and revolutionize breeding programs in most species. Genomic methods for predicting merit of livestock are being adopted and implemented more rapidly than any other selection technologies. This paper describes the limitation posed by pedigree and performance information, and the manner in which genomic information offers promise to change that circumstance. The current status of genomic approaches and possible future directions will be briefly reviewed.

Genetic progress

The purpose of artificial selection is to achieve controlled improvement of a population. The annual rate of genetic progress (ΔG) is dictated by the rate of improvement per generation (Hazel, 1943), and the generation interval (L) or time taken for the parental generation to be replaced by their selected offspring. The improvement per generation is dictated by the amount of genetic variation (σ_g), the intensity of selection (i) and the accuracy of selection (r). The accuracy of selection is the correlation that would be observed if it were possible to relate true and estimated genetic merit. In practice, that correlation can be derived from a statistical basis, without recourse to the true, unknown breeding values. The well-known formula for annual genetic gain is

$$\Delta G = \frac{ir\sigma_g}{L} \qquad [1]$$

We need not concern ourselves with the details that selection is typically not for a single characteristic but for some form of aggregate merit involving a number of traits that may be favorably or, more commonly, unfavorably correlated, and that the components of the equation (i, L and r) commonly differ by sex and pathway of selection. Our interest is in manipulating the breeding scheme to achieve a high rate of annual gain in a cost-effective manner, whereby the benefits of the improvement greatly exceed the costs of the breeding program. This is most easily achieved when the measurement and selection activities are focused on a small nucleus or seedstock sector of the industry that provides germplasm to meet the demands of the entire industry. Reproductive advances such as artificial insemination have allowed this to happen in dairy cattle, whereas a multiplier sector has been used in natural mating beef cattle populations, pigs and poultry. In the latter two industries the short generation intervals and higher prolificacy can be exploited to minimize genetic lag between the nucleus and commercial sectors.

A naïve approach to increase gain would be to practice intense selection by choosing only a small proportion of available candidates and using them widely, collecting lots of information on the selection candidates or their relatives to achieve accurate prediction of merit, and carrying out selection no later than puberty in order to minimize the generation intervals. Sadly, this approach is seldom practical because achieving high accuracy in a large number of selection candidates typically has a high cash cost, and would delay selection for traits that cannot be measured by puberty, prolonging generation intervals. Effective improvement programs have evolved in most species to carefully balance selection intensity, accuracy of selection and generation interval in relation to the cost and value of genetic gain. In dairy cattle, with sex-limited lactation traits, bulls have routinely been progeny tested before widespread use. In contrast, broiler breeders can phenotype selection candidates for many of the important traits by puberty, enabling rapid improvement without delaying generation intervals to increase selection accuracy.

Conventional pedigree and performance-based selection

Fundamental to an understanding of selection is the model equation for phenotypic performance, which simply defines phenotype as the additive result of genotype and residual effects, perhaps influenced by some systematic non-genetic factors or nuisance effects such as flock/year, age or sex. The goal for prediction is to use available information, such as pedigree and performance records, to derive an estimate of the genetic merit that is in some sense best. The best estimator might be one that minimizes the variance of the prediction errors (i.e. the differences between the true and the estimated merit) or maximizes the correlation between true and estimated merit. It turns out that in circumstances where the genotypic and residual effects are normally distributed, the two definitions of best are obtained by the same approach, and the estimator is a linear function of all the available sources of information. That is, merit is estimated by adjusting each phenotypic record by a particular estimate of systematic non-genetic effects, and then weighting the resultant deviations across traits and relatives with weights derived specifically for each individual and trait. Most of the information comes from the nearest relatives and from highly correlated traits, so these sources have weights with larger magnitude than do other sources.

The average accuracy of prediction based on particular sources of information is a function of the sources and respective weights. The square of the accuracy is referred to as the reliability (r^2) in some livestock industries, and that value is of interest as it can be interpreted as the proportion of variation in true merit that can be accounted for using the information at hand. Another interpretation is that $1\text{-}r^2$ is the proportion of genetic variance in merit that cannot be explained given the available information.

The limitation of conventional approaches

The weights that are used to combine information from alternative sources are a complex function of the additive relationships between the animals with records. The additive relationships obtained from pedigree information reflect the expected or average relationship. For example, the relationship between non-inbred unrelated parents and their offspring is one-half, as is the relationship between full-sibs of non-inbred unrelated parents. However, given a finite number of genes influencing a trait, the actual relationship between a pair of full sibs might average one-half, but some full sibs could be more related and others less related to each other. An extreme example would be identical twins, where all the genes are identical, giving a relationship of unity between non-inbred twins. Any departure between average and actual relationships cannot be inferred from the pedigree alone.

The additive merit of a selection candidate without its own records or any offspring can be estimated from knowledge of the merit of its parents from

$$r^2_{offspring} = (r^2_{sire} + r^2_{dam})/4 \qquad [2]$$

Even with near perfect information, the upper limit is $r^2 \leq 0.25$ if one parent is known and $r^2 \leq 0.5$ if both parents are known. These values correspond to $r \leq 0.5$ or $r \leq 0.7$, respectively, limiting gain per generation to 50 - 70% of what could be achieved if candidates could be perfectly assessed by selection age.

Many of the traits that are economically important in breeding programs cannot be measured by puberty, nor in both sexes (e.g. milk yield, egg size), or cannot be measured without sacrificing the animal as a breeding prospect (e.g. meat tenderness, disease resistance), or waiting until the end of its life (e.g. longevity). Many others are simply too expensive to measure on every candidate (e.g. feed intake). Further, even when the selection candidate can be measured early in life, the value of that information is limited by the heritability of the trait. Individual breeding values for low heritability traits cannot be accurately assessed without measuring large numbers of progeny. The net effect is that reliability of estimated overall merit is typically sub-optimal at puberty.

The biological explanation for the 0.50 ceiling on r^2 is apparent when the genetic merit of an individual is expressed as the average of its parents (PA), plus a term representing its deviation from mid-parent value (ϕ), referred to as Mendelian sampling (MS). That is

$$g_{individual} = 0.5\, g_{sire} + 0.5\, g_{dam} + \phi_{individual} \qquad [3]$$

The coefficients of 0.5 on the merit of the sire and dam can be justified by the fact that livestock species have chromosomes in pairs, and only one member of each pair is passed on to the offspring. In the absence of any information other than pedigree, we must assume a random or average half sample is inherited. This formula has implications on the contributions of various sources to the observed variation. Consider a random mating unselected population. We might reasonably expect such a population to exhibit the same genetic variance from one generation to another, and in both sexes. That is, we expect

$$\mathrm{var}(g_{individual}) = \mathrm{var}(g_{sire}) = \mathrm{var}(g_{dam})$$

It is a statistical fact that the variance of a random variable scaled by a constant is the square of the constant times the variance of the unscaled random variable. That is, for a constant k,

$$\mathrm{var}(kX) = k^2\, \mathrm{var}(X) \qquad [4]$$

Applied to [3], assuming random mating giving zero covariance between the sire and dam, and zero covariance between sire (or dam) and the Mendelian sampling term, it is apparent that the choice of sire contributes 0.5^2=0.25 genetic variance to the next generation, as does the choice of dam, implying that $\mathrm{var}(\phi) = 0.5$ in order for genetic variation to be maintained. If there were no variation due to Mendelian sampling, the genetic variance would halve each generation and soon disappear as mutations are somewhat rare events.

The biological mechanisms for the deviation from parent average is the creation of new chromosome combinations from crossing-over between existing homologous pairs, and the chance sampling of one member from each chromosome pair, accumulated over all the chromosome pairs.

The goal in predicting genetic merit of selection candidates would be to achieve high accuracy by puberty, or preferably even earlier if costs are incurred in managing selection candidates until puberty. This cannot be achieved by performance recording, except for that subset of economically-relevant traits that have a high heritability and can be measured cheaply by puberty. In the broiler industry, this may not pose as much of a limitation as it does in the layer industry.

The genomic promise

Knowledge of the genes responsible for variation in traits would allow a different approach to genetic evaluation. Rather than assuming an offspring inherited a random half sample of its genome from each parent, we could inspect the DNA and perfectly characterize the inherited fraction. We would not even need to know the parents. This approach would not require phenotypic observations and could be done at birth or perhaps earlier. This is the current status of some inherited diseases and many other monogenic traits in livestock. Much of the molecular work undertaken over the last 2-3 decades has had the goal of identifying genes that account for a significant proportion of variation in economically important traits that are typically polygenic rather than monogenic in nature.

It is unrealistic to expect that we could identify every polymorphism, many of which will be of no consequence, nor screen them all to find the few responsible for inherited variation. The 1,000 genome project in humans is currently identifying about 500,000 new single nucleotide polymorphisms (SNP) in every individual sequenced. Without knowledge of all the polymorphisms, we must be content to find genomic clues that indicate the presence of causal genes. This is akin to detecting planets by observing light reflected off them at some time in the past. In the context of the genome, we rely on a phenomenon known as linkage disequilibrium (LD) to provide evidence of important genomic regions. Various strategies exist to create or exploit LD.

The well-known Hardy Weinberg law applies to large random-mating populations and indicates that in the absence of selection, migration, mutation and drift, the genotypic proportions can be predicted from allele frequencies, and vice versa. Mendel established a law of independent assortment, or linkage equilibrium (LE), which might be universally true if every gene were on its own chromosome, but does not account for physical linkage between nearby genomic locations on the same chromosome. A typical chromosome has about one recombination event per meiosis, which can quickly break down association that might exist between distant genes on the same chromosome due to selection, migration, mutation etc. Imagine a new mutation or the migration of an individual into a population carrying a novel mutation. That mutation would tend to be preserved within the context of nearby alleles sharing the same DNA strand, until a recombination event occurred between them. Alleles that are closer to the mutation will take longer for their linkage to break down by recombination. The ability of an allele to act as a surrogate that can be used to predict some other particular allele is measured by LD. One unit for measuring LD is the correlation between alleles, or the correlation between the genotypes (number of copies of particular alleles) at two genomic locations.

Loci that are on different chromosomes are expected to exhibit LE at the population level, at least in the absence of selection that creates complications such as the Bulmer effect (Bulmer, 1971). Loci nearby on the same chromosome are more likely to exhibit pairwise LD, with the amount of LD tending to increase as physically closer pairs of loci are compared. However, even very close loci may exhibit LE, for example if they are ancient polymorphisms and recombination events have broken up their historical relationship. In order to find every causal mutation, we would need to have a lot of markers.

Early studies to detect QTL typically used micro-satellite markers (Spelman *et al.*, 1996) that can be highly polymorphic, having many alleles at some loci. Some studies took advantage of family structures to create LD. Within every half-sib family there is LD even for markers that exhibit LE at population level, so within family analyses such as the grand-daughter design (GDD) used in dairy cattle (Weller et al., 1990) can use sparse marker genotypes to exploit LD. Other studies used crosses between distant lines or breeds that result in F1 individuals that have one chromosome in each pair from each of the lines or breeds. There will be long-range LD in the F2 animals, which can be exploited using flanking markers to test for the presence of putative QTL at various locations between the markers. The high cost of genotyping resulted in most GDD and F2 experiments having too few animals to identify any but the largest QTL.

These circumstances all changed with the recent sequencing of the bovine and other livestock species. Those endeavors generated many SNP markers and provided a reference sequence. A subset of known bovine SNP were selected for genomic coverage and minor allele frequency to produce an Illumina 50k SNP chip, and most other livestock species have followed with public or private high-density panels with similar numbers of markers. Rather than exploiting LD due to family structure, these panels provide opportunities to exploit ancestral LD, at least for that fraction of the QTL that happen to be in LD with the markers on the panel. The technology that enables simultaneous genotyping of all the SNP on the panel make these chips more cost-effective than using micro-satellite markers, and much larger populations of up to 10,000 animals have been genotyped, although there are also many small studies with 1,000 or fewer individuals.

The current status of genomic approaches

Regressing phenotype or breeding value on QTL genotype would estimate the additive effect of a QTL. However, since the actual location of the QTL are unknown, nor are the QTL genotypes, this is not an option, but the regression of phenotype or breeding value on SNP marker genotypes can be calculated. A SNP in perfect LD with one QTL would show a steeper regression than other SNPs with less LD with that QTL. Such regressions could be undertaken on every SNP. In the human area, regressions are typically carried out one SNP at a time, from a hypothesis-testing framework. In livestock, our interest is not so much in finding significant SNP, but in accurately predicting merit from as many SNP as are required, regardless of whether or not they reach statistical significance.

It is not possible to simultaneously fit more fixed SNP effects than there are observations. Stepwise least squares procedures could be used to progressively add the most informative SNP, to derive an informative subset. However, least squares approaches are known to overestimate effects, particularly when the overall power of the experiment is low. In contrast, methods that shrink estimates tend to be more reliable. Two methods of shrinking estimates are to fit random rather than fixed regressions, or to fit mixture models. Both these approaches are commonly used in genomic predictions.

If the QTL locations were known, along with the most informative SNP in those regions, then relationships between selection candidates and historical animals could be derived from these SNP in order to obtain accurate predictions of merit for the selection candidates without the phenotypic records. However, the QTL locations are not yet known, but could be assessed from the joint regression of performance on the SNP genotypes.

Regression of performance on all SNPs simultaneously can be achieved by fitting the effects as random. That requires knowledge of the variance ratio appropriate for each SNP effect. One option is to fit the same variance ratio for every SNP, which is known as ridge regression. That variance ratio could be estimated from the data by various means. Meuwissen *et al.* (2001) refer to this method as BLUP, whereas Fernando and Garrick (2009) refer to it in a Bayesian context as Bayes C0. Another option is to fit a different variance ratio for each SNP, allowing the estimates of some loci to be shrunk more than others. A Bayesian method for such an analysis was developed by Meuwissen *et al.* (2001) who called the method Bayes A. However, it is unlikely that every SNP would be in LD with a QTL, so a more appealing method might be one which allows some fraction of the loci to have zero effect. Meuwissen *et al.* (2001) developed such a method in the context of a mixture model, which they referred to as Bayes B. One problem with their method is that it required the mixture fraction (π) to be known. Kizilkaya *et al.* (2010) introduced the concept of a mixture model within the framework of ridge regression, describing that method as Bayes C. That method with π=0 is Bayes C0 or ridge regression. Fernando and Garrick (2009) further extended that method to simultaneously estimate the mixture fraction from the data, a method they refer to as Bayes Cπ.

The regressions tend to overfit the so-called training data used in the analysis; therefore quantifying the accuracy of the predictions cannot be done from that same analysis. Cross-validation where the training data is partitioned into subsets, one used for training and another used for validation is one method for quantifying the accuracy. Another alternative is to validate in an independent dataset.

Simulated data with a finite number of QTL has demonstrated that models like Bayes B that exploit the correct mixture fraction result in more reliable predictions than methods like Bayes A or Bayes C0 that fit very SNP. Further, estimating the mixture fraction from the data gives better genomic predictions than using an improper mixture fraction. Simulation studies have shown correlations between genomic predictions and underlying genotype of 0.7-0.9 (Meuwissen *et al.*, 2001), accounting for 50-80% of total genetic variance. It is tempting to assume such predictions do not discriminate between parent average (PA) and Mendelian sampling (MS) and estimate both components equally well.

Early whole genome analyses of the North American Holstein population (VanRaden *et al.*, 2009) reported the PA reliability (r^2) of animals without records or offspring to average 0.19 across traits and the genomic prediction to improve on that value by a further 0.18 increase. In the international collaborative analysis of Brown Swiss performance, Jorjani and Zumbach (2010) compared the PA predictions from conventional evaluations vs. the genomic evaluations from 50k SNP panels with the subsequent performance of progeny tested daughters four years later. Those analyses also demonstrated an increase in reliability by 0.18. Some of that increase in predictive ability is likely due to improved prediction of PA. If we were to assume all of it was due to MS, and MS accounted for 50% of genetic variance, then it would seem that these genomic predictions are predicting up to 36% genetic variance.

There are fewer published reports of genomic predictions in beef cattle. Analyses of US Angus bulls based on their published expected progeny differences (EPD) for a range of traits resulted in correlations between two-thirds data used in training and one-third used for validation as in Table 1 (from Garrick, 2009). In that study, the AI bulls were randomly allocated to three subsets according to the sire of the bull, such that paternal half-sibs were not represented in more than one of the subsets. The pooled correlations between genomic and realized performance ranged from 0.5-0.7, accounting for 25-50% genetic variance.

Table 1: Correlations between 50k genomic prediction and realized performance for validation of Angus sires in independent Angus datasets for backfat (BFat), calving ease direct (CED) and maternal (CEM), carcass marbling (Marb), carcass ribeye area (REA), scrotal circumference (SC), weaning weight direct (WWD) and yearling weight (YWT).

Trait	Train 2 & 3 Predict 1	Train 1 & 3 Predict 2	Train 2 & 3 Predict 3	Overall[1]
BFat	0.71	0.64	0.73	0.69
CED	0.65	0.47	0.65	0.59
CEM	0.58	0.56	0.62	0.53
Marb	0.72	0.73	0.64	0.70
REA	0.63	0.63	0.60	0.62
SC	0.60	0.57	0.50	0.55
WWD	0.65	0.44	0.66	0.52
YWT	0.69	0.51	0.72	0.56

[1] Overall correlation estimated by pooling the estimated variances and covariances from each separate validation

A recent publication by Habier *et al.* (2010a) partitioned the German Holstein population into 'training sets' in order to control the maximum pedigree-based additive genetic relationship between any bull in validation and all bulls in training. This partitioning was repeated in four scenarios to vary the level of relationship. Random partitioning resulted in additive relationships as high as 0.6 between training and validation bulls. Restricting the maximum relationship to 0.49 produced partitions that prevented parent-offspring relationships or splitting of full-sibs across training and validation subsets. Restricting the maximum relationship to 0.249 prevented grand-parental and half-sib relationships across training and validation subsets. A further scenario prevented maximum additive relationships from exceeding 0.1249. Creation of these scenarios required that some bulls be excluded from both the training and validation subsets. Interestingly, these scenarios had little impact on the average maximum relationship between training and validation subsets that remained at about 9% for the first three scenarios. The results in terms of correlations are in Table 2, for predictions based on 1,048 training bulls using conventional pedigree analysis (P-BLUP), and for methods using genomic relationship matrices with equal (Bayes C0) or heterogeneous SNP weighting (Bayes B). Clearly the genomic predictions outperform pedigree-based methods, justifying their continued implementation, but the reduction in predictive power for the 0.1249 scenario is alarming for the use of genomic predictions for traits that are not routinely phenotyped every generation, as is envisioned for beef cattle traits associated with reproduction, feed intake, disease and eating quality.

Table 2. **Correlations (ρ) between genomic predictions based on samples of 1,048 German Holstein training bulls and observed performance in validation subsets with training and validation animals partitioned to control the maximum additive relationship (a_{max}) between any validation bull and all training bulls.**

	a_{max}			
	0.65	**0.49**	**0.249**	**0.1249**
P-BLUP	0.51	0.51	0.45	0.21
Bayes C0	0.58	0.60	0.50	0.32
Bayes B	0.62	0.62	0.55	0.12

Further validation analyses have been undertaken using North American Holsteins for a small (1,000 bull) or large (4,000 bull) training set comprising animals born after 1994, validated in animals born before 1975 (Habier *et al.*, 2010b). Those results (Table 3) fail to account for more than 0.52^2=28% genetic variance. However, the validation bulls would have been assessed from progeny performance in management circumstances quite different from today, so both heterogeneous variance and genotype-environment interaction could have contributed to the reduction in predictive ability.

Table 3: **Correlations between North American Holstein 50k genomic predictions from 1,000 or 4,000 training bulls born after 1994 and realized performance for ancestral bulls born before 1975.**

	Training bulls	
Trait	1,000	4,000
Milk	0.42	0.44
Fat	0.48	0.52
Protein	0.15	0.18
Somatic Cell Count	0.14	0.28

Validation of genomic predictions in other breeds provides a worst-case scenario in terms of predictive ability. Across-breed predictions could perform poorly because of dominance, epistasis, genotype-environment interactions, variation in LD among breeds, among other reasons. Training analyses based on North American milk yields from 8,512 Holstein bulls resulted in correlations of 0.194 in 742 Brown Swiss bulls and 0.198 in 1,915 Jersey bulls from Bayes A, and 0.141 in Brown Swiss and 0.244 in Jersey from Bayes B. Training in two of the three breeds and validating in the third resulted in correlations of 0.077 in Brown Swiss, 0.197 in Jerseys and 0.253 in Holsteins. Linkage cannot be contributing to these across-breed predictions, only LD, and that accounts for no more than 10% of genetic variance.

Any improvement on the accuracy of PA predictions provides opportunities for improved breeding schemes. These results clearly indicate that genomic techniques can increase predictive ability and therefore have an immediate role in breeding schemes. The performance of genomic predictions based on analysis of 4 generations of a Hy-line layer population are shown in Table 4, where validation was undertaken in generation 5, the offspring of generation 4 from the training population (Wolc *et al.*, 2010).

Table 4. Validation of conventional pedigree evaluation (P-BLUP) and predicted genomic breeding values used to estimate the merit of layer selection candidates at an early or late selection stage based on their parent average (PA) genotypes or their own individual genotypes for egg production (PD), egg weight (EW), shell quality (PS), albumen height (AH), weight of first 3 eggs (E3), color of first 3 eggs (C3) and yolk weight (YW).

Stage	Method	PD	EW	PS	AH	E3	C3	YW
Early	P-BLUP	0.18	0.45	0.22	0.34	0.38	0.45	0.29
	Bayes Cπ -PA	0.33	0.37	0.32	0.38	0.33	0.48	0.31
	Bayes Cπ	0.36	0.57	0.38	0.55	0.57	0.55	0.39
Late	PBLUP	0.42	0.60	0.44	0.57	0.68	0.67	0.52
	Bayes Cπ-PA	0.42	0.57	0.50	0.62	0.60	0.68	0.45
	Bayes Cπ	0.53	0.69	0.51	0.73	0.74	0.66	0.49

The early adoption of genomic prediction in livestock has not been to improve accuracy on previously difficult to predict traits, but to improve the accuracy at a young age of the easily predicted traits. Further, the predictive ability is most reliable in offspring of the training animals, implying that for the near future, each seedstock population must have its own training analyses for every trait and that phenotypic data will need to be continually collected in order to provide for ongoing training in successive generations.

Likely future directions

Enormous potential remains for increasing the predictive ability from genomic data to levels closer to that which can be obtained in simulated data. Research is urgently required to further investigate methods in which some of this potential might be exploited in the near term. This might involve the use of higher-density SNP panels (e.g. 500k or 1m SNPs), the use of haplotypes, and joint analysis that models both linkage and LD relationships. Further, the cost-effective use of this technology warrants the use of cheaper lower density panels for screening non-parents. One approach for using low-density panels would require genotypes of selection candidates for high-density markers to be imputed from high-density information on their parents and low-density information on the individuals (Habier *et al.*, 2009). That approach has particular appeal in poultry breeding and is the subject of current research.

Zusammenfassung:

Revolutionäre Methoden genetischer Leistungssteigerung

In den vergangenen 50-100 Jahren wurden bei landwirtschaftlichen Nutztieren durch Selektion bemerkenswerte Leistungssteigerungen erzielt, allerdings mit Unterschieden zwischen Tierarten und der Bereitschaft, Gebrauch von neuen Erkenntnissen der Tierzuchtforschung zu machen. Die Basis züchterischer Verbesserung bleibt immer - die Auswahl überdurchschnittlicher Kandidaten als Eltern der nächsten Generation. In diesem Rahmen hat sich über die Jahre einiges geändert. Die Selektionswürdigkeit wird nicht mehr nach äußeren Kriterien beurteilt, sondern nach messbaren Abweichungen vom definierten Zuchtziel, häufig ausgedrückt in einem Selektionsindex, der alle wirtschaftlich wichtigen Kriterien in einer Zahl zusammenfasst. Rechentechnische Methoden, um Informationen korrelierter Merkmale von Verwandten zu einem möglichst genauen Gesamtzuchtwert zusammenzufassen, wurden fortlaufend verfeinert. In aller Regel gibt es aber technische Grenzen für die Genauigkeit der Zuchtwert-schätzung, die erst mit der Anwendung genomischer Selektion überwunden werden können. Das ist in der Tat eine revolutionäre Entwicklung, und die Anwendung genomischer Methoden der Zuchtwertschätzung macht rasche Fortschritte. In dieser Übersicht werden die Grenzen herkömmlicher Selektion aufgrund von Leistungsprüfung mit Familienformation dargestellt und es wird erklärt, wie genomische Informationen helfen, diese Grenzen zu überwinden.

References

Bulmer, M.G. (1971): The effect of selection on genetic variability. Am Nat, 105: 201-211.

Fernando, R.L. and D.J. Garrick (2009): GenSel - User manual for a portfolio of genomic selection related analyses. Animal Breeding and Genetics, Iowa State University, Ames. http://taurus.ansci.iastate.edu/gensel

Garrick, D. J. (2009): The nature and scope of some whole genome analyses in US beef cattle. Proceedings of the Beef Improvement Federation 41st Annual Res. Symp. April 30 - May 3, 2009. Sacramento, California, USA. 41:92-102.

Habier, D., R.L. Fernando and J.C.M. Dekkers (2009): Genomic selection using low-density marker panels. Genetics 182:343-353.

Habier, D., J. Tetens, F.-R. Seefried, P. Lichtner and G. Thaller (2010a): The impact of genetic relationship information on genomic breeding values in German Holstein cattle. Genet. Sel. Evol., 42:5.

Habier, D., R.L. Fernando, K. Kizilkaya and D.J. Garrick (2010b): Extension of the Bayesian alphabet for genomic selection. Proc. 9th World Congress on Genetics Applied to Livestock Production. *In press*.

Hazel, L.N. (1943): The genetic basis for constructing selection indexes. Genetics 28:476-490.

Jorjani, H. and B. Zumbach (2010): Preliminary results from validation tests. Interbull Workshop on the Use of Genomic Information in Genetic evaluations. Paris, March 4-5, 2010. *In press*.

Kizilkaya, K., R.L. Fernando and D.J. Garrick (2010): Genomic prediction of simulated multibreed and purebred performance using observed fifty thousand single nucleotide polymorphism genotypes. J. Anim. Sci. 88: 544-551.

Meuwissen, T.H.E., B.J. Hayes and M.E. Goddard (2001): Prediction of total genetic value using genome-wide dense marker maps. Genetics 157: 1819-1829.

Spelman, R. J., W. Coppieters, L. Karim, J. A. M. van Arendonk and H. Bovenhuis (1996): Quantitative trait loci analysis for five milk production traits on chromosome six in the Dutch Holstein- Friesian population. Genetics 144: 1799-1808.

VanRaden, P. M., C.P. Van Tassell, G.R. Wiggans, T.S. Sonstegard, R.D. Schnabel, J.F. Taylor and F.S. Schenkel (2009): Invited review: Reliability of genomic predictions for North American Holstein bulls. J. Dairy Sci. 92:16-24.

Weller, J.L., Y. Kashi and M. Soller (1990): Power of daughter and granddaughter designs for determining linkage between marker loci and quantitative trait loci in dairy cattle. J. Dairy Sci. 73:2525-2537.

Wolc, A., C. Stricker, J. Arango, P. Settar, J. Fulton, N. O'Sullivan, D. Habier, R. Fernando, D.J. Garrick, S.J. Lamont and J.C.M. Dekkers (2010): Breeding value prediction for early production traits in layers using pedigree and marker based methods. Proceedings of the 9th World Congress on Genetics Applied to Livestock Production. In press.

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Genome-wide Selection in Poultry

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Introduction

Feeding the world's growing population with high-quality food is the core aim of agricultural production. In view of the growing world population, this challenge will continuously become larger, especially in the big cities of developing countries, where most of the increase of 80 million annually is expected. Production of eggs in these regions will be of increasing importance as a relatively cheap source of animal protein. In contrast to poultry meat, which is traded between continents in large volume, eggs are produced typically for consumption in the region and traded across borders in significant volume only in Europe.

In addition to conventional selection criteria like egg production, feed conversion and egg quality, traits related to animal welfare have become more important in Europe and North America. To improve these traits requires additional and very cost-intensive data recording. Novel recording systems have to be developed to test chickens in non-cage environments and husbandry systems accepted by welfare legislation.

Continued genetic progress can be expected within the available gene pool of highly efficient white-egg and brown-egg lines, based on conventional selection on key traits. However, the laws of diminishing returns also apply in poultry breeding, and efforts to optimize performance testing, selection and reproduction have to be intensified to assure competitive rates of progress. Incorporating additional traits in the complex breeding goal and selection routine will automatically increase the total cost of data recording and lower the rate of progress for all other traits, without generating a measurable return on investment for the breeding company. They are therefore challenged to look for more precise, quicker and/or less expensive methods to evaluate individual and family differences in relevant criteria.

As numerous simulation studies have shown, genomic selection can contribute significantly to the enhancement of breeding progress. In layer breeding, genomic selection has been successfully applied in practice to identify and eliminate the FMO3 gene which used to cause off-odor in brown-shelled eggs (HONKATUKIA *et al*. 2005).

There is no convincing evidence to date demonstrating that genomic selection can in fact improve all major traits in a complex index at competitive cost. The following review will address the current status and perspectives of genomic selection in layer breeding in more detail.

Genome-wide Selection

Many theoretical advantages of genomic selection have to be weighted against the substantial expenditure. In addition to launching costs for the establishment of the method in each line or gene pool, there are also substantial costs for typing all candidates in each generation of selection. There is a theoretical potential for savings in performance testing (e.g. due to shorter testing periods and earlier selection decisions). However, in the learning process of the first several generations, there will be no possibility to economize performance testing, because the effective contribution of genomic selection depends on complex genotyping and the correlation between phenotypic parameters and markers.

The establishment of genomic selection in all lines and application to select between and within families requires that performance testing continues for all economically relevant characteristics.

The accuracy of this conventional testing, i.e. “phenotyping”, determines the success of genomic selection carried out later. Using a broad calibration and genome-wide typing with SNPs will hopefully indicate regions associated with specific traits. Until we know more, each line has to be analyzed individually, and the parameters estimated from one line cannot be simply used in another line. Commercial hybrids usually constitute 4-way crosses, i.e. the cost of genotyping quadruples before results can be expected in the field. Furthermore, the 4 lines for a white-egg breeding program have nothing in common with the 4 lines for a brown-egg breeding program of the same primary breeder. The calibration process therefore has to be carried out at least 8 times. After the first genomic selection and reproduction of cross-line offspring, we can begin to measure the selection response in comparison with conventional selection.

This comparison will provide information to assess the additional benefit of genomic selection. To reduce the cost for typing, the set of markers can be readjusted after this initial phase and reduced to the most informative regions. With a small line-specific SNP-Chip, the cost of routine genotyping can be substantially reduced.

For poultry, genomic selection is expected to contribute primarily to more accurate breeding value estimation in broiler breeding, in layer breeding also to a shorter generation interval. These two factors will combine to speed up the annual rate of progress in breeding.

The benefits of genomic selection should eventually become apparent in terms of lifetime productivity and lower susceptibility to diseases. Furthermore, genome analysis can help to describe the current gene pool more accurately and to optimize effective population size without sacrificing selection intensity, while focusing on short-term breeding progress. Based on simulation studies, it has been calculated that breeding progress can be increased by 20-40 % annually by extensive application of genomic selection is (DEKKERS, 2009; AVENDAÑO *et al.*, 2009).

At present, two approaches are being used in the application of genomic selection. In addition to studies based on genome-wide SNP detection within several lines, so-called “case control studies” are of particular interest. Case control studies are used to analyze specific metabolic disorders or susceptibility to diseases, using DNA samples from infected birds and compared to those from non-infected birds. Since all birds originated from the same genetic groups, were re-produced at the same time, reared and exposed to the same infection pressure, differences in susceptibility can be traced back to possible genetic differences.

In layer breeding, the selection among full brothers at an early stage and the prediction of persistency of egg production and egg quality are of major interest for genomic selection. Males are selected traditionally based on the performance of their sisters and female relatives of previous generations. Therefore, full brothers have identical breeding values, although their real genetic potential varies greatly, as will be demonstrated by their progeny. If all males were raised and complete families of full brothers selected, the inbreeding would increase dramatically. Therefore, only few sons per dam are presently being raised, and the number of sons selected per sire is restricted. The objective with genomic selection would be to keep as many sons per family at hatch and to reduce them to available rearing capacity by within-family selection on the basis of marker information.

First experiments using a DNA chip with 40,000 SNPs have begun. The calibration data consist of performance parameters from three or more generations. The first generation of offspring has been reproduced in 2009 from sires and dams selected exclusively on genomic breeding values, ignoring their phenotypic performance. The regression of offspring performance on parent performance will provide information on the accuracy of genomic selection and realized genetic gain.

Potential for application of genomic selection

Compared to other farm animals, performance testing of layers at the pure line stage is relatively cheap per individual hen, especially if they can be used for reproduction during the testing phase by means of artificial insemination. The cost of genomic selection is presently estimated at approximately 200 € per bird for a 40,000-SNP marker panel. There is no chance for savings in performance testing anywhere near this amount.

A practical question which needs to be answered is how best to combine selection indexes based on phenotypic and genomic data. Both are supposed to combine all available information in an optimal manner, with appropriate attention to accuracy and source of information (pure-line or cross-line data). The additional cost of genetic improvement would eventually have to be paid by customers, who will only be prepared to accept a price increase if and when a significant increase in productivity of the commercial layer has been confirmed. The substantial launching cost will have to be pre-financed by the breeding company in any case, and predicted results will not be sufficient to argue the need for a price increase.

As positive examples for effective marker-assisted selection in poultry, we can point to (1) the elimination of fishy odor in brown-shelled eggs caused by a mutation on the 8th chromosome, (2) reduced susceptibility to Marek's disease and (3) reduced susceptibility to infection by *E. coli*. Hopefully selection against feather-pecking will also benefit from marker assisted selection (FLISIKOWSKI *et al.*, 2009) in the near future.

Practical experience with genomic selection suggests that the time from discovery to successful practical application is usually underestimated. The breeding company Aviagen started in 2006 and 2007 with chips containing 6,000 and 12,000 SNPs and is currently working with a chip containing 40,000 SNPs to increase the marker density in promising regions and to discover more informative regions. Cobb Vantress, in close collaboration with Hendrix Genetics, began a large-scale project in broiler breeding in 2008.

Conclusion

The poultry industry recognizes the advantages of continued genetic improvements, which add to profitability at the commercial level without requiring any additional investments or changes of organization. Offering future generations of parents at the same price would be advantageous for the buyer, but for reasons described above not realistic to expect. To recover the additional cost of genetic improvement, breeding companies have to try to sell parents in larger numbers which will intensify the competition among the few primary breeders for layers and broilers dominating the world market. Genomic selection provides information which can already be used in growing animals without performance testing. This increases the speed and accuracy of selection decisions. The prerequisite for the application is, however, upstream performance testing for all traits of commercial interest. Therefore, phenotypic performance recording must first be established for new traits before markers can be applied. Selection on molecular markers is no miracle to improve new traits directly, it is only an additional tool with the potential to increase the effectiveness of breeding without manipulating the genome of the birds. The breeding goals have to be defined and rates of progress predicted in order to offer the commercial poultry industry realistic expectations of future improvements. Short-term efforts to realize improvements in the areas of management and husbandry, hygiene and disease prevention, and last but not least to optimize nutrition, should not be relaxed while expecting too much from genomic selection too soon.

For laying hens, we expect continuing progress in each generation in terms of persistency of production and egg quality, feed efficiency, health, behavior and adaptability to different housing systems. Traits related to hen welfare will receive increasing attention in testing and selection. The high level of productivity already achieved, with rate of lay exceeding 90% for many months, is no reason to question whether further progress can be achieved. Testing in different housing systems and under field conditions will remain important while further developing genomic selection with company-specific DNA chips. With these tools, selection will become more complex and costly, but also help to tailor different strain crosses to specific needs of egg producers in different parts of the world market.

Summary

Primary breeders of egg-type chickens are making a significant contribution to supply highly efficient layers to commercial egg producers, who in turn produce high quality animal protein at low cost to the growing world population. Genomic selection has the theoretical potential to further improve the effectiveness of breeding, but so far the practical benefits of these strategies have not been verified in commercial poultry breeding. Possible savings in performance testing cannot cover the increasing cost of genotyping. Company-owned SNP-chips have been developed and are being utilized in both layer and broiler breeding. First practical results are expected in 2010. The focus is on shortening the generation interval and to select between full brothers (with identical conventional breeding value) for sex-limited traits. Case control studies, where individuals with extreme phenotypic differences e.g.

in terms of specific disease resistance can be identified, are used to search for SNP-markers which may help to select more effectively for vitality.

Zusammenfassung

Für die Versorgung der zunehmenden Weltbevölkerung leistet die Zucht von Legehennen einen nachhaltigen Beitrag. Verbesserte Selektionsmethoden wie z.B. die genomische Selektion bieten dafür gute Ansätze. Der praktische Nutzen konnte aber bisher noch nicht in kommerziellen Geflügelzuchtprogrammen nachgewiesen werden. Offensichtlich können den steigenden Kosten keine wesentlichen Einsparungen in der Leistungsprüfung gegenübergestellt werden. Im Bereich der Legehennen- und Broilerzucht werden firmeneigene SNP-Chips eingesetzt. Erste Ergebnisse werden für 2010 erwartet. Eine Verkürzung des Generationsintervalls und eine Selektion innerhalb männlicher Vollgeschwistergruppen bei geschlechtsgebundenen Merkmalen werden angestrebt. Anhand von Fall-Kontroll-Studien sollen Tiere mit extremen Abweichungen im Phänotyp als Grundlage für die Etablierung von SNP-Markern zur Selektion auf bessere Vitalität herangezogen werden.

Literature

AVENDAÑO, S., K. WATSON AND A. KRANIS (2009): Genomics in Poultry Breeding: the cost of doing business. Proc. EEAP Meeting Barcelona, 2009.

DEKKERS, J. (2009): Implementation of Genomic Selection in Egg Layer Chickens, Joint ADSA-CSAS-ASAS Annual Meeting, #34843.

FLISIKOWSKI K., H. SCHWARZENBACHER, M. WYSOCKI, S. WEIGEND, R. PREISINGER, J.B. KJAER AND R. FRIES (2009): Variation in neighbouring genes of the dopaminergic and serotonergic systems affects feather pecking behaviour of laying hens. Animal Genetics 40(2): 192-199.

HONKATUKIA, M., K. REESE, R. PREISINGER, M. TUISKULA-HAAVISTO, S. WEIGEND, J. ROITO, A. MÄKI-TANILA, J. VILKKKI (2005): Fishy taint in chicken eggs is associated with a substitution within a conserved motif of the FMO3 gene. Genomics 86: 225-232.

PREISINGER, R. (2010): Genomische Selektion beim Geflügel. Züchtungskunde 82: 9-13.

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New selection traits from group housing systems

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Introduction

With the increasing use of floor housing systems in the layer industry, the adaptability of laying hens under these management conditions has become of special interest for primary breeders. The breeding goal is to produce as many saleable eggs as possible per hen housed in a laying period, i.e. clean eggs to be collected in the nest and not misplaced somewhere else in the system. As a working hypothesis, it can be assumed that genetic differences exist between and within lines in pre-laying behaviour, search for a nest, duration of stay in the nest and the resulting distribution of oviposition throughout the day. Differences in the nesting behaviour may affect the number of nest boxes necessary to minimise floor eggs. Commercial egg producers prefer hens which e.g. actively use different tiers in an aviary system, visit the nest box only shortly before oviposition, lay the egg quickly and then leave the nest as soon as possible to make room for other hens.

To analyse relevant behaviour parameters, it is first of all necessary to develop a reliable technical system which permits the recording of reliable individual data under practical conditions. At the Experimental Station of the TU Munich-Weihenstephan, the “Weihenstephan funnel nest box” was developed for this purpose. The function of this single nest box can has been described by THURNER et al. (2005).

Figure 1: Experimental unit with 48 single nest boxes for individual testing in floor housing systems

The most important nesting behaviour trait, nest acceptance, is largely influenced by training and management, beginning during the rearing period. After transfer to the production house, adjustment of the laying nests to the requirements of the hens and frequent collection of misplaced eggs are important to minimise misplaced eggs. After a test flock has been properly trained and started production, data can be collected to answer the question: to what extent is the variation in nesting behaviour genetically determined and can thus be used as basis for genetic improvement?

Previous studies based on time-consuming video analysis of a limited number of hens or labour-intensive trap nests suggest that the behaviour (social arrangement) of the hens in small groups differs from typical large commercial flocks. If data are collected for genetic improvement, it is essential that the testing conditions simulate conditions in commercial units.

Research on nesting behaviour in floor systems

Since 2005, the Institute for Agricultural Engineering and Animal Husbandry of the Bavarian State Research Centre for Agriculture and Lohmann Tierzucht GmbH have cooperated in developing nest boxes for individual laying performance and nesting behaviour of laying hens at the experimental station Thalhausen. With the aid of the Weihenstephan Funnel Nest Box (FNB), the daily egg number, nest acceptance, exact oviposition time and the duration of stay in a nest box are automatically recorded for each hen in flocks of up to 360 layers. Transponder technology is used in combination with a specifically developed single nest box. Based on this recording system, genetic parameters for behaviour and performance traits in group housing systems were estimated and families with desirable performance profile selected for line improvement in the primary breeding program of Lohmann Tierzucht.

Figure 2: View of a Weihenstephan Funnel Nest Box

Selection criteria for nesting behaviour

Nest acceptance

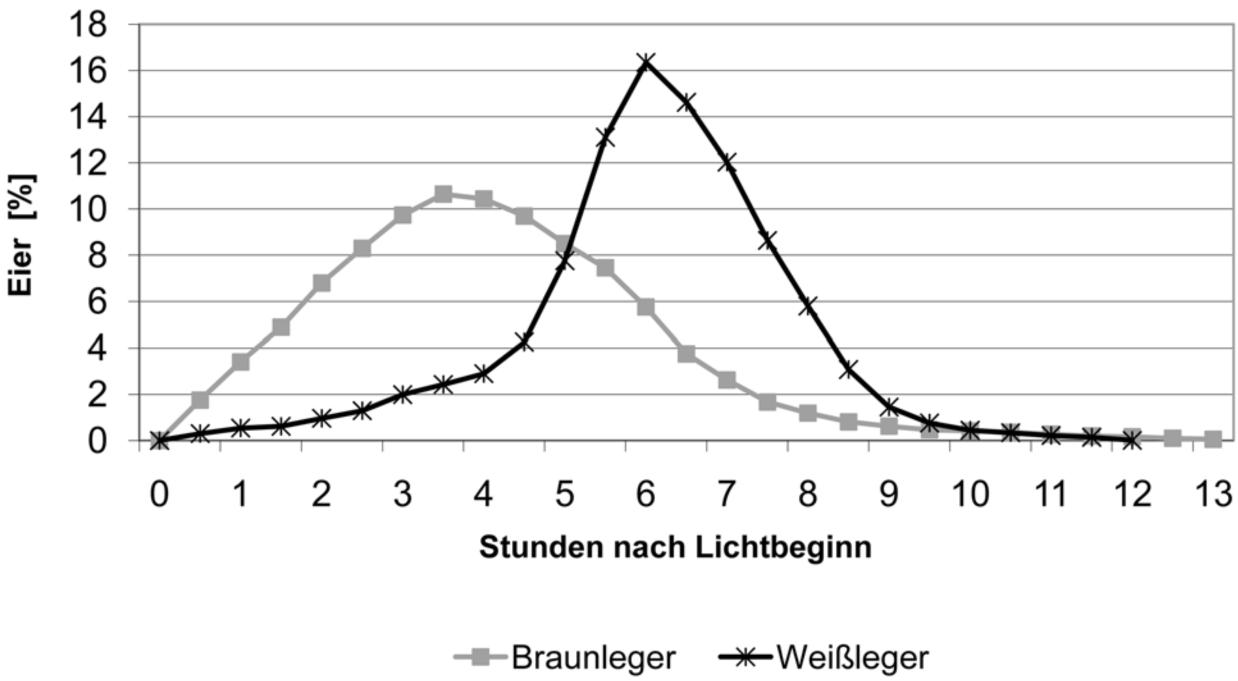
In analysing components of nesting behaviour, main focus is on nest acceptance, i.e. counting the number of "saleable" eggs laid in the nest. At the experimental station Thalhausen, distinctly different white-egg and brown-egg strains of Lohmann origin were performance-tested in pens for 360 hens, equipped with Funnel Nest Boxes. Daily nest visits were recorded with oviposition time for each hen during a period of up to one year. On the basis of the recorded number of nest eggs per hen, a family breeding value can be estimated for egg production, taking into account nest acceptance, which is then combined with traditional selection criteria in a selection index.

Figure 3: A hen in three phases: entering the nest, staying inside and leaving the nest.

Oviposition time

Based on individual records of the exact oviposition time we were able to compare the laying pattern of different lines of brown-egg and white-egg layers. It was found that most brown eggs were laid about two hours after the lights were turned on, whereas a high percentage of the white-egg layers started looking for a nest three hours after the beginning of the light day. The brown-egg layers had already reached the maximum of their daily production three hours after the lights came on, the white-egg laid most of their eggs six hours after the light day began. As shown in figure 4, the White Leghorn line concentrated the nest visits in two hours, whereas the brown-egg line spread the nest visits over four hours. Such a short time period in which most of the eggs were laid has also been found by many other authors as e.g. LILLPERS (1993) and ZAKARIA *et al.* (2005), but the time of the day for this period differed. While ZAKARIA *et al.* (2005) observed broiler breeder flocks in the morning, LILLPERS (1993) noticed that the early afternoon was a main egg laying period for a flock of White Leghorns housed in individual cages.

Figure 4: Distribution of oviposition time during the day for two different strains



Duration of stay in the nest

The narrow range of white-egg layers in terms of their oviposition time, combined with longer nest occupation per egg laid, means that more nest space is needed compared to the brown-egg layers to avoid misplaced eggs due to unavailability of nests when needed. While the brown-egg layers stayed an average of 30 minutes in the nests, the white-egg layers spent 45 minutes in the nest for each oviposition. Shorter occupation times for white layers, in a similar range as reported by ICKEN *et al.* (2009) for brown layers, were found by ZUPAN *et al.* (2008). Nest visits without oviposition were mainly observed at the beginning of the laying period, when hens have the habit to explore their new environment. Nest visits without oviposition lasted an average of 10 minutes for brown layers and nearly half an hour for white layers.

Table1: Average oviposition time and duration of stay in the nest for brown and white layers

trait	brown layer	white layer
oviposition time	08:00	09:45
duration of stay with oviposition	30 min	45 min
duration of stay without oviposition	10 min	28 min

Reducing the duration of stay in the nest would be desirable, if this can be achieved by selection. Apart from less investment for nests, soiling of nests and eggs could be reduced. However, possible negative correlations have to be kept in mind: hens which try hard to find a nest, may also want to stay longer in it. The final objective must be to collect of maximum number of saleable eggs from hopefully happy hens!

Time interval between ovipositions

As a component of total egg production, the variation in difference between two ovipositions could also be analysed from the detailed data. In table 2, individual hens of four different flocks were classified by their mean time interval in a laying sequence. We were surprised to find that up to 22 % of the hens laid two eggs with normal shells within less than 24 hours. Conspicuously, these hens did not have the highest laying performance. They laid fewer eggs than the hens which belong to the second category with an average time interval between 24 hours and 24 hours and 15 minutes. The relation between both traits is not linear, but our results tend to support the earlier observation of ATWOOD (1929), who found in a flock of 172 laying hens that shorter time intervals were associated with longer clutch lengths and higher total egg production. Later investigations of BEDNARCZYK *et al.* (2000) on more than 2000 layers also support these relationships and suggest that clutch traits may be effectively used in the selection index for laying hens.

Table 2: Percentage of hens in different time interval categories and corresponding laying performance

flock	mean time interval of laying sequences [hh:mm]							
	< 24:00		24:00 to 24:15		24:15 to 25:00		>25:00	
	hens [%]	rate of lay [%]	hens [%]	rate of lay [%]	hens [%]	rate of lay [%]	hens [%]	rate of lay [%]
1	3	70	70	79	22	70	3	43
2	22	79	63	80	12	67	2	27
3	20	63	47	72	18	58	4	24
4	9	57	74	72	11	67	1	9

Conclusion

With the Weihenstephan Funnel Nest Box, individual egg recording in floor housing systems has been made possible. The data collected provide interesting information about the oviposition time and duration of stay in the nests. Used for pedigreed hens from a primary breeding program, the data can be integrated in the current breeding program. Lohmann Tierzucht has started to add parameters of laying behaviour to conventional selection criteria, i.e. egg production, feed efficiency and egg quality, to adapt the performance profile of white-egg and brown-egg layers to future world market demands.

Zusammenfassung

Das Weihenstephaner Muldennest zeigt, dass eine Leistungsprüfung von Legehennen in alternativen Haltungssystemen möglich ist, und liefert darüber hinaus interessante Informationen bezüglich des Eiablagezeitpunktes und der Nestaufenthaltsdauer, die von wirtschaftlicher Relevanz sind. Da diese Form der Leistungsprüfung an Tieren mit Abstammung und individueller Kennzeichnung erfolgt, können die Daten direkt in das laufende Zuchtprogramm integriert werden. Damit stellt die Lohmann Tierzucht die Selektion der Zuchttiere auf eine noch breitere Basis. Neben der Futterverwertung und Eiqualität steht die Zahl verkaufsfähiger Nesteier ganz oben in der Prioritätenliste.

Reference

ATWOOD, H.; 1929: Observations concerning the time factor in egg production. Poultry Science 8: 137-140.

BEDNARCZYK, M., K. KIECTZEWSKI, T. SWACZKOWSKI; 2000: Genetic parameters of the traditional selection traits and some clutch traits in a commercial line of laying hens. Archiv für Geflügelkunde 64:129-133.

ICKEN, W., S. THURNER, D. CAVERO, M. SCHMUTZ, G. WENDL und R. PREISINGER; 2009: Analyse des Nestverhaltens von Legehennen in der Bodenhaltung. Archiv für Geflügelkunde 73:102-109.

LILLPERS, K. (1993): Oviposition patterns and egg production in domestic hen. Dissertation, Swedish University of Agricultural Science, Department of Animal Breeding and Genetics, Uppsala.

THURNER, S., G. WENDL, R. PREISINGER, G. FRÖHLICH, S. BÖCK und R. WEINFURTNER (2005): Entwicklung eines automatischen Legenestes zur einzeltierbezogenen Erfassung von Verhaltens- und Leistungsparametern bei Legehennen in artgerechter Gruppenhaltung. In: Aktuelle Arbeiten zur artgemäßen Tierhaltung 2005, KTBL-Schrift 441, 274-283. ISBN:3-7843-2189-5.

ZAKARIA, A.H., P.W. PLUMSTEAD, H. ROMERO-SANCHEZ, N. LEKSRISOMPONG, J. OSBORNE and J. BRAKE (2005): Oviposition pattern, egg weight, fertility, and hatchability of young and old broiler breeders. Poultry Science 84: 1505-1509.

ZUPAN, M., A. KRUSCHWITZ, T. BUCHWALDER, B. HUBER-EICHER and I. ŜTUHEC (2008): Comparison of the prelaying behaviour of nest layers and litter layers. Poultry Science 87: 399-404.

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"Temperature training" during the last days of incubation: a new method to improve poultry performance

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Introduction

Incubation climate can significantly influence hatchability, chick quality, and later performance in poultry. One of the most important factors is the incubation temperature (Decuypere and Michels, 1992). Changes of only 1°C from the optimum have a major impact, for instance, on hatching results in turkeys (French, 1994), but the strength of this influence depends on the time frame used and the duration of changes in incubation temperature during embryogenesis (French, 2000). Further, during 'critical periods' of early development incubation climate may cause life-long modifications in body functions (Tzschentke and Plagemann, 2006).

Questions of interest are, whether the development of body functions can be improved by short-term prenatal temperature stimulation and which time window of the incubation period is optimal for the application of such stimulations. In poultry, the final incubation period is characterized by well-developed body functions (e.g. temperature regulation, Tzschentke 2007) as well as dramatic changes in the quality of regulatory processes (Tzschentke and Plagemann, 2006). On the one hand, environmental stimulation could improve the maturation and adaptability of body functions ('training effect', Nichelmann and Tzschentke, 2002). The end of the incubation period seems to be a 'critical developmental period' so that long-lasting effects of temperature stimulation on body functions and, finally, on the post-hatching performance can be expected. Results from a previous study with a small sample of broiler chicks indicate that short-term variation in incubation temperature during the last 4 days of incubation may have a long-lasting stimulating influence on broiler performance at age of slaughter (Janke *et al.*, 2006).

So far, different incubation programs for broiler chicks and other poultry species have not included modification of daily incubation temperature as a possibility to stimulate the development of body functions and/or to increase post-hatching adaptability to different environmental conditions and thereby to improve performance. To test our hypothesis that short-term variation in incubation temperature during the last days of incubation can improve hatching results and have long-lasting effects on the performance in poultry, studies were carried out in different modern poultry breeds under various experimental conditions and are still in progress. The questions of interest are: (1) which pattern of temperature stimulation is optimum, (2) are differences between strains or sexes in their response to 'temperature training' significant and (3) to what extent are breeder age and egg storage time affecting the outcome of prenatal 'temperature training'?

In this paper we report how commercial male and female broiler chicks (ROSS 308) responded to (1) a mild short-term and (2) chronic increase in incubation temperature at the end of incubation (day 18 until hatching). The traits studied under experimental conditions were hatchability, sex ratio at hatch (secondary sex ratio), chick quality and growth performance to age at slaughter.

Materials and methods

Incubation

In 6 trials a total of 9,883 hatching eggs of ROSS 308 broilers from breeders aged between 30 to 50 weeks were incubated from days 1 to 17 under normal incubation temperature (37.2 – 37.4 °C). On day 18 the eggs were transferred to hatchers with three different temperature programs:

- 37.2 – 37.4 °C (control),
- 38.2 – 38.4 °C (chronic warm incubation),
- 38.2 – 38.4 °C for 2 hours daily (short-term stimulation, 'temperature training').

After analysis of the results from the first two trials with three incubation treatments, we used only two different incubation treatments (short-term warm stimulation vs. control) in trials 3 to 6, because this method seemed to be the best to improve hatchability and later performance.
In all trials, the one-day-old chicks were sexed. Random sampling of chicks (30 males and 30 females from all incubators) were analysed by the Pasgar©score (vitality, navel, legs, beak, and belly). The highest chick quality has a score of 10 and one point is subtracted for each abnormality recorded in one of the above-mentioned five criteria (www.pasreform.com).

Growing period until slaughter age

Day-old chicks from incubation trial 2 were used for a follow-up growth trial. A total of 120 male and 120 female chicks from every incubator were randomly assigned to 20 pens with 12 chicks each (10 pens per sex) and grown to 35 days of age. The chicks were kept at temperatures recommended by the breeding company: 35–34 °C on days 1-2, 33-32 °C on days 3 and 4, 30 °C on days 5 to 7, 29 °C during week 2, 26 °C during week 3, 22 °C during week 4, 20 °C during week 5.

Feed (composition as shown in table 1) and water were provided *ad libitum*. Body weight and feed intake were recorded on days 1, 14, 21 and 35. Body weight was recorded individually and analysed on per pen. Feed was weighed back weekly per pen. One bird per pen with close to average body weight of this pen was slaughtered at the end of the trial (10 males and 10 females per treatment group) to determine carcass composition. Weights of total breast meat (without skin), complete right leg, liver, heart, gizzard, spleen and sum of abdominal and viscera fat were individually recorded. All parts were expressed as percentage of body weight.

Table 1: Feed formulation in the growth trial with nutrient content (g/kg)

Ingredient	Trial 1
Wheat	200.0
Corn	336.1
Soya bean meal	376.7
Soya oil	42.9
Di-calcium-phosphate	22.0
Calcium carbonate	4.6
Sodium chloride	3.2
DL-methionine	2.8
L-lysine-HCl	1.7
Premix [1)]	10.0
Dry matter [2)]	903
Crude protein [2)]	208
ME, MJ/kg [3)]	12.8
Lysine [4)]	12.5
Methionine+Cystine [4)]	9.6

[1)] Vitamin- mineral premix provided per kg of diet: Fe, 32 mg; Cu, 12 mg; Zn, 80 mg; Mn, 100 mg; Se, 0.4 mg; I, 1.6 mg; Co, 0.64 mg; retinol, 3.6 mg; cholecalciferol, 0.088 mg; tocopherol, 40 mg; menadion, 4.5 mg; thiamine, 2.5 mg; riboflavin, 8 mg; pyridoxine, 6 mg; cobalamin, 32 µg; nicotinic acid, 45 mg; pantothenic acid, 15 mg; folic acid, 1.2 mg; biotin, 50 µg; choline chloride, 550 mg

[2)] Analysed values;

[3)] Calculated values (WPSA; 1985);

[4)] Calculated values

The results from six hatching trials and one growth trial were analyzed statistically by one-way analysis of variance, using the following model for all traits:

$$y_{ij} = \mu + a_i + e_{ij}$$

where
μ = overall mean
a_i = treatment group (hatcher, temperature regime)
e_{ij} = error term.

The significance of differences was evaluated by the Student-Newman-Keuls Test ($P \leq 0.05$), using SAS operating system (Version 9.1, 2002/03).

Results and Discussion

Influence of "temperature training" on hatchability and chick quality

The results from incubation trials 1 and 2 showed that chronic warm incubation (+ 1 °C during the last 4 days of incubation) had no negative effect on hatchability (96.4% and 94.1% in the experimental group vs. 96.2% and 94.6% in the control) as well as chick quality. But only short-term warm stimulation (+ 1°C for 2 h per day during the last 4 days) improved hatchability by more than 1.5%. The results of trials 1 and 2 were confirmed in trials 3-6: short-term warm stimulation during the last 4 days of incubation improved hatchability of broiler chicks significantly (Tab. 2). Further, in all of the 6 incubation trials short-term warm stimulation consistently changed the sex ratio in the direction of more males: the ratio of 52.4% males to 47.6% females was significantly different from the control group (50.2% males to 49.8% females).

Other authors who studied the influence of short-term warm loads during incubation on hatching results used different warm-loads (39-39.5 °C) for different lengths of time (3-24 h per day during incubation days 16-18). The results were contradictory (e.g. Moraes *et al.*, 2004; Yahav *et al.*, 2004; Collin *et al.*, 2005). In our study we used a milder and shorter warm load during a later period (day 18 to hatch) of embryonic development for the application of 'temperature training'.

In poultry embryos, peripheral and central nervous thermoregulatory mechanisms as well as other body functions are well developed at the end of incubation (Tzschentke, 2007). Therefore no negative side effects would be expected after mild temperature variations during this stage. During the final stages of embryonic development, body functions develop feedback mechanisms, which change the quality of regulatory processes dramatically. This seems to be a 'critical period' in the development of body functions (Tzschentke and Plagemann, 2006).

Our hypothesis is that environmental 'training' of bodily functions during the last days of incubation can be a tool to improve their maturation and reactivity to environmental variations life-long and thus improve adaptability, vitality, health and productivity of the chickens throughout their life cycle.

Chick quality sensitively reflects incubation problems and is recommended as an indicator in the evaluation of incubation conditions (Boerjan, 2004). For evaluating the chick quality of day-old chicks (www.pasreform.com) the Pasgar©score was used. With this score the vitality of individual chicks can be evaluated using morphological parameters as well as the chicks' alertness (reflexes). In all trials, the Pagar©score was higher than 9 (of maximum 10) for male and female chicks, in some trials suggesting a beneficial effect of short-term stimulation with increased temperature. However, the mean value of the Pasgar©score was 9.6 for all groups, with no difference between sexes or temperature treatment (Tab. 2).

Table 2: Summary of results from 6 incubation trials: means and standard deviations (9883 hatching eggs set; 5.7 % infertile; egg weight 62.2 ±.2.4 g)

Parameter	Control	4 days, 2 h, +1°C
Eggs per hatcher	4323	3710
Hatched live chicks, %	94.1 ±1.7	95.4 ±2.6
Female chicks, %	49.8 ab ±2.0	47.6 b ±1.4
Male chicks, %	50.2 ab ±2.0	52.4 a ±1.4
Pasgar©score		
Female chicks	9.6 ±0.3	9.6 ±0.3
Male chicks	9.6 ±0.2	9.6 ±0.3

a; b; c – Means with different letters differ significantly

We interpret the consistently increased male:female ratio after short-term stimulation as a clear sign for improved chick vitality. It is known that male embryos are more sensitive to environmental factors than female embryos (Bogdanova and Nager, 2008, Catalano *et al.*, 2008). Related to this fact, our hypothesis is that prenatal 'temperature training' with short-term warm loads improves chick vitality, especially in the late-term male embryos. For the male chicks of ROSS 308 broilers used in our study the short-term warming resulted in a significant increase in hatchability due to the fact that relatively more male chicks hatched than in the control group.

Influence of 'temperature training' on performance until slaughter age

In the Growth Trial, the feed intake of male broilers from all three incubation groups was significantly higher during the entire fattening period than for the females. The 24 h chronic warm incubation during the last 4 days of incubation had no negative effect on the later performance of male or female broilers. But only prenatal short-term warm stimulation improved the performance until slaughter age, especially in the male broilers (Tab. 3). Daily feed intake of male broilers in the short-term warm stimulated group was higher from the first day in comparison to the control and the chronic warm incubated group. The male broilers from the short-term warm stimulated group gained 64.8 g per day to 35 days of age and reached a final weight of 2336 g, which was a significant increase by 2.9% over the control group. Feed conversion of the male but also female broilers from short-term prenatal warm stimulation was improved in comparison with the females of the control and chronic warm incubated groups.

Table 3: Performance of male broilers - Incubation trial 2 (Mean, SD)

Treatment Group	1 - control		2 - chronic warm		3 - 2h warm per day	
Age, days	Mean	SD	Mean	SD	Mean	SD
	Feed intake, g/d					
1 - 14	35.0	0.9	35.4	0.6	35.9	0.7
15 - 21	89.9	3.4	93.0	2.4	92.5	3.6
22 - 35	155.3	4.0	155.3	4.8	156.3	4.5
1 - 35	93.3	3.2	94.3	2.4	95.2	3.0
	Body weight gain, g/d					
1 - 14	28.7	0.6	29.2	0.9	29.6	0.7
15 - 21	66.8	3.8	68.3	2.3	69.6	3.4
22 - 35	94.4 b	4.0	95.0 b	3.1	97.9 a	2.6
1 - 35	62.2 b	2.9	63.0 ab	2.0	64.8 a	2.0
	Feed conversion, kg feed/kg gain					
1 - 14	1.22	0.02	1.22	0.02	1.22	0.01
15 - 21	1.35	0.04	1.36	0.03	1.33	0.04
22 - 35	1.65	0.08	1.64	0.05	1.60	0.03
1 - 35	1.50 a	0.04	1.50 a	0.02	1.47 b	0.02
	Body weight in g					
14	445	45	453	50	455	44
21	919	100	937	118	942	118
35	2270 b	203	2292 ab	243	2336 a	191

a; b - Means with different letters differ significantly

The long-lasting stimulating effect of the prenatal 'temperature training' with short-term warm load on the performance at the end of the fattening period can be explained similarly to that already described for the hatching results. Besides 'training effects', which appears to improve the vitality and health especially in male broiler chicks, a stimulating effect on muscle development can be assumed. In embryos as well as chicks of meat-type poultry mild heat exposure stimulates skeletal muscle growth due to an immediate increase in satellite cell proliferation and accelerated differentiation (Halevy *et al.*, 2001, 2006a,b; Maltby *et al.*, 2004). Further, if applied during developmental 'critical periods' environmental influences can change the programming of respective body functions (Tzschentke and Plagemann, 2006), so that 'temperature training' can also induce life-long alterations in the regulation of food intake and body weight.

The carcass analyses based on the small sample of ten male and ten female broilers from each group did not reveal any difference due to hatching egg treatment. No differences could be found in the carcass composition (meat, organs, fat) of both sexes between the groups with different incubation temperature. For marketing it is especially important that body fat content is not increased by changes in the incubation environment.

The complete results from the male and female chickens of this study were published recently (Tzschentke and Halle, 2009).

Conclusions

An incubation temperature profile including short-term temperature variation can improve broiler performance. The final incubation period seems to be a very sensitive and relatively safe period for 'temperature training'; this also holds for chronic elevated temperature. However, different goals (e.g. improvement of body weight or improvement of temperature adaptation) need different temperature manipulation. Short-term 'temperature training' of the embryos improved post-hatch performance under our conditions, whereas chronic temperature changes during the last days of incubation may improve adaptation to high temperature during the fattening period. The effect of temperature manipulations at the end of incubation is sex-specific and may also depend on the strain of chickens used, breeder age (Yalcin *et al.*, 2005) and egg storage conditions.

Summary

Incubation climate can significantly influence hatchability, chick quality, and later performance in poultry. One of the most important factors is the incubation temperature. For practical application of prenatal 'temperature training' the questions of interest are: (1) which pattern of temperature variation is the optimum one, (2) are there differences in the effect of prenatal 'temperature training' between poultry breeds and lines as well as between male and female animals, and (3) what is the influence of factors like breeder age and egg storage time on the outcome of prenatal 'temperature training'? In an exemplary study carried out in male and female broiler chicks (ROSS 308) the influence of a mild (1°C over standard) short-term (2 h daily), as well as chronic (over 4 days) increases in incubation temperature at the end of incubation (day 18 until hatch) on hatchability, sex ratio of the hatched chicks (secondary sex ratio), chick quality and performance until age of slaughter was investigated. Chronic as well as short-term increases in incubation temperatures by 1 °C over standard during the last 4 days of incubation had no negative effect on hatchability, chick quality and post-hatching performance. Only short-term warm stimulation ('temperature training') improved hatchability by more than 1.5% and induced a significantly higher percentage of hatched male chicks in all experimental series. At slaughter the male broilers incubated under short-term warm stimulation reached the highest final live weight and a significantly better feed conversion in comparison with males from the control as well as males from the chronic warm incubated group. These results suggest that 'temperature training' with short-term temperature variation during the last days of incubation can be applied to improve poultry performance (applied for European Patent EP2105048, published September 30, 2009).

Zusammenfassung

Das Brutklima beeinflusst signifikant das Schlupfergebnis, die Kükenqualität und die spätere Leistung des Geflügels. Einer der bedeutendsten Klimafaktoren ist die Bruttemperatur. Für die praktische Anwendung eines pränatalen 'Temperatur-trainings' sind u.a. folgende Fragen zu beantworten: (1) Welche Form der Temperaturvariation ist optimal? (2) Treten Unterschiede hinsichtlich des Effekts eines pränatalen 'Temperaturtrainings' zwischen verschiedenen Geflügelarten, Zuchtlinien sowie männlichen und weiblichen Tieren auf? (3) Welchen Einfluss üben Faktoren wie das Alter der Elterntiere und die Dauer der Lagerung der Bruteier auf den Erfolg des pränatalen 'Temperaturtrainings' aus? In einer Beispielsstudie an männlichen und weiblichen Broilern (ROSS 308) wurden der Einfluss einer milden (1°C über dem Standard) kurzzeitigen (täglich 2 h) sowie einer chronischen (über 4 Tage) Erhöhung der Bruttemperatur am Ende der Brutphase (Tag 18 bis Schlupf) auf die Schlupfrate, das Geschlechtsverhältnis der geschlüpften Küken (sekundäres Geschlechtsverhältnis), die Kükenqualität und die Leistung bis zum Schlachtalter untersucht. Chronische und kurzzeitige Erhöhung der Bruttemperatur in der Endphase der Inkubation beeinflussten das Schlupfergebnis, die Kükenqualität und die spätere Leistung der Tiere nicht negativ. Jedoch nur die kurzzeitige Warmstimulierung ('Temperaturtraining') führte zu einer Verbesserung der Schlupfrate um mehr als 1,5% und in allen Versuchsserien zu einer signifikanten Erhöhung des Anteils geschlüpfter männlicher Küken. Die pränatal kurzzeitig warmstimulierten männlichen Broiler erreichten im Schlachtalter ein signifikant höheres Schlachtgewicht und eine signifikant verbesserte Futterverwertung im Vergleich zu den männlichen Tieren der Kontrolle und der chronisch warm erbrüteten Gruppe. Die Ergebnisse zeigen, dass ein Bruttemperaturprofil mit kurzzeitiger Temperaturvariation ('Temperaturtraining') in den letzten Bruttagen die Leistung des Geflügels deutlich verbessern kann (eingereicht zum Europäischen Patent 2105048, publiziert 30. September 2009).

References

BOERJAN, M. (2004): Maximising chick uniformity, performance and vitality. World Poultry, 20: 18-20.

BOGDANOVA, M.I. & R.G. NAGER (2008): Sex-specific costs of hatching last: an experimental study on herring gulls (Larus argentatus). Behavioral Ecology and Sociobiology, 62: 1533-1541.

CATALANO, R., T. BRUCKNER & K.R. SMITH (2008): Ambient temperature predicts sex ratios and male longevity. PNAS, 105: 2244-2247.

COLLIN, A., M. PICARD & S. YAHAV (2005): The effect of duration of thermal manipulation during broiler chick embryogenesis on body weight and body temperature of post-hatched chicks. Animal Research, 54: 105-111.

DECUYPERE, E. & H. MICHELS (1992): Incubation temperature as a management tool: a review. World´s Poultry Science Journal, 48: 28-38.

FRENCH, N.A. (1994): Effect of incubation temperature on the gross pathology of turkey embryos. British Poultry Science, 35: 363-371.

FRENCH, N. (2000): Effect of short periods of high incubation temperature on hatchability and incidence of embryo pathology of turkey eggs. British Poultry Science, 41: 377-382.

HALEVY, O., A. KRISPIN, Y. LESHEM, J.P. MCMURTRY & S. YAHAV (2001): Early age heat exposure affects skeletal muscle satellite cell proliferation and differentiation in chicks. American Journal of Physiology-Regulatory Integrative and Comparative Physiology, 281: 302-309.

HALEVY, O., M. LAVI, & S. YAHAV. (2006a): Enhancement of meat production by thermal manipulations during embryogenesis of broilers, in: YAHAV, S. & TZSCHENTKE, B. (Eds.) New insights into fundamental physiology and peri-natal adaptation of domestic fowl, pp. 77-87 (Nottingham University Press).

HALEVY, O., S. YAHAV & I. ROZENBOIM (2006b): Enhancement of meat production by environmental manipulations in embryo and young broilers. World´s Poultry Science Journal, 62: 485-497.

JANKE, O., B. TZSCHENTKE & I. HALLE (2006): Does variation in incubation temperature increase broiler chicken performance? Proceedings of the European Poultry Conference, Verona, (on CD).

MALTBY, V., A. SOMAIYA, N.A. FRENCH & N.C. STICKLAND (2004): In ovo temperature manipulation influences post-hatch muscle growth in the turkey. British Poultry Science, 45: 491-498.

MORAES, V.M.B., R.D. MALHEIROS, V. BRUGGEMAN, A. COLLIN, K. TONA, P. VAN AS, O.M. ONAGBESAN, J. BUYSE, E. DECUYPERE & M. MACARI (2004): The Effect of timing of thermal conditioning during incubation on embryo physiological parameters and its relationship to thermotolerance in adult broiler chickens. Journal of Thermal Biology, 29: 55-61.

NICHELMANN, M. & B. TZSCHENTKE (2002): Ontogeny of thermoregulation in precocial birds. Comparative Biochemistry and Physiology, Part A, 131: 751-763.

TZSCHENTKE, B. (2007): Attainment of thermoregulation as affected by environmental factors. Poultry Science, 86: 1025-1036.

TZSCHENTKE, B. & I. HALLE (2009): Influence of temperature stimulation during the last 4 days of incubation on secondary sex ratio and later performance in male and female broiler chicks. British Poultry Science 50: 634-640.

TZSCHENTKE, B. & A. PLAGEMANN (2006): Imprinting and critical periods in early development. World´s Poultry Science Journal, 62: 626-637.

VOGT, H. (1986): WPSA-Energieschätzungsgleichung. Working Group No. 2 „Nutrition" of the European Federation of WPSA Report of the Meeting, World's Poultry Science Journal, 42:189-190.

YAHAV, S., A. COLLIN, D. SHINDER & M. PICARD (2004): Thermal manipulation during broiler chick embryogenesis: effects of timing and temperature. Poultry Science, 83: 1959-1963.

YALCIN, S., S. ÖZKAN, M. CABUK, J. BUYS, E. DECUYPERE & P.B. SIEGEL (2005): Pre- and postnatal conditioning induced thermotolerance on body weight, physiological responses and relative asymmetry of broilers originating from young and old breeder flocks. Poultry Science, 84: 967-976.

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"White Gold" and Aral Sea disaster – Towards more efficient use of water resources in the Khorezm region, Uzbekistan

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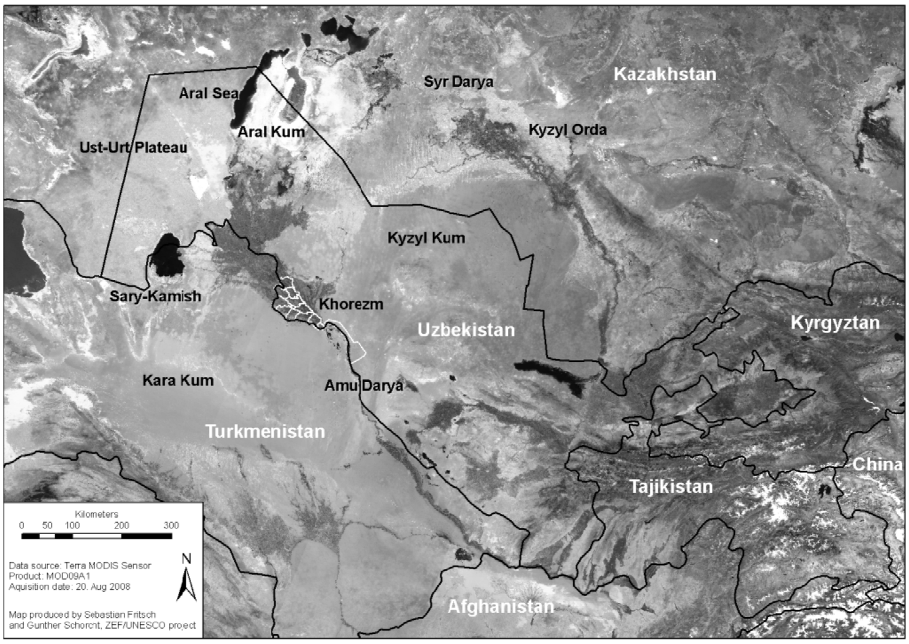
Introduction

Khorezm, one of the twelve provinces of Uzbekistan, is one of the oases of the great historic civilizations of Central Asia, fed by the ancient river Oxus, today the Amu Darya. For at least 3000 years, waters from the Amu Darya and Syr Darya rivers supported thriving agricultural communities in the Aral Sea Basin (ASB) (Figure 1). But during the Soviet era (1924-1991), and particularly since the late 1950ies, the Amu Darya and Syr Darya rivers were tapped excessively and used in immense irrigation systems to secure the production of cotton, the "White Gold". The area of irrigated land in the ASB almost doubled, from 4.5 million ha in the fifties to 7.9 million ha in 2006. This dramatic expansion of the irrigation system resulted in substantial decrease in water inflow to the Aral Sea from 43 km^3 $year^{-1}$ in 1960s to only 9 km^3 $year^{-1}$ during 2001-2005. Consequently, within decades the Aral Sea shrunk from being the fourth largest freshwater lake in the world (6.8 million ha surface area) to less than 20% of its former surface. By 2006 the sea's level had dropped 23 m, the volume decreased by 90%, and the salinity rose to more than 100 g l^{-1} (Micklin 2007). The desiccation of major parts of the Sea continues to threaten ecosystems and livelihoods, particularly in the circum-Aral region.

Figure 1 Map of the Aral Sea Basin (ASB) with the location of Khorezm province in Uzbekistan.



It is extremely doubtful that the Aral Sea could be restored to its pre-1960s level, size, and ecological state, as this would necessitate a 51% reduction in annual irrigation withdrawals of 105 km^3. Given the dependency of the two primary irrigation water users in the basin (Uzbekistan and Turkmenistan) on cotton production as a major source of foreign currency revenues, significant reductions in irrigated area and water use are highly unlikely in the near to mid-term future. Providing solutions to the adverse environmental, economic, and social impacts of the Aral Sea disaster is of crucial importance for the sustainable development of the basin. However, "creeping environmental problems" such as the Aral Sea desiccation require "creeping solutions" (Glantz, 2008) i.e. incremental steps that can be taken to improve the resource-use efficiency and environmental as well as socio-economic conditions.

In this context, the BMBF[1] funded ZEF/UNESCO project *"Economic and Ecological Restructuring of land and Water Use in the Region Khorezm, Uzbekistan* (www.uni-bonn.de/khorezm)", was initiated to develop comprehensive, science-based concepts and technologies for improving economic efficiency and ecological sustainability of agricultural land and water use in the Khorezm region.

In this paper, we analyse the socio-technical aspects of the irrigation water management system in Khorezm and discuss options for improving the efficiency, equity, and sustainability of water use in the region. This article is partly based on Oberkircher, L., Tischbein B., Hornidge, A.-K., Schorcht, G., Bhaduri, A., Awan, U.K., Manschadi, A.M., forthcoming: *Reconceptualising Water Management in Khorezm, Uzbekistan / Recommendations towards IWRM, ZEF Working Paper Series. Vol. 54. Bonn: Zentrum für Entwicklungsforschung.*

Key characteristics of the Khorezm region

Khorezm is located in the northwest of Uzbekistan about 250 km south of the present shores of the Aral Sea (Figure 1). It covers an area of about 6,800 km² of mostly dry arid desert of which roughly 270,000 ha can be used for irrigated agriculture. The region is characterised by a continental arid climate with hot and dry summers and cold winters with an annual average precipitation of less than 100 mm. Hence, agricultural production and rural livelihoods in Khorezm rely entirely on irrigation water supply from the Amu Darya river.

Of the 1.5 million of Khorezm's population (as of 2008), over 70% resides in rural areas and is mostly engaged in cotton, wheat and rice production either as private farmers (fermers), peasants (dehqons), workers on private farms, or a combination of the latter two. Unemployment rates are high and about 28% of the population live below the poverty line of 1 US$ per day (Müller 2006).

Irrigated crop fields in the Khorezm province, Uzbekistan (photo courtesy of Dr. G. Rücker)

[1] German Federal Ministry of Education and Research

Agricultural production system

Agriculture is the most important sector of the economy in Khorezm accounting for about 67% of the total regional GDP. At present, cotton, wheat, rice and fodder maize dominate the crop portfolio of the farmers in the region. Other crops such as potatoes, vegetables, fruits, grapes and melons are produced mainly on rural household plots for home consumption or sale on local markets (Bobojonov 2008).

Cotton plays by far the largest role in the regional economy – it occupies 50% of the irrigated cropland, consumes about 40% of the total water supply to the region, contributes as much as 16% to the GDP, and earns virtually 99% of the total export revenues of Khorezm (Rudenko 2008).

Cultivation of winter wheat was introduced in 1992 as part of the national programme for achieving food self-sufficiency. Consequently, the area under wheat in Khorezm increased to about 20% particularly at the expense of fodder crops such as lucerne (Djanibekow 2008), which was widely cultivated in rotation with cotton during the Soviet Union period. Rice is the third important crop in Khorezm and almost one-third of rice in Uzbekistan is produced in this region. In spite of occupying only 10% of the cropped area, rice consumes around 30% of the total water supply because it is grown in paddies; small basins which are flooded with water throughout most of the growing season from April-May until September.

One of the key characteristics of agriculture in Khorezm is that cotton and winter wheat are produced under the so-called 'state-order' or 'state-procurement' system (Djanibekow 2008). This means at the onset of each cropping season, the state government imposes production 'quotas' and determines the area on each farm that has to be allocated to cotton and winter wheat. The government also sets the prices, and organizes the processing and export (in the case of cotton). In turn, it ensures the supply and delivery of water, diesel, fertilisers, and other required inputs via state-controlled agencies (Rudenko 2008). The input prices are entirely determined by the central government and there is no private input market. The amount of cotton and winter wheat that farmers must deliver is determined by the soil fertility of their fields, expressed as soil bonitet. The local government officials use the soil bonitet index to determine the crop yields that the farmers must obtain from each individual field. Based on this system of area- and production-based quota for cotton and wheat, the irrigation management organisations at national, regional, and local level determine crop water requirements and develop delivery plans for each cropping season (see below).

The prescription of the strategic crops cotton and winter wheat by the national administration is exclusively restricted to the fermers (private farmers). These family-based agricultural enterprises have evolved following a series of land reforms initiated by the Government of Uzbekistan to decollectivise the former state and collective farms. The fermers have lease contracts of up to 50 years because agricultural land has remained state property. In 2007, there were 17000 fermers in the Khorezm region with an average farm size of about 10-40 ha (Djanibekow 2008). Despite the ongoing lease contracts, farmland was recollected by the state at the end of 2008 and once more redistributed in bigger plots to roughly one fourth of the former fermers (Djanibekow *et al.* 2010). In the course of land reforms, subsistence farmers (dehqons) were also granted a private plot of about 0.13 irrigated hectares (called tamorka). This was in addition to the garden plot of 0.12 ha (around the house). These tamorka plots account for about 20% of the irrigated land in Khorezm and play an important role in the production of fodder, vegetables, and fruits.

Irrigation water management

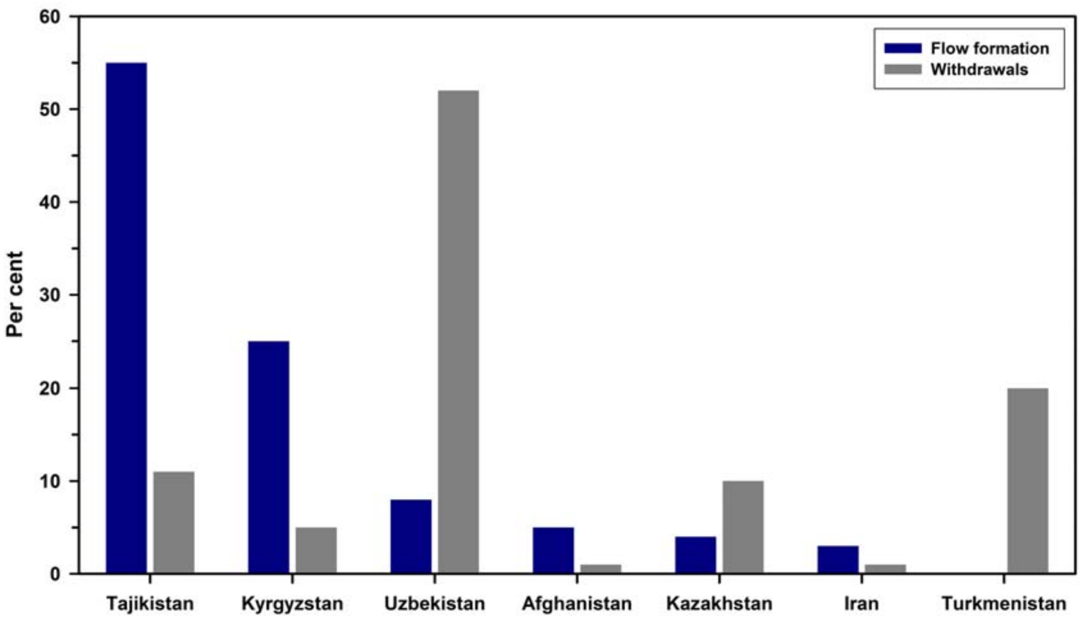
Officially, about 3.5 - 5 km³ of water per year are diverted from the Amu Darya river to irrigated fields in Khorezm through a dense network of 16000 km irrigation channels. During the severe droughts such as in 2000, 2001, and 2008, however, water supply to the region can be reduced by 40%. The water arriving in Khorezm is partly stored in the Tuyamuyun water reservoir, and its volume is rationed depending on the monthly water demand in the region. More than 95% of the water is used for agricultural purposes. Irrigation water is conveyed to the fields in open, predominantly non-lined channels resulting in substantial losses due to percolation, seepage, evaporation, and overflow to the drainage system. In 2005, a year with sufficient water supply, nearly 60% of incoming water ended up in the drainage system flowing directly to desert sinks (Conrad *et al.* forthcoming).

Control gates for regulating water distribution on a main canal system in Khorezm
(photo courtesy of Dr. A.M. Manschadi)

Vulnerability to fluctuations in water supply

Due to its tail-end situation along the Amu Darya river, the Khorezm region highly reliest on the upstream areas for water supply. The Amu Darya's water is derived from the mountain ranges of the Pamirs and Tien-Shan located in the upstream countries Kyrgyzstan, Tajikistan and Afghanistan. About 85% of the total surface water resources in the ASB (117 km³) is formed in the territories of these countries. The major water users in the basin, however, are the downstream nations Uzbekistan, Turkmenistan, and Kazakhstan, which together account for 82% of water consumption. Uzbekistan alone consumes about 50% of the total river flow in the basin (Figure. 2).

Figure 2 Average annual water flow formation and withdrawals in the Aral Sea Basin (data source: Micklin 2007)



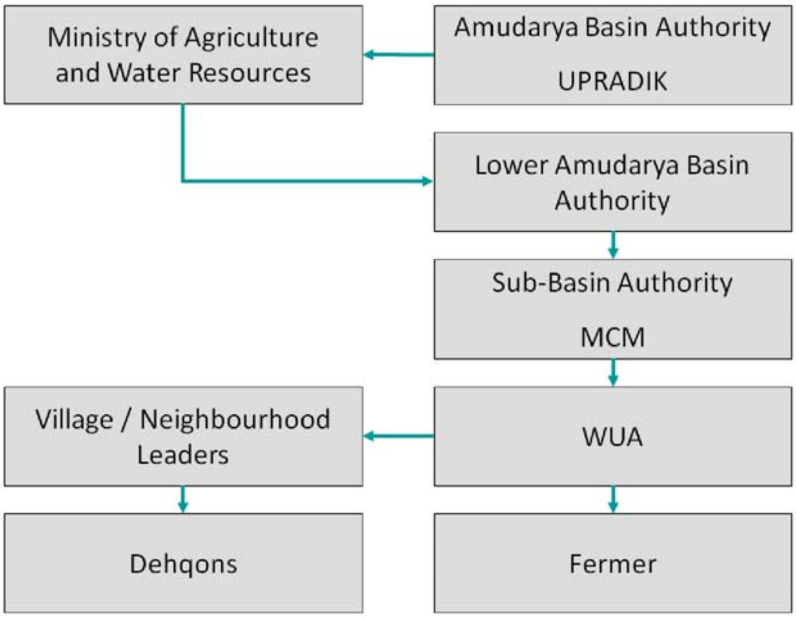
This distinct spatial pattern of water formation–water consumption in the ASB has been the source of tensions over water use among and within the riparian states. Under the Soviet Union, a centrally-planned system of water quotas regulated water allocation among the five Central Asian republics with the overall aim of providing irrigation water to downstream regions in the summer for maximising the cotton production. In exchange for water, the energy-scarce upstream republics (Kyrgyzstan and Tajikistan) received fossil fuels (coal and gas) at subsidised prices to generate electricity and heat in the winter. The breakdown of Soviet Union and establishment of independent Central Asian republics in 1990 ended this centrally-managed "water-energy nexus". Higher post-Soviet market prices for oil and gas together with unreliable delivery and the pursuit of energy security in the upstream countries increased the demand for hydropower in upstream republics. As a result, large quantities of water are released by up-stream countries in winter to generate heat and electricity during peak demand season, so that less water is available during the summer irrigation season in downstream regions. While Central Asian countries have so far managed to avoid open conflicts over water, the situation might spin out of control in the future. Climate change, accelerated melting of glaciers, and changes in temperature and rainfall patterns will undoubtedly increase the occurrence of extreme weather events such as frost, droughts, and floods in the region. In 2008, for instance, a severe drought combined with an extremely cold winter resulted in a compound water-energy-food crisis and serious political tensions in Central Asia. Furthermore, the downstream water availability is likely to worsen if Afghanistan stabilises and begins to develop new irrigation schemes and uses more water (planned are at present about 800000 ha). Therefore it is likely that the downstream regions, such as Khorezm province, will particularly be affected by decreasing water availability.

Water management practices

The Soviet heritage and the extensive irrigation and drainage systems established over decades of central planning created a strong "path dependency" of the agricultural production on central administrative systems. The state quota for cotton and wheat is the major determinant of the irrigation water management process in the Khorezm region. As supply of water is a key factor for the fulfilment of production quotas, a number of state organisations on different administrational levels (Figure 3) are formally responsible for the allocation and delivery of water from the off-takes along the Amu Darya river to the entrance of the Water User Associations (WUAs). Allocation hereby refers to the assignment of so called water limits to different units within the irrigation network. These limits are determined through water requests based on irrigated area, planted crops, and the respective irrigation state norms. These are passed on and aggregated on various organisational levels from the dehqon (peasant) and fermer (private farmer) via the WUA, UIS (sub-basin irrigation system authority), and BUIS (lower Amu Darya basin irrigation system authority) to the national Ministry of Agriculture and Water Resources (MAWR). The allocation of limits is carried out vice-versa from MAWR downwards, and water quantities are allocated among different water management units on each level (Martius *et al*. 2009). Therefore, the national MAWR is the ultimate authority responsible for the planning and allocation of irrigation water in Uzbekistan. It collaborates on transboundary water management, i.e. allocation of annually agreed water volumes to Uzbekistan, with the Interstate Commission for Water Coordination of Central Asia (ICWC) with Kazakhstan, Kyrgyzstan, Tajikistan, Turkmenistan and Uzbekistan as members and Afghanistan as consulting partner.

The physical delivery of water and the operation and maintenance of the irrigation and drainage network is also the responsibility of state water management organisations, mainly the Main Canal Management (MCM) units of UIS. However, due to inadequate human, financial, and technical resources, they are largely unable to provide the required services. This has resulted in poor functioning of the irrigation and drainage infrastructure and hampered the provision of measurement and communication equipment, which are essential for timely, reliable, and equitable water supply. Similarly, the on-farm irrigation and drainage network has been deteriorating, since the WUAs, which are formally responsible for maintenance and operation of infrastructure within their boundaries, fail to fulfil their obligations. WUAs were introduced between 2000 and 2005 in Khorezm along the administrative boundaries of the former collective farms. Due to the lack of a state budget, WUAs rely on fee collection from water users for covering the operation and maintenance costs of the irrigation system. The water users, however, are reluctant to pay the irrigation service fees because (i) WUAs are organised in a top-down hierarchical manner without active participation of water users, (ii) small farming units (up to 1 ha cropland) are not represented by WUAs, and (iii) poor performance of WUAs with respect to dependability and equity of irrigation water delivery particularly in water-scarce years. Hence, the WUAs have so far failed to become effective organisations for farm-level water supply management.

Figure 3 Water allocation through limits (Veldwisch 2008)



While, formally, water delivery should match the water allocations, the actually delivered water quantities depend on many factors, only one of them being the official limits. In fact water users apply different strategies to access water outside the formal functioning of the water management organisations (Veldwisch 2008). Such strategic practices are a deviation from the formal rules of water management in Khorezm, reveal a strong agency of the actors involved, and follow their own set of informal institutions. One example is the use of small, mobile pumps to lift water into field canals, which is formally considered illegal theft of water, but is informally a wide-spread practice (Oberkircher and Ismailova forthcoming). Also the individual instead of collective actions explain the increasing number of water conflicts between members especially during water scarce years. Thus by catering to individuals' water demands, water delivery according to strategic practice (both with the help of technical means or social relations) is a deviation from the formal water management institutions but at the same time effectively compensates inadequacies of the formal water management organisations – at least for influential agents (Oberkircher *et al* 2010).

Despite frequent malfunctioning of the water management system and widespread deviation (strategic practices), the formal institutions appear to be surprisingly resilient. An explanation might be found in the continuous processes of strengthening and reproducing the system through discursive practices of the actors involved (Oberkircher *et al.* 2010). The deviant actors spend considerable effort and resources on the discursive compensation of their behaviour. When in the case study WUAs fermers deviate from the rule that cotton as a state crop should be irrigated before the cash crop rice, they tended to state in any official conversation that cotton needs to be irrigated first. Whereas individuals take water management actively into their own hands and pursue their own interest, their statements suggest that 'water management is up to the state'.

Opportunities for improving irrigation system management

Considering the inefficient use of water resources and the likely decrease of water supply in the future, the current irrigation management system in Khorezm can certainly not be considered sustainable. Implementation of an innovative, efficient, and participatory irrigation management system may help in not only saving considerable quantities of water but also in improving agricultural production and rural development in an equitable and sustainable manner. Such a concept should be based on the bio-physical and socio-economic systems that characterise the region, the types of practices that actors in water management apply, and the different scales of water management, namely the state water management level, the WUA level, and the farmer and field level.

Regional water management level

In most years, Khorezm receives ample quantities of water. Under this abundance, the WMOs are able to distribute more water than theoretically required by the crops to compensate for institutional and technical deficiencies. In water-scarce years, however, water allocation and delivered quantities mostly do not match. Individual agents, such as district governors (hokims), state water managers, WUA chairmen or influential fermers, pursue their own interest in water, either for commercial agriculture, for satisfying the demands of clients or for fulfilling the state production targets, and thereby accumulating social capital and stabilising their position within the state hierarchy. Furthermore, inadequate maintenance and rehabilitation of the irrigation and drainage network and deterioration of water regulation and measurement infrastructure contribute to inappropriate and inequitable water delivery. In addition, the official water requests and limits include only officially registered paddy rice production (about 16500 ha each year), but not rice produced on areas freed from the state order or as second crop after wheat. Consequently, almost all WUAs regularly exceed their limits and pay fines generally recovered from rice growing fermers. This gap in the official water planning facilitates individual agents to pursue their interests, with rice cropping being both the most profitable as well as the most secretively handled business of fermers. The following tools and options will help to create and improve transparency and accountability in the water management system, thereby facilitating a near-time allocation of water according to demand by water users and water limits.

Introducing a board of WUA chairmen

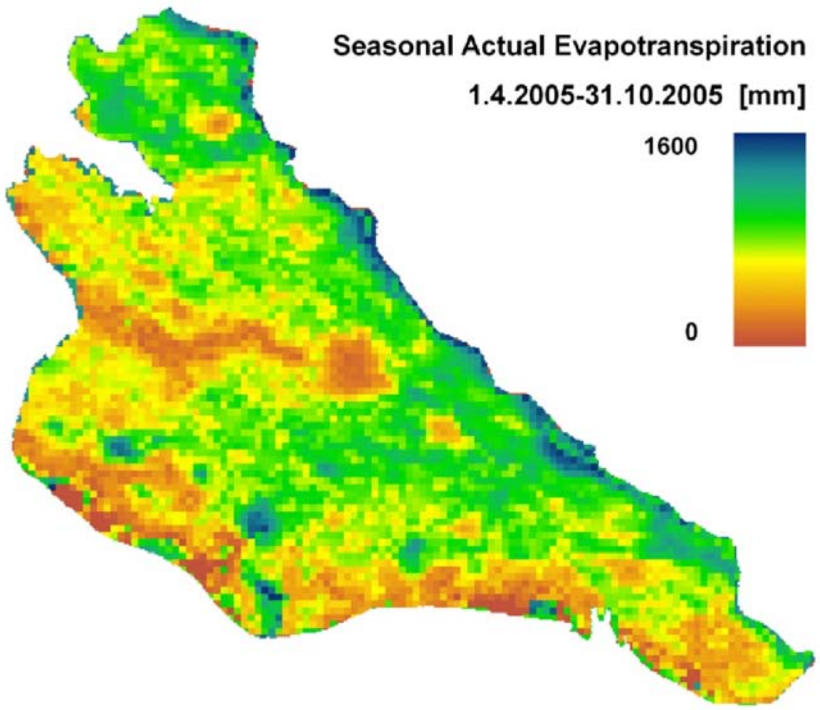
Similar to experiences from other irrigated systems in ASB (Abdullaev *et al.* 2009), a first step might be to assemble the representatives of WUAs along a main canal system in the so-called Unions of Water Users (UWU), and to introduce 'joint governance boards' between the UWUs and state water managers. By bundling the influence of the individual chairmen, the UWUs will form an important counterbalance to the state in water management and be able to hold the state water managers accountable for their decisions and practices.

Technical tools and models

A number of simulation models and tools were developed in the project to monitor, analyse, and identify deficiencies in the irrigation water management system at the regional level. These monitoring systems, land classification tools, and indicator packages are based on GIS, remote sensing, and water/crop models. Such tools allow the estimation of actual evapotranspiration both within and among the irrigation seasons. They can be used to localize temporal water stress, and to show spatio-temporal disparities of water supply inside Khorezm (Figure 4).

In combination with hydrological measurements, remote sensing based tools enable tracing actual water distribution to crop fields and highlighting infrastructural problems in the irrigation network (Conrad et al. forthcoming). In 2005, which represents a typical year with high water supply, less than 30% of the irrigation water was consumed by the crops during the main irrigation phases. The other 70% did not reach the field and can therefore be assigned to canal losses of low field application efficiencies (see below).

Figure 4 **Khorezm map of actual evapotranspiration based on MODIA remote sensing data (1 km ground resolution) (Conrad 2007)**



At the level of main canal system, a spatially-explicit irrigation water distribution model was developed to optimise irrigation water distribution and enhance water productivity in the entire canal system covering 22,850 ha of irrigated land. This model is currently being applied and evaluated by the local irrigation managers.

Modifying the state order system for cotton

While the state procurement mechanism for cotton will not be abolished in the near future, there are options to render this system easier and make it more attractive to the producers. For instance, a more flexible state procurement mechanism can be introduced, which sets the cotton production targets for individual farmers rather than area quotas. Even a multiyear production plan can be introduced with fixed amount of cotton production targeted over more years rather than one single year. These modifications of the state procurement system will provide incentives to farmers to increase cotton production per unit area (i.e. fulfilling cotton quotas on smaller area), which will enable them to allocate more of their land to crops with higher profits. In such a modified state order system, farmers will be able to make their own decisions on adoption of those innovative technologies that not only enhance crop production but also help improving soil fertility, such as diversification of crop portfolio, introduction of new crop rotations, and implementation of soil and water conservation practices (Djanibekov et al. forthcoming).

Increasing local processing of cotton fibre

As the state-ordered cotton production is the major consumer of water in the region, any reduction in the area of this crop will undoubtedly result in using water saving. Given that only 10% of total cotton production is processed locally in Khorezm, one economically viable option to reduce cotton area is the development of textile industry and export of value-added products. Findings of a detailed study on the cotton value chain indicated that increasing cotton fibre processing into yarn by only 10% would allow achieving the same regional export revenue while reducing the area sown to cotton by 30000 ha, saving 228 million m³ of water, and removing about 6 million USD in explicit subsidies (Rudenko 2008). Thus, developing a strong cotton textile industry is one of the key factors for improving the economic viability, rural employment, water productivity, and ecological sustainability of the Khorezm region.

Integrating rice irrigation into water planning

To be able to keep track of water in the irrigation system and make decision-making on rice irrigation water more transparent, we suggest that rice on fermer land, which is freed from the state order, should be included in the formal water allocations and in the water fees of the WUAs as a separate position. The same applies to an earlier decision-making on the cropping of rice as a second crop after wheat.

Water user association (WUA) level

Due to insufficient human and financial resources and lack of water users' participation in decision-making processes regarding water distribution, the WUAs in the region are generally unable to establish flexible, on-demand, and equitable irrigation scheduling systems. In addition, there is insufficient coordination and communication of irrigation activities among the water users, WUA members, and higher-hierarchy state water management institutions. These structural deficiencies together with infrastructural deficits, such as missing or non-appropriate diversion and measurement structures, lead to substantial water losses. Results of our two-year study in an irrigation sub-unit (850 ha) revealed that on average 16.5 Mio m^3 water (2700 mm) was diverted to this area. About 4.2 Mio m^3 (700 mm) was used in pre-season leaching of salts. The remaining water (2000 mm) is more than double the amount recommended for the irrigation of cotton. Our estimations of technical irrigation efficiency of approx. 30% and a field application efficiency of 45% reflect that a high share of these water quantities do not reach the crops. These findings are in line with our estimations of irrigation water losses at the regional level based on remote sensing. Our research on social mobilisation in one case study WUA has shown that even if water users are granted the possibility and space to get involved, they are very hesitant to do so. The common governance structures people are used to are top-down with centralised command and control mechanisms. WUAs rely on good relations with state representatives to represent water users upwards and lobby for water. In return, interference into WUA-activities at the WMO-level, by district and regional governors can be observed regularly. Governance of water in a participatory way does not come natural to fermers, dehqons or the local elites (Hornidge et al. 2009). Experience and knowledge on how to conduct elections and meetings or how to ensure transparency are often not available. Instead of using the WUA as platform to show agency, farmers rely on their patron-and-client networks as described above. This leads to a side-stepping of the WUA and further weakens its possibilities to function adequately.

The problems at WUA level have led to a situation in which neither the water users, nor the WUA staff, nor the state water management staff were satisfied with the resulting water management. During the water scarce year of 2008, dissatisfaction of all involved led to incessant complaints to the WMOs. This in turn may have been the reason for consolidating farm land in 2008, which resulted in significant reduction in the number of water users, and thereby easing water management at WUA level, although the total area to be irrigated did not change. The following options can help improving the performance of WUAs.

Raising funds: Development of WUAs as business units

Even the currently low water and membership fees will not be paid without a reform of the incentive structures for water users to cover the costs of the WUA. As shown above, the principal functions of WUAs include water distribution, cost-recovery, governance and representation. However, a WUA may work as a business unit and have a range of ancillary functions such as the provision of management services, training, extension, and insurance or short term credit to individuals or rural communities or farmers. Such additional functions will decrease the marginal cost of the production and will motivate farmers to pay for water. Once fees are regularly paid, the WUA can provide more additional functions for societal benefits using the resources raised. If the users perceive benefits generated from such functions, they will be further motivated to pay the fees. The WUA and its members should follow the principal of reciprocal accountability, i.e. if a water user does not pay for services he does not receive water, and, in turn, the WUA is not paid if it fails to provide the required services.

Increasing participation: Empowerment and social mobilisation

A joint experiment of researchers, fermers and WUA staff in WUA Ashirmat has shown that social mobilisation implemented by well-known and accepted people can increase awareness among water users of the WUA's existence and work (Hornidge *et al.* 2009). However, this is only the first step towards higher participation in the WUA's activities. To create room for water users to communicate their needs and represent their interests, it is necessary for the WUA to conduct regular meetings, so that water users' needs are acknowledged and ownership of the WUA is developed. Stratification among the water users which is related to geographical location should be reduced by introducing canal managers for different parts of canals, so that e.g. tail-enders have representation mechanisms to secure their water access. Dehqons should also be included in the information and decision-making processes.

Improving the technical performance: Water measurement devices and irrigation scheduling models

One of the critical factors for transparent, equitable, and timely distribution of water to individual farmers' fields is the availability of water measurement and monitoring devices, such as gauging rods (water-depth rods), flow-meters on pumping stations, and flow-measuring flumes and weirs at all off-takes into fields of fermers and dehqons. These devices at the WUA level are generally out-dated and imprecise or non-existent. Establishing the appropriate water measurement system on all water sources and entering the information in a common database accessible to WUA members and water users are the pre-requisites for improving the efficiency and productivity of irrigation management system.

Once the necessary water monitoring infrastructure is in place, irrigation scheduling models can be used to optimise water distribution to crop fields. By accounting for short-term variations in weather conditions, these models allow a flexible and on-demand irrigation scheduling during the crop season, which results in enhanced water productivity. Detailed modelling studies in the project suggest that matching the timing of irrigation events to actual crop water demand, instead of the presently widely-practiced norm-based irrigation scheduling, can save 25% of water without incurring any yield penalty.

Farmer and field level

The water management problems discussed for the WUA level make adequate water delivery (timing and quantity) highly uncertain. This unreliability of supply is reflected in practices of tail-end fermers who tend to over-irrigate once water is available or block drains and collectors to raise the groundwater level, so that groundwater can contribute to crop water requirements. While this makes sense in the fermers' situation to cope with uncertainty of water supply in the short-term perspective, it also has long-term negative impacts, such as secondary soil salinisation, which is a widespread phenomenon in the region.

In addition, irrigation scheduling in Khorezm is based on norms which were developed in the 1960s. Since then, soil characteristics and groundwater levels have changed, farm size and the number of farmers/water users have altered, cropping patterns have diversified, and new modelling tools to calculate season-specific crop water requirements and estimate irrigation efficiencies have been developed. Consequently, these norms, while remaining helpful tools for estimating large-scale total crop water requirements, fail to provide flexible and on-demand irrigation scheduling, which is required for improving water productivity of cropping systems. Furthermore, awareness on water-saving and adoption rate of water-efficient irrigation technologies are generally low. Inefficient use and wastage of water can be attributed to a range of factors including lack of land tenure security, area- and production-based state quota on cotton and wheat, highly subsidised water supply, unreliable water delivery, lack of economic incentives, and technical deficiencies in irrigation and drainage infrastructure.

To improve the efficiency of water use at farm and field level, we suggest here some technical options that should go alongside changes in the institutional setting that create awareness on water-saving as well as space for innovation.

Optimizing the water application process

Due to the flat topography, double-sided irrigation is a highly suitable application method in Khorezm. Results of field experiments suggest that, compared to the conventional furrow irrigation technique, double-sided irrigation technique can save 15% water. Furthermore, the increase in salt accumulation at the end of the furrows due to low irrigation water application and high capillary rise could be halved, and as a consequence cotton yield increased by 0.5 t ha^{-1} (Paluasheva 2005). Improvement in operation and design of the furrow technique (optimizing application discharge; introducing surge flow; laser land levelling) allows to raise the application efficiency from currently 45% to 65%.

Adequate irrigation scheduling

As an alternative to the widespread use of outdated irrigation norms, scheduling of irrigation events based on simulation models of crop-soil-water dynamics can help better matching of water supply to crop demand. Our simulation studies revealed that a flexible and on-demand irrigation scheduling, instead of the norm-based approach, provides a water saving potential of 20% without causing reduction in crop yields. The excessive volumes of water applied for pre-season salt leaching (700 mm) can also be significantly reduced by site-specific methods that account for actual soil salinity in the crop root zone. Combining modern soil salinity monitoring devices, such as the electromagnetic induction device EM-38, and models of soil salinity dynamics (for instance the HYDRUS model) can reduce the leaching water use significantly (Akramkhanov *et al.* 2008; Forkutsa *et al.* 2009).

Water pricing

The question of whether introducing water pricing may impact water conservation in Khorezm was examined using a modelling approach (Bobojonov 2008). The simulation results indicated that a direct pricing for irrigation water indeed bears the potential for increasing water use efficiency of about 5-6 % and for increasing the financial resources of the water management organizations. However, to recover the costs of the entire system, prices would need to be as high as 9 USD per thousand m^3 for irrigation water. However, introducing a price for water only makes this mechanism unattractive under the present state procurement system. Various findings (Bobojonov 2008, Rudenko and Lamers 2006) recurrently warn for an over-optimism and estimated a reduced impact when introducing isolated measures. Thus, water pricing would yield much more effects when flanked by other measures such as an easing of the state procurement system and allowing crop diversification.

Strengthening water inspection

In 2009, WUA Ashirmat introduced local water inspectors (Ul Hassan *et al.* 2010) – a promising innovation which should be followed by other WUAs too. Local water inspectors should cooperate with the district inspectors of Uzsuvnazorat and make use of its formal sanctioning mechanisms. It can be expected that the local inspectors will feel more bound to their fellow villagers than to the state organisation Uzsuvnazorat and would try to avoid a control function with sanctioning mechanism. However, the mere existence of water inspection on local level and social sanctioning mechanisms would have a strong impact on water-saving awareness on the local level.

Build capacity and promote water-saving

In addition to these control mechanisms, Uzsuvnazorat together with local water inspectors should widen their functions and adopt a more prominent role in the education of water users on water wastage and water-saving. Concrete water-saving measures are currently only promoted through the annual Pakaz meetings of fermers in which state representatives communicate agricultural norms and regulations, which occasionally also relate to water-saving (i.e. shorter furrows). Awareness-raising campaigns as well as capacity building during and in addition to the state trainings should be conducted more systematically and frequently. By continuously being alerted about water-saving and by acquiring the skills and techniques to practice it, water users would be able to develop ownership of water-saving instead of considering water management and water-saving 'up to the state' only.

Summary and conclusion

In this paper, we provided an analysis of irrigation water management in the province Khorezm of Uzbekistan and presented recommendations on how water management can be improved.

The analysis takes into account the local context of water management and derives its results from an interdisciplinary analysis of socio-economic, technical, and bio-physical aspects. We have described three different types of practices, which actors involved in water management apply: formal practices, strategic practices, and discursive practices. We also outlined the infrastructural deficiencies in irrigation and drainage network and their implications for unreliable and inadequate water delivery. We have concluded that the three types of practices and the poor status of water conveyance and monitoring systems shape water management in Khorezm. In our recommendations to improve the irrigation management, we have analysed water management at different levels from the state water management level to the farmer and field level. We concluded that transparency, accountability, and participation matter and need to be improved to create an enabling environment for improving the efficiency of the irrigation system. Furthermore, the irrigation infrastructure and technical efficiency should be improved. There is a huge water saving potential in the region without adversely affecting the agricultural productivity. Our key message is that the technical and institutional improvement options have to go hand in hand as they rely on each other to achieve an efficient, equitable, and sustainable irrigation system.

Zusammenfassung

„Weißes Gold" und Aralsee-Syndrom: Ansätze für eine effizientere Wassernutzung in der Provinz Khorezm, Usbekistan

Die Bewässerungswirtschaft in der am Unterlauf des Amu Darya gelegenen usbekischen Provinz Khorezm weist eine Reihe von Defiziten auf, die nicht nur für viele Be- und Entwässerungssysteme im Aralsee-Einzugsgebiet typisch sind, sondern die auch in der Summe in den vergangenen Jahrzehnten zu dem Aralsee-Dilemma geführt haben. Ineffiziente Wassernutzung, unzureichende Erfüllung der Anforderungen der Wassernutzer und gravierende Belastungen der Boden- und Wasserressourcen kennzeichnen die Situation in Khorezm. Die erwarteten Auswirkungen der Klimaänderung und eine zukünftig verstärkte Nutzung der Wasserressourcen im oberen Teil des Amu Darya-Einzugsgebiets begründen die Befürchtung, dass die in Khorezm verfügbaren Wasserressourcen zukünftig knapper werden und stärkeren Schwankungen ausgesetzt sein werden. Damit steht die Bewässerungswirtschaft in Khorezm (und im gesamten Einzugsgebiet des Aralsees) vor der Herausforderung, die Wasser- und Landnutzung auf ökonomische Effizienz, ökologische Nachhaltigkeit und soziale Verträglichkeit umzustrukturieren.

Da die Ursachen für die bestehenden Defizite in einem Mix aus technischen, institutionellen und sozio-ökonomischen Ursachen liegen, kann der Umstrukturierungsprozess nur dann dauerhaft erfolgreich sein, wenn entsprechende, Ursachen-orientierte Ansätze auf allen relevanten Ebenen der Wasser- und Landnutzung kombiniert werden. Auf der Grundlage intensiver und mit lokalen Partnern durchgeführten Felduntersuchungen sowie Modellierungen wurden im Rahmen des vom BMBF finanziell geförderten ZEF/UNESCO-Projektes institutionelle, ökonomische und technische Komponenten erarbeitet und als Umstrukturierungskonzepte für die Ebene der Region, der Wassernutzer-gemeinschaften (WUAs) und der Wassernutzer entwickelt. Fernerkundungs- und GIS-gestützte Verfahren zur Landnutzungsklassifikation und zur Bestimmung der aktuellen Evapotranspiration liefern wertvolle Daten für die regionale Wasserbilanzierung, die Beurteilung der Bewässerungsdurchführung, die Bewässerungsplanung sowie deren operationeller Verbesserung.

Ein Modell zur Optimierung der Wasserverteilung für den Versorgungsbereich eines Hauptkanals wird derzeit mit lokalen Wassermanagern in der Praxis getestet. Die Berücksichtigung des Reisanbaus in der Wasserverteilungsplanung lässt eine verbesserte Annäherung der bisherigen offiziellen Wasserverteilung an die Realität erwarten. Die Umsetzung von Wasserverteilungsplänen kann dadurch gesteigert werden, dass die Repräsentanten der Wassernutzergemeinschaften stärker in die Planung der regionalen Wasserallokation eingebunden werden. Eine schrittweise Flexibilisierung der zentralen Planung des Baumwollanbaus ist geeignet, die Eigenverantwortung der Landwirte zu stärken und auf diesem Wege ein Produktions- und Innovationspotenzial zu mobilisieren. Durch Modellsimulationen wurde nachgewiesen, dass ein Ausbau der Baumwollverarbeitung sowohl die Wertschöpfung verbessert als auch die Boden- und Wasserressourcen schont. Auf der Ebene der WUAs gilt es, die Partizipation der Wassernutzer durch Maßnahmen der sozialen Mobilisation zu verbessern und die Service-Funktionen um genossenschaftliche Elemente zu erweitern, um auf diesem Wege die Akzeptanz der WUAs in der Bevölkerung zu steigern. Die Anwendung flexibler Bewässerungssteuerungsmodelle erlaubt eine bessere Erfüllung des räumlich-zeitlich variablen Bedarfs. Um die Implementierung daraus resultierender Wasserverteilungspläne zur ermöglichen, ist eine Rehabilitierung der hydraulischen Bauwerke insbesondere im Hinblick auf die Mess- und Dosiereinrichtungen notwendig. Die im Projekt auf der Feldebene entwickelten Modelle erlauben es, situations-spezifische Bewässerungskennwerte zu ermitteln. In Verbindung mit Ansätzen für eine verbesserte Wasseraufleitung wird der Landwirt in die Lage versetzt, das Bewässerungswasser effizienter und produktiver zu nutzen. Die Einführung von Verbrauchs-abhängigen Wassergebühren, die Stärkung bestehender Kontrollinstanzen und die intensivierte Vermittlung von Techniken der Wassereinsparung stellen geeignete Maßnahmen dar, das vorhandene Potenzial für einen effizienten Umgang mit der Ressource Wasser stärker als bisher zu nutzen.

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References

Abdullaev, I., Kazbekov, J., Manthritilake, H., Jumaboev, K., 2009: Participatory water management at the main canal: a case from South Ferghana Canal in Uzbekistan. Journal of Agricultural Water Management 96(2): 317-329.

Abdullaev. I, Nurmetova. F, Abdullaeva. F, Lamers., J.P.A., 2008: Socio- technical aspects of water management in Uzbekistan: Emerging water governance issues at the grass root level. In: Rahaman, M.M. and O. Varis (eds.): Central Asian Water. Water & Development Publications - Helsinki University of Technology, Helsinki, Finland, 89-103.

Akramkhanov, R., Sommer, A., Martius, C., Hendrickx, J.M.H., Vlek, P.L.G., 2008: Comparison and sensitivity of measurement techniques for spatial distribution of soil salinity. Irrigation and Drainage Systems, 22: 115-126.

Bobojonov, I., 2008: Modeling Crop and Water Allocation under Uncertainty in Irrigated Agriculture. A Case Study on the Khorezm Region, Uzbekistan. PhD thesis.University of Bonn, Germany.

Conrad, C., 2007: Estimation of water productivity in irrigated river basins using remote sensing and geoinformation techniques: a case study in Khorezm river basin, Uzbekistan. PhD thesis, University of Würzburg, Germany.

Conrad, C., Dech, S.W., Hafeez, M., Lamers, J.P.A, Tischbein, B., (submitted): Remote sensing and hydrological measurement based irrigation performance assessments in the upper Amu Darya Delta, Central Asia. Physics and Chemistry of the Earth.

Djanibekov, N., 2008: A Microeconomics Analysis of Farm Restructuring in the Khorezm Region, Uzbekistan. PhD thesis.University of Bonn, Germany.

Djanibekov, N., Bobojonov I., Lamers, J.P.A., 2010: Land consolidation for increasing cotton production, but sufficient for triggering rural development in Uzbekistan? In IAMO Studies on the Agricultural and Food Sector in Central and Eastern Europe (eds): Kelly Labar, Martin Petrick and Gertrud Buchenrieder (In press).

Djanibekov, N., Rudenko, I., Lamers, J.P.A, Bobojonov, I., forthcoming: State-Ordered Cotton Production in Uzbekistan, Central Asia: Two Sides of the 'White Gold' Medal. Case prepared for Cornell University.

Forkutsa, I., R. Sommer, R., Shirokova, Y., Lamers, J.P.A., Kienzler, K., Tischbein, B., Martius, C., Vlek, P.L.G., 2009: Modeling irrigated cotton with shallow groundwater in the Aral Sea Basin of Uzbekistan: II. Soil salinity dynamics. Irrigation Science 27(4): 319-330.

Glantz, M. H., 2008: Sustainable development and creeping environmental problems in the Aral Sea region. In M. H. Glantz (Ed): Creeping Environmental problems and Sustainable Development in the Aral Sea Basin. Cambridge University Press, pp 1-25.

Hornidge, A.-K., M. Ul Hassan, et al., 2009: 'Follow the Innovation' - A joint experimentation and learning approach to transdisciplinary innovation research. ZEF Working Paper Series No. 39.

Martius, C., Froebrich, J., Nuppenau, E.-A., 2009: Water resource management for improving environmental security and rural livelihoods in the irrigated Amu Darya Lowlands. In: Brauch, H.G., Oswald Spring, Ú., Grin, J., Mesjasz, C., Kameri-Mbote, P., Chadha Behera, N., Chourou, B., Krummenacher, H. (eds.): Facing global environmental change: environmental, human, energy, food, health and water security concepts 4. Berlin/Heidelberg /New York: Springer, 749-762.

Micklin, P., 2007: The Aral Sea Disaster. Annu. Rev. Earth Planet. Sci., 35: 47-72

Müller, M., 2006: A general equilibrium approach to modeling water and land use reforms in Uzbekistan. PhD thesis. Center for Development Research (ZEF), University of Bonn.

Oberkircher, L., Tischbein B., Hornidge, A.-K., Schorcht, G., Bhaduri, A., Awan, U. K., Manschadi, A. M., 2010: Reconceptualising Water Management in Khorezm, Uzbekistan / Recommendations towards IWRM, ZEF Working Paper Series. Vol. 54. Bonn: Zentrum für Entwicklungsforschung.

Oberkircher, L. and B. Ismailova, forthcoming: On Pumps and Paradigms: Water Scarcity and Technology Adoption in Uzbekistan. Society & Natural Resources, Scholar One.

Paluasheva, G., 2005: Dynamics of soil saline regime depending on irrigation technology in conditions of Khorezm oasis. In: Proceedings of the International Scientific Conference: 'Scientific Support as a Factor for Sustainable Development of Water Managment': Taraz, Khazakhstan, 20-22 October 2005 (in Russian)

Rudenko, I., 2008: Value chains for rural and regional development: The case of cotton, wheat, fruit, and vegetable value chains in the lower reaches of the Amu Darya River, Uzbekistan. PhD thesis. Center for Development Research (ZEF), University of Bonn.

Ul Hassan, M. et al., 2010: Improving the Performance of the Ashirmat Water Users Association through Social Mobilization and Institutional Development. ZEF/UNESCO Khorezm project's internal documentation. Work package 720: Follow the Innovation.

Veldwisch, G.J., 2008: Cotton, Rice & Water: Transformation of Agrarian Relations, Irrigation Technology and Water Distribution in Khorezm, Uzbekistan. PhD thesis. University of Bonn, Germany.

Technological and health aspects of drinking water for broiler chickens

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Introduction

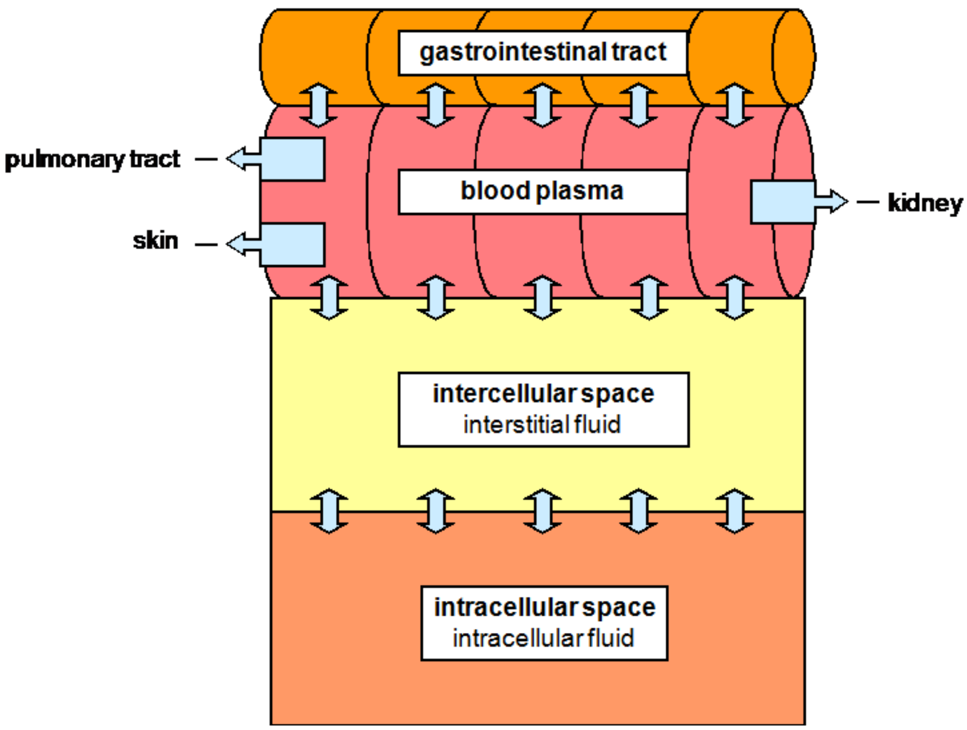
Water is a feedstuff and the most important nutrient for animals (Kamphues *et al.*, 2002; 2007). For this reason particular attention must be paid to satisfying the animals' needs, to water line technology and to the quality of the drinking water. If water is not provided in sufficient quantity and quality this can lead to drops in performance and even endanger animal health. Water quality is not only significant for the health of the animals but, in the case of food-supplying animals, also for the protection of consumers against zoonotic pathogens, e.g. *Salmonella spp.* and *Campylobacter spp*. and other harmful substances.

In October 2009 Lohmann Animal Health GmbH & Co. KG held a two-day workshop on the subject of „Water supply in poultry farming – a key factor for health and performance“. The aim of the workshop was to gather information on the water supply of broiler chickens whereby the knowledge gained at the workshop should be passed on to others.

Regulating the water balance

Water is needed as a diluent, transport medium and for maintaining internal cell pressure and body temperature. Water is excreted primarily via urine and faeces but also in the form of steam via the skin and respiratory tract. In addition to drinking water, water is also available to the animals via feedstuffs and via metabolic water generated by metabolic processes. Water uptake is triggered off by the feeling of thirst. Thirst is triggered off by an increased osmolality of body fluids (fig. 1). Dehydration (exsiccosis) is a hypertensive hypovolumaemia to which an intra-cellular dehydration is connected. The centre of thirst is situated in the hypothalamus. In the event of shortage of fluid the antidiuretic hormone

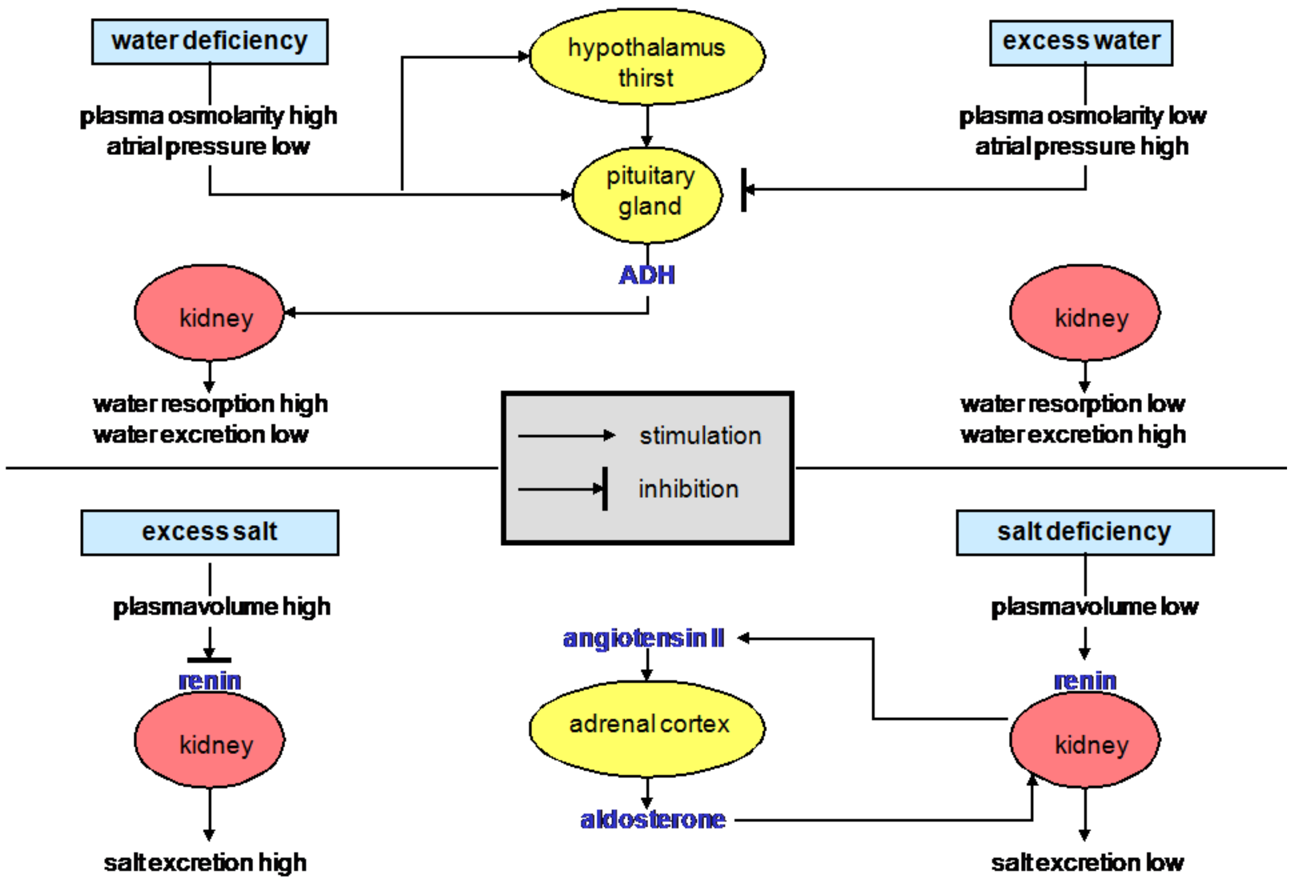
Figure 1: Regulating the water supply



(ADH) is released. This causes the kidneys to retain more water and excrete less. In the event of water excess the release of the hormone ADH and the feeling of thirst are inhibited and thus the amount of water excreted by the kidneys increased. The concentration of sodium in the blood plasma in connection with the renin-angiotensin-aldosterone system is also involved in the regulation of water supply. A reduction in sodium concentration leads to a reduction in the plasma volume. The renin-angiotensin-aldosterone system is thus set into operation. It results in an increased resorption of sodium in the kidneys and an increase in plasma volume.

The water taken up is resorbed in the small intestine via osmotic activity. 70% of the body mass of broiler chickens is water (Wilson, 2009). Of this around 50 % is located in the intracellular space and 20 % in the extracellular space which is subdivided into intravasal space and extravasal space (fig. 2). The exchange of water between the fluid spaces is caused by differences in concentration with the aim of achieving osmotic balance.

Figure 2: Fluid spaces



Water requirements

The animals must be supplied daily with a quantity and quality of water according to their needs (table 1). Water deficiency results in a reduction in feed uptake and thus in reduced weight gain, to dehydration / exsiccosis and eventually to death if more than one tenth of water content is lost. Growing animals take up more water than they excrete (Wilson, 2009).

There is particular correlation between the amount of feed taken up (dry mass) and water consumption (Langhans *et al.*, 1995). Furthermore, the ambient temperature and feed composition also have an influence on water consumption. The water requirements of broiler chickens expressed in ml per animal and day are calculated on the base of the age of the animals multiplied by factor 6 at optimum ambient temperatures and factor 7 at high temperatures (Wilson, 2009). At temperatures over 20 °C the water requirements rise by 6 % per °C. Not only the ambient temperature but also the temperature of the water itself influences water consumption. Water temperature below 5 °C and above 44 °C leads to lower consumption (Wilson, 2009). Of course the taste of water also influences water consumption.

First indicators of an inadequate water supply are a decrease in feed consumption and performance. The causes can be broken self-drinkers, broken or blocked water lines and insufficient flow rates. A flow rate of 50 to 80 ml per nipple and minute are required for an adequate water supply – this corresponds to a handful of water in 10 s (Wilson, 2009).

There is also a correlation between the amount of water taken up and the wetness of the litter. Attention must be paid that the water supply is not decreased just to prevent the litter material from becoming too wet.

Table 1: Drinking water requirements of broiler chickens (KTBL-Heft 83, 2009)

Day of life	ml per animal and day
7th day	58 to 65
14th day	102 to 115
21st day	149 to 176
28th day	192 to 216
35th day	232 to 261
42nd day	274 to 308
49th day	309 to 374
56th day	342 to 385

Origin of the drinking water

Drinking water for broiler chickens comes either from the communal network or from the farm's own well. Until it is fed into the farm water lines, the water from the communal network is of drinking water quality which is guaranteed by prescribed monitoring and treatment. The quality of the water from the farm's own well corresponds to the quality of groundwater if it is not treated prior to being fed into the farm and/or drinking system. Based on the results of water analyses the decision must be made whether any treatment is necessary.

Once the water has been fed into the farm, in particular into the drinking lines, changes can occur meaning that the quality of the water fed into the system does not correspond to the quality of the water taken up by the animals. Changes occurring to the water after it has been fed into the water lines of the farm can have a number of reasons, e.g. resulting from medication or additives being added to the water or due to contamination of the drinking system following maintenance work (Seemann, 2009).

Drinking water system

The first part of the drinking water system is the connection to the communal network or to the own supply system (farm's own well). This is followed by the pipe to the houses in which the equipment for water preparation and dosing equipment for the addition of medication is located. From there the water is directed into the houses where the height-adjustable drinking water lines with nipple drinkers are installed. There are nipple drinkers with and without drip cup. There should be 1 drinker for every 12 to 15 animals and the distance between the drinkers should not be more than 2 to 3 m (Wilson, 2009). An important factor is the height of the nipple drinkers above the litter which must be adapted to the size of the animals. Nipple-drinker systems are low-pressure systems. There is a reduction in pressure from 3 to 0.01 to 0.04 bar (Meyer, 2009). It should be avoided that the water stands for longer periods in the water lines which happens in the case of low consumption (e.g. after placement) as this encourages the precipitation of ingredients, the multiplication of bacteria and the formation of biofilm (Kamphues *et al.*, 2007). Biofilms are ecosystems of microorganisms of different families which cohabit in an extracellularly formed polysaccharide-protein layer and are thus difficult to combat using antimicrobial agents (Eecke, 2009; Watkins, 2009).

Even if the water is primarily of good quality there is a danger of contamination of the drinking water in the event of technical defects in the drinker system. Open drinkers must thus be protected against contamination. During the continual monitoring of the drinker systems the following aspects must be observed (Müller and Schlenker, 2007):

- Presence of a sufficient number of drinkers
- Availability to all animals
- Sufficient water pressure, even during peak requirements
- Presence of water meters to monitor water consumption
- Frost protection in winter
- Prevention of contamination in the drinkers
- Access to the drinker systems should be possible for the farm workers to carry out function control and cleaning

Drinking water quality

Water quality is determined by the presence of microorganisms which cause disease and of substances which are toxic or influence the taste.

There are no legal requirements for the quality of drinking water in animal husbandry such as those for the quality of drinking water for human consumption. Drinking water for animals in the EU is incorporated into the term “feedstuffs” (Regulation (EC) no. 178/2002 of the European Commission and the Council of 28th January 2002). In order to support the objectives in this regulation the Feed Hygiene Regulation (regulation (EC) no. 183/2005 of the European Commission and the Council dd. 12th January 2005) was enacted. The stipulations laid down therein came into force on 1st January 2006. According to this the drinking water for animals must be of such quality that it is suitable for the animals concerned. The legislator restricts the legal requirements to general safety requirements. However, it is advisable to orientate the requirements for animal drinking water to the Drinking Water Ordinance (2001). Drinking water for animals should be free from pathogens and free from harmful substances in toxic concentrations and should also have a pleasant smell and taste. Pathogens and harmful substances do not only affect the health of the animals but also the sanitary harmlessness of the food produced from them. In addition to a variety of recommendations for drinking water quality (e.g. Müller and Schlenker, 2007; Eecke, 2009) there is also a framework issued by the Federal Ministry for Nutrition, Agriculture and Consumer Protection (BMELV, 2007) on the legal evaluation of water as a feedstuff. The title is “The hygienic quality of drinking water for animal husbandry” („Hygienische Qualität von Tränkwasser“).

This requires the microbiological quality of water fed into the system to be free from Salmonella and Campylobacter and as far as possible free from *Escherichia coli*. The colony count of aerobic bacteria is used as the orientation value for measuring microbial contamination (table 2). These orientation values should not only apply to the water fed into the system, but above all to the water outlets in the houses. The imprecise indication for *Escherichia coli* should be interpreted in such a way that not more than 10 cfu are present in 100 ml water.

The BMELV has issued orientation values for the evaluation of physico-chemical parameters of drinking water taking food and feed safety into consideration (table 3). Physico-chemical factors can lead not only to damage to health but also to changes in the taste of water and deposits in the water pipes which on the one hand promotes the formation of biofilm and on the other hand can result in the animals taking up an insufficient quantity of water.

Knowledge on the meaning of physico-chemical parameters for the health and performance of broiler chickens is incomplete. A pH-value of water of below 5.9 should be detrimental to performance; a pH value over 8 reduces the disinfectant effect of chlorine (Watkins, 2009). High concentrations of minerals

Table 2: Reference values of the Federal Ministry for Nutrition, Agriculture and Consumer Protection (BMELV, 2007) for the evaluation of the biological quality of drinking water

Parameter	Reference value for drinking water for animal consumption (BMELV)	Remark	Limits for drinking water (TrinkwV) (Drinking Water Ordinance) for human consumption
Salmonella	0 cfu/100 ml	Zoonotic pathogen	No indication
Campylobacter	0 cfu/100 ml	Zoonotic pathogen	No indication
Escherichia coli	preferably 0 cfu/10 ml	Indicator germ*	0 cfu/100 ml
Coliform bacteria	No indication	Indicator germ*	0 cfu/100 ml
Enterococci	No indication	Indicator germ*	0 cfu/100 ml
Colony count	10000 cfu/ml (20 °C) 1000 cfu/ml (37 °C)	Microbial contamination	22 °C: 100 resp. 1000 cfu/ml ** 36 °C: 100 cfu/ml

* Indicator acc. to the Drinking Water Ordinance for the presence of pathogens and/or faecal contamination
** at 22 °C 1000 cfu/ml in small systems (giving up to 1000 m³ water per annum), 100 cfu/ml in systems giving more than 1000 m³ per annum

in hard to very hard water (range of hardness III and IV) due to calcium- and magnesium carbonate and sulphates, nitrates or chlorides lead to precipitations in the water lines and impairment to the function of the nipple drinkers (Kamphues *et al.*, 2007; Watkins, 2009; Wilson, 2009). Precipitations on the walls of the pipes not only restrict the lumen, but also promote the colonization of bacteria and other microorganisms and thus the formation of biofilm. The electrical conductivity is determined by the concentration of electrolytes. Values above 500 µS/cm are indicators for depositions of sodium, potassium and chloride (Kamphues *et al.*, 2007). A content of sodium chloride below 1000 mg/l does not cause any problems. Values exceeding this level reduce feed consumption. Iron promotes bacterial growth. Particularly when using a farm's own well, the groundwater can be contaminated with fertilizer and liquid manure containing nitrate and nitrite which can lead to reduced weight gain (Watkins, 2009). Complexing substances in the water can influence the efficacy of medication and vaccines applied via the drinking water (Kamphues *et al.*, 2007).

Treatment of drinking water

Particularly when using a farm's own well the treatment of the drinking water can be necessary. The main treatments are the filtration of the water, softening and reducing the germ count. Cleaning and disinfection of the water lines to remove deposits (precipitations) and biofilm also belong to water treatment.

There are filters which can be backwashed and replacement filters. Filtration is necessary to remove particles of dirt such as sand and to remove iron and manganese oxide following the oxidation of iron and manganese (de-ironing, demanganisation). Softening is required less in cold water pipes than in warm water pipes and is mainly carried out using ion-exchange resin. If the germ count is too high (in excess of the values shown in table 2) disinfection should be carried out by adding e.g. chlorine compounds or active oxygen. Only products approved in accordance with the Drinking Water Ordinance or with feed laws or with the Biocide Regulation (2002) should be used for the purpose (Kamphues *et al.*, 2009). UV-radiation can also be used for disinfection (Meyer, 2009).

Table 3: Reference values of the Federal Ministry for Nutrition, Agriculture and Consumer Protection (BMELV, 2007) for the evaluation of the physico-chemical quality of drinking water

Parameter	Reference value for drinking water for animal consumption (BMELV)	Disorders	Limits for drinking water (TrinkwV) for human consumption
pH-value	5 to 9	<5 corrosion in the water lines	6,5 to 9,5
Electric conductivity (µS/cm)	<3000	Diarrhoea, taste impaired	2500
Soluble salts (g/l)	<2,5	Thirst	No indication
Oxidability (mg/l)	<15	Contamination with oxidable substances	5
Ammonium (mg/l)	<3	Contamination	0.5
Arsenic (mg/l)	<0.05	Health disorders, low performance	0.01
Lead (mg/l)	<0.1	Nervous symptoms, low performance	0.01
Cadmium (mg/l)	<0.02	Unknown	0.005
Calcium (mg/l)	<500	Diarrhoea, precipi-tations in pipes	No indication
Chloride (mg/l)	<250	Wet faeces	200
Iron (mg/l)	<3	Influence on taste, deposits in pipes, antagonist to other trace elements	0.2
Fluorine (mg/l)	<1,5	Bone disorders	1.5
Potassium (mg/l)	<250	Wet faeces	No indication
Copper (mg/l)	<2	Diarrhoea	2
Manganese (mg/l)	<4	Influence on taste, deposits in pipes	0.05
Sodium (mg/l)	<250	Wet faeces	200
Nitrate (mg/l)	<200	Methaemoglobin formation	50
Nitrite (mg/l)	<30	Methaemoglobin formation	0.5
Mercury (mg/l)	<0,003	General disorders	0.0001
Sulphate (mg/l)	<500	Laxative effects	240
Zinc (mg/l)	<5	Low performance	No indication

A particular problem is the prevention and removal of deposits, air pockets and biofilms in the water lines. The lines should be flushed up to three times a week with 1.5 to 3 bar pressure, whereby automatic equipment is available for this purpose (Meyer, 2009; Wilson, 2009). Thorough cleaning and disinfection by flushing and/or rinsing the water lines which have been emptied of drinking water and are open at the end must take place during the service period as preparation for placement with new chicks.

Monitoring drinking water quality

If the water is taken from the public network the quality of the drinking water is only guaranteed up to the point where the water is fed into the farm (house installation). If wells are available in an animal production unit solely for providing drinking water for the animals, monitoring of water quality is not prescribed. Thus, on using farm's own wells there is a danger of contaminating the flock with microorganisms and harmful substances. It is thus recommended that well water be analysed at least once a year prior to feeding it into the farm system. Both drinking water from the public network and water from the farm's own well can become contaminated in the water lines of the farm or house, e.g. due to open supply tanks, due to the addition of medication and vaccines and by the nipple drinkers. Water can stagnate in the water lines during the service period thus leading to high bacterial counts and biofilm formation particular at high temperatures. Biofilms can come loose and contaminate the water with huge amounts of microorganisms, which can also include pathogens. Biofilms, however, can also secrete metabolic products which have a negative effect on the smell and taste of the drinking water. It is therefore important to differentiate between the quality of the drinking water fed into the supply system and the quality of the water taken up by the animals.

The quality of the drinking water at the end of the drinker lines in the house should be determined once or twice a year. In case of strong deviation from the drinking water quality, appropriate samples should be taken to clarify whether the contamination is being caused in the farm and at what points, or whether it is being brought into the farm from the farm's own well. An inspection of the microbiological quality of the drinking water should also be carried out following cleaning and disinfection of the drinker system.

During sampling care must be taken that the results are not falsified due to incorrect sampling. Before taking a sample the water must run for 5 minutes. A min. of 250 ml is needed for microbiological analyses, whereby the sample should be sterile if at all possible (flame the tap) and filled into a sterile bottle to prevent any secondary contamination of the water. To clarify deviations in drinking water quality or to check the cleaning and disinfection of the drinker system the sampling, place of sampling and time of sampling must be adapted to the matter concerned. For chemical analyses 2 l of water must be brought cooled to the laboratory as quickly as possible and kept in the refrigerator until the analysis is carried out. The analysis should take place within 6 hours if possible.

Various test instruments are available for on-site inspection of the quality of drinking water for human and animal consumption.

Administration of substances via drinking water

In 2009 BMELV published a guideline on the oral application of veterinary drugs for productive livestock via feed or drinking water. Upon each treatment of food-producing animals the efficacy of the use of veterinary drugs, the prevention of undesired side-effects, user safety, food safety, consumer protection, animal welfare and the prevention of spreading antibiotic resistance must be taken into consideration when making the decision. The veterinarian must ascertain that the oral application of the required dose can be ensured for each animal. For administration via drinking water only those drugs may be used which are approved for this type of application. The pyhsico-chemical properties of the water must be taken into consideration (e.g. water hardness, pH-value, iron- and calcium content). In order to prevent any impairment to the drugs and in particular to live vaccines certain demands must be made on the quality of the drinking water – drinking water suitable for human consumption should be used. The entry of unused medicated water into the environment must be prevented to avoid any risks of resistance. The dosing apparatus must be in technically good order. The veterinarian must provide the livestock owner with written instructions on the administration of oral drugs. Vaccines, antibiotics, minerals, vitamins, amino acids, phytopreparations and organic acids can be administered via water. By acidifying the drinking water Thorp (2009) achieved improved weight gain and lower mortality in broiler chickens. Thorp further reported about similar results following administration of health products containing vitamins, minerals, amino acids, glucose, organic acids and herbal substances via drinking water. The administration of vitamin C is said to reduce heat-induced stress during catching.

Summary

Drinking water for animals is a feedstuff and the most important nutrient. A shortage of water in the organism quickly leads to a decrease in feed consumption, a reduction in growth, disturbances in metabolism and finally to death. Biological and chemical ingredients can affect the health of the animals and the consumer.

The importance of drinking water for the performance and health of the animals is often underestimated. The article addresses the issue of regulating the water balance of the animals, the water requirements, drinking system technology, water quality and the maintenance and monitoring thereof, with particular emphasis being placed of broiler chickens. It further deals with the possibility of administering various substances and preparations via drinking water. Knowledge gained at a workshop on the subject „Water supply in poultry farming – a key factor for health and performance" is included in the article. The workshop with international guests was organised by Lohmann Animal Health and took place in October 2009.

Literature

Biozidverordnung (2002): Verordnung über die Zulassung von Biozidprodukten und sonstige chemikalienrechtliche Verfahren zu Biozid-Produkten und Biozid-Wirkstoffen (Biozid-Zulassungs-verordnung-ChemBiozid ZulV), BGBl I 2002, 2514-2524.

BMELV (2007): Hygienische Qualität von Tränkwasser. Orientierungsrahmen zur futtermittel-rechtlichen Beurteilung.

BMELV (2009): Leitfaden des BMELV vom 19. Juni 2009 über die orale Anwendung von Tierarznei-mitteln im Nutztierbereich über das Futter oder das Trinkwasser.

van Eecke, J. (2009): Biofilm in water systems of poultry houses. Lohmann Animal Health, workshop „Water supply in poultry farming – a key factor for health and performance", Cuxhaven, 20-21 October 2009.

Kamphues, H. and I. Schulz, (2002): Praxisrelevante Aspekte der Wasserversorgung von Labor- und Nutztieren. Übers. Tierernährung, 30, 65-107.

Kamphues, J., R. Böhm, G. Flachowsky, M. Lahrssen-Wiederholt, U. Meyer and H. Schenkel (2007): Empfehlungen zur hygienischen Beurteilung von Tränkwasser für lebensmittel-liefernde Tiere unter Berücksichtigung der gegebenen rechtlichen Bedingungen. Landbauforschung Völkenrode, 57, 255-272.

KTBL-Heft 83 (2009): Wasserversorgung in der Geflügelhaltung. Kuratorium für Technik und Bauwesen in der Landwirtschaft.

Langhans, W., R. Rossi and E. Schürrer (1995): Relationship between feed and water intake in ruminants: digestion, metabolism, growth and resorption. Proc. Soc. Nutr. Physiol. 3, 199-216.

Meyer, W. (2009): Water systems technology for poultry. Lohmann Animal Health, workshop „Water supply in poultry farming – a key factor for health and performance".

Müller, W. and G. Schlenker (2007): Kompendium der Tierhygiene. 3. Auflage, Lehmanns Media, Berlin.

Seemann, G. (2009): Water supply under extreme hygiene requirements. Lohmann Animal Health workshop „Water supply in poultry farming – a key factor for health and performance".

Thorp, B. (2009): Administration of substances via drinking water systems. Lohmann Animal Health, workshop „Water supply in poultry farming – a key factor for health and performance".

TrinkwV (2001): Verordnung über die Qualität von Wasser für den menschlichen Verbrauch vom 21. Mai 2001 (Trinkwasserverordnung-TrinkwV) , BGBl I 2001, 959 –969.

Verordnung (EG) Nr. 178/2002 zur Festlegung der allgemeinen Grundsätze und Anforderungen des Lebensmittelrechts, zur Errichtung der Europäischen Behörde für Lebensmittelsicherheit und zur Festlegung von Verfahren zur Lebensmittelsicherheit vom 28. Januar 2002. Amtsblatt der Europäischen Gemeinschaft: Rechtsvorschriften 2002 (L31) vom 1.2.2002.

Verordnung (EG) Nr. 183/2005 des Europäischen Parlaments und des Rates vom 12. Januar 2005 mit Vorschriften zur Futterhygiene. Amtsblatt der Europäischen Gemeinschaft: Rechtsvorschriften 2005 (L 35) vom 8.2.2002.

Watkins, S. (2009): Water quality in poultry farming. Lohmann Animal Health, workshop „Water supply in poultry farming – a key factor for health and performance“.

Wilson, M. (2009): Water management in broilers. Lohmann Animal Health, workshop „Water supply in poultry farming – a key factor for health and performance“.

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Field results using autogenous vaccines in commercial layers to reduce *E. coli* infection

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Introduction

Bacterial infections are of great economic importance in the poultry industry worldwide. Particularly in the chicken layer industry recent reports of colibacillosis, erysipelas and fowl cholera in Western Europe implicate a resurgence of these diseases (ZANELLA *et al.* 2000, VANDEKERCHOVE *et al.* 2004, MAZAHERI *et al.* 2006). This might be attributed to a change from cage housing systems to alternative housing systems like aviaries and free-range systems and an accompanied increase of infectious pressure (MAZAHERI *et al.* 2006).

To prevent bacterial diseases in the egg production industry, vaccination is of increasing relevance as it encounters problems due to growing antibiotic resistance and an increased consumer demand concerning food safety (VAN OIRSCHOT 1994, WHITE *et al.* 2002). Furthermore, vaccination can avoid withdrawal periods in marketing eggs following antibiotic treatment. As commercial vaccines are frequently not efficient due to the high antigenic variability of the disease causing organism (BRAGG, R.R. 2002, SMART *et al.* 1993) or as commercial vaccines are not available for certain diseases like erysipelas in chickens, autogenous vaccines can offer sophisticated alternatives.

Adjuvants used in vaccine preparations for layers are in general either aluminium hydroxide or different mineral oils in different phase combinations. Each adjuvant has advantages and disadvantages. An aluminium hydroxide gel adjuvant vaccine usually causes little local reaction and the antibody titre increases quite rapidly after injection (MOREIN *et al.* 1996). Unfortunately, this titre also decreases rapidly in almost the same manner, thus requiring a second injection. A mineral oil adjuvant will release the antigen slowly and respond directly to antigen-presenting macrophages, resulting in antibodies being able to have a longer persistence than aluminium hydroxide gel adjuvant vaccine. Thus, often only a single application is required. On the other hand, the oil responsible for the slow release might cause local reactions (DEGUCHI *et al.*, 1998, FUKANOK *et al.*, 2000, MOREIN *et al.*, 1996, REID *et al.* 1987).

Field records from layers have been used to estimate the effect of different adjuvants on the protection level after vaccinating against *E.coli* during rearing.

We conducted a retrospective analysis of field records to elucidate the influence of the adjuvant in the autogenous vaccine on hen-housed production and mortality, based on weekly records from five farms with up to 9 years of data per farm. Differences due to the autogenous vaccine were estimated from a total of 81 flocks, using variance analysis with the program package SAS (SAS, 2004).

Material and Methods

Farms, housing systems and genotypes

The data included in the analysis were recorded on five farms of different egg producers. Each farm had two separate houses with up to nine flocks with complete weekly records for this study. The number of hens per flock varied between about 8,000 and 43,000 hens with an average of 20,000 hens per flock. Two farms were equipped with aviaries, three used free-range systems. Three different genotypes, two brown and one white-egg layer strain were included in the study. Since the type of layer was highly confounded with farm and housing system, the genotype was not considered in further analysis. Each house was treated as separate experimental unit. The general time trend was measured as linear regression.

Autogenous Vaccines

Each flock was vaccinated during rearing with a trivalent autogenous vaccine containing relevant strains of the bacterial pathogens *Escherichia coli, Pasteurella multocida* and *Eryselpelothrix rhusiopathiae*.

The last two flocks per farm were vaccinated intramuscularly once with an autogenous vaccine produced by Lohmann Tierzucht (LTZ) at an age of 14 weeks, following several flocks on the same farms vaccinated twice with an autogenous vaccine produced by another company at 14 and 16 weeks of age. The LTZ vaccine was blended with mineral oil as adjuvant and the other vaccine used aluminium hydroxide as carrier substance to enhance the immune response.

The distribution of flocks over system, farm, house and vaccination programs in rearing is shown in table 1.

Table 1: Number of tested flocks per environment, farm, house and type of vaccine

housing system	farm	house	number of tested flocks carrier substance oil	$Al(OH)_3$	total
free range	1	1	2	7	
		2	2	6	
	2	1	2	5	
		2	2	5	
	3	1	2	6	
		2	2	6	47
aviary	4	1	2	7	
		2	2	7	
	5	1	2	6	
		2	2	6	34
number of tested flocks			20	61	81

Statistical Analysis

The weekly records for mortality and rate of lay from 22 to 69 weeks of age were combined into twelve 4-week periods for analysis purposes. To estimate the time trend, consecutive flocks in each house were assigned a serial number, which was included in the model. In total, up to 12 four-week observations of 81 flocks were analysed in the model that considers the following fixed and random effects as well as a linear regression.

Fixed effects:
1. housing system (HS)
2. house (H) within HS
3. vaccination (V)
4. interaction between HS and V
5. interaction between H and V within HS
6. age (A) in terms of 4-week period
7. interactions between V and A
8. week within four-week period (W)

Linear regression:
9. sequence of the flocks (S)

Random effects:
10. flock (f) within HS, V, and
11. random error

Results

As expected, age (A) effects, represented by the four-week observations, were highly significant, but also the sequence of the flocks influenced both traits, mortality and hen housed rate of lay. Additionally, highly significant were the considered interaction between vaccination and age as well as the week within the period of a four week observation (W(A)) on laying performance. The highest F-value was estimated for the effect age, which therefore has the strongest influence on both traits.

Table 2: Test of significance for the considered effects on laying performance and mortality per hen housed

Effect	Laying performance per hen housed [%]		Mortality per hen housed [%]	
	F-value	Significance	F-value	Significance
(1) HS	3.85	n.s.	0.23	n.s.
(2) H (HS)	1.66	n.s.	1.79	n.s.
(3) V	0.27	n.s.	0.48	n.s.
(4) HS*V	0.42	n.s.	0.00	n.s.
(5) H*V(HS)	0.31	n.s.	0.36	n.s.
(6) A	326.56	***	50.72	***
(7) V*A	3.17	***	0.62	n.s.
(8) W (A)	9.13	***	1.29	n.s.
(9) S	18.45	***	11.74	***

The estimated regression coefficients for the sequence of the flocks are for both traits significant. From flock to flock, rate of lay per hen housed increased by 1.7 %, and mortality decreased by 0.03 % per week. In a full laying year of 52 weeks this is an increase of about 5 eggs per year and 1.5% less mortality.

Vaccination

As shown in table 1, two flocks per house were vaccinated with an oil based autogenous vaccine of Lohmann Tierzucht (oil) and a variable number of flocks vaccinated with an autogenous vaccine using aluminium hydroxide (Al(OH)3) as carrier from another company. Although the two types of vaccines were sequentially tested, the Least-Squares estimates for vaccine effects shown in table 2 comparing the two vaccines should be free of the general time trend, which was included as linear regression in the model. Under ideal experimental conditions, the comparisons would have been simultaneous and with untreated controls.

Housing system

Significant differences between flocks due to the vaccine used can also be seen when looking at the interaction effect between housing system and vaccine type. The Least Squares mean rate of lay was lower when an $Al(OH)_3$ based vaccine (76.3 %) was used than an oil-based vaccine (77.5 %) was inoculated. The same positive tendency was estimated for mortality and can be read in table 3. Weekly mortality is relatively high, with 0.30 % and 0.27 % for the two types of vaccines, but favours the oil-based LTZ vaccine consistently in both types of housing by a margin of .03 % or 1.56 % per year. The different standard errors of the estimates reflect the fact that more flocks were vaccinated with the $Al(OH)_3$ based vaccine before changing to the LTZ vaccine with oil suspension.

Table 3: LS-Means for hen housed rate of lay and mortality

effect			LS means [standard error] laying performance per hen housed [%]	mortality per hen housed [%]
vaccination		oil	77.49 [1.80]	0.27 [0.04]
		$Al(OH)_3$	76.32 [0.80]	0.30 [0.02]
housing system *vaccination	free range	oil	75.58 [2.05]	0.28 [0.04]
	free range	$Al(OH)_3$	75.36 [0.99]	0.31 [0.02]
	aviary	oil	79.41 [2.35]	0.26 [0.05]
	aviary	$Al(OH)_3$	77.28 [1.17]	0.29 [0.02]

The LS-Means for the two vaccination groups in four-week periods (not shown in detail here) indicate that the main benefit of the oil-based LTZ vaccine is expressed after peak production: insignificant +0.1 % per week from 22-33 weeks of age, but a significant difference of + 1.7 % per week from 34-69 weeks of age. During the last four-week period, average rate of lay per hen housed was 57.6 % versus 62.0 %. Similar results were found for cumulative mortality, which is included in the trait hen-housed rate of lay.

Significant differences between housing systems and farms with the same housing system suggest considerable potential for further improvement of farm management irrespective of the housing system and vaccination program. Farm 5 achieved the best performance of all farms, averaging nearly 10 % above the results of farm 2.

Discussion

Consumer demand for safe food and awareness of potential problems due to antibiotic resistance on the one hand and the preference for eggs from non-cage systems on the other hand present a challenge for egg producers and poultry veterinarians to control bacterial infections by vaccination (VAN OIRSCHOT 1994, WHITE *et al.* 2002). Prevention and control of bacterial infections in laying hens by means of commercial vaccines is often hampered by a lack of efficiency due to the high antigenic variability of the pathogens. Therefore, autogenous vaccines are used instead (BRAGG 2002, WAMBURA 2010). They offer the unique possibility to cover bacterial strains currently responsible for disease on a specific farm. The efficiency of autogenous products is highly influenced by a large number of factors like pathogen detection, typing and selection, regular “update” of the vaccine with the strains currently causing disease as well as infectious pressure in the flock.

In this study we tried to elucidate the influence of the adjuvant on the efficiency of autogenous vaccines for laying hens in the field. We therefore conducted a retrospective analysis of performance data provided by five different egg producers with two comparable houses each. In all 10 houses, the last two flocks of layers were vaccinated with an autogenous trivalent vaccine produced by Lohmann Tierzucht and blended using mineral oil as adjuvant (oil), following previous vaccination of several flocks with a comparable vaccine of a competitor using aluminium hydroxide as adjuvant ($Al(OH)_3$). Weekly rate of lay and mortality was expressed in twelve 4-week periods from 22 to 69 weeks of age for statistical analysis.

To get a reliable estimate of differences due to the vaccine used, we ignored strain of layers (confounded with house within farm and housing system) in the model; other unknown differences due to farm management and nutrition were minimized by using both vaccines on each farm in consecutive flocks. Ideally, both types of vaccines should have been used simultaneously in the two houses per farm and repeated over two years to get unbiased estimates of the vaccination effect. To estimate the effects of vaccination as free as possible from the general time trend, we included the test year in the model as linear regression over the whole observation period. The regressions of hen-housed rate of lay and weekly mortality on years estimate the combined effects of selection for improved adaptability to non-cage systems and improved environment due to cumulative experience on each farm with a given housing system. The estimated difference of the performance following the oil-based vaccination compared to the alternative vaccine previously used should be free of the general time trend and understood as additional effect on all farms in the last two flocks.

The results found under these conditions indicate that commercial layers vaccinated once during rearing with an autogenous mineral oil based bacterin outperform layers vaccinated twice with an aluminium hydroxide based autogenous vaccine. This implies that an oil-suspension vaccine provides better protection than the aluminium hydroxide based vaccine, which is in accordance with previous studies (MURTHY *et al.*, 2007, BENNECKE 2008, JACOBS *et al.* 1992). One reason for this result might be the longer duration of the immune response. Duration of immunity is one of the key prerequisites for vaccines used in layers, as laying periods last up to one year after immunisation and a booster vaccination during the laying period is technically more than difficult. Mineral oil adjuvants lead to a prolonged release of antigens, respond directly to the macrophages and thereby display a longer lasting antibody titre level than aluminium hydroxide gel adjuvant vaccines (FUKANOKI *et al.* 2000).

Several other factors may affect the efficiency of a vaccine like the antigen quantity per dose as well as the production procedure of the antigen. As the vaccine with the aluminium hydroxide as adjuvant was produced by a different producer and their production scheme is not known, our conclusions are limited to the adjuvants, which is the only objective distinguishing feature between the two vaccines.

In conclusion, in this retrospective study the mineral oil based autogenous vaccine produced by Lohmann Tierzucht lead to better performance of commercial layers in comparison to vaccination with an aluminium hydroxide based autogenous vaccine. Furthermore, better performance was achieved with a single immunisation compared to the labour- and cost-intensive double immunisation required by the aluminium hydroxide based vaccine.

Zusammenfassung:

Praxisergebnisse mit dem Einsatz autogener Impfstoffe zur Verringerung von *E. Coli* bei Legehennen in Bodenhaltung

Bei der Aufbereitung von bestandsspezifischen Impfstoffen setzen verschiedene Hersteller unterschiedliche Adjuvantien (Trägerstoffe) zur Verstärkung der Immunantwort ein. In der Regel sind dies verschiedene Mineralöle oder Aluminiumhydroxid ($Al(OH)_3$), deren Einfluss auf die Wirkung des Impfstoffes in diesem Beitrag untersucht wurde. Die hierzu ausgewerteten Felddaten berücksichtigen Leistungsdaten von 81 Legehennenherden, die sich auf fünf Betriebe mit jeweils zwei Häusern - drei Freilandbetriebe und zwei geschlossene Bodenhaltungen mit integrierter Voliere - aufteilen. Die letzten beiden Herden eines Hauses wurden einmalig mit einem bestandsspezifischen Impfstoff der Firma Lohmann Tierzucht geimpft, dessen Adjuvanz eine Ölsuspension ist. In allen anderen 61 Herden wurde den Legehennen hingegen zweimalig ein bestandsspezifischer Impfstoff mit $Al(OH)_3$ als Trägerstoff verabreicht.

Unter Berücksichtigung verschiedener Einflussfaktoren zeigen die einmalig geimpften Legehennen im Vergleich zu den mit $Al(OH)_3$ als Trägerstoff geimpften Hennen eine bessere Legeleistung je Anfangshenne (77,5 % vs. 76,3 %) sowie eine niedrigere Verlustrate (0,27 % vs. 0,30 % pro Woche). Ähnlich positiv zeigt sich die Wirkung des Öladjuvanz bei einer Betrachtung innerhalb der beiden Haltungssysteme. Eine Verknüpfung der Effekte Betrieb und Haltungssystem macht jedoch deutlich, dass die besseren Leistungsergebnisse in der Bodenhaltung in erster Linie auf den Betriebseffekt zurückzuführen sind. Die Unterschiede in der Legeleistung betragen von Betrieb zu Betrieb bis zu 10 %. Ferner verstärkt die Betriebsentwicklung im Verlauf der neun Beobachtungsjahre die Überlegenheit des Öladjuvanz gegenüber $Al(OH)_3$, durch dessen Einsatz in den letzten beiden Beobachtungsjahren. Der geschätzte Regressionskoeffizient zeigt von Herde zu Herde eine Steigerung

in der Legeleistung um 1,7 % und eine Abnahme der Verluste um 0,03 %. Das entspricht einer jährlichen Steigerung der Legeleistung je Anfangshenne um 5 Eier und einer Senkung der Verlustrate um 1,5 % als kombinierter Effekt genetisch verbesserter Anpassung an Bodenhaltungsbedingungen, Erfahrung der Betriebsleiter mit den besonderen Haltungsbedingungen und verbesserter Impftechnik. Die verbesserten Leistungsergebnisse in Kombination mit nur einmaliger und dadurch weniger zeit- und kostenaufwendiger Immunisierung sprechen für einen bestandsspezifischen Impfstoff mit Ölsuspension als Adjuvanz.

References:

BENNECKE, K., 2008: Vergleichende Untersuchungen zur protektiven Wirkung einer Streptococcus suis Ganzzell- und einer Subunitvakzine. Diss., Tierärztliche Hochschule Hannover.

BRAGG, R.R., 2002: Isolation of serovar C-3 Haemophilus paragallinarum from Zimbabwe: a further

indication of the need for the production of vaccines against infectious coryza containing local isolates of H. paragallinarum. Onderstepoort. J. Vet. Res. 69: 129- 132.

DEGUCHI, K., T. HONDA, K. MATSUO, H. FUJIKAWA, T. IWAMOTO and Y. SAKANOUE, 1998: Influence of inoculation site of combined oil-adjuvanted vaccine on the antibody response in chickens. J. Vet. Med. Sci. 60: 831-835.

FUKANOKI, S., K. MATSUMOTO, H. MORI and R. TAKEDA, 2000: Relation between antigen release and immune response of oil adjuvanted vaccines in chickens. J. Vet. Med. Sci. 62: 571-574.

JACOBS. A.A.C., W. CUENEN, and P.K. STROM, 1992: Efficacy of a trivalent Haemophilus paragallinarum vaccine compared to bivalent vaccines. Vet. Microbiol. 32: 43-49.

MAZAHERI, A., H.C. PHILIPP, H. BONSACK AND M. VOSS, 2006: Investigations of the vertical transmission of Erysipelothrix rhusiopathiae in laying hens. Avian Diseases 50(2): 306-308.

MOREIN, M., M.V. ERIKSSON, A. SJOLANDER and K.L. BENGTSSON, 1996: Novel adjuvants and vaccine delivery systems. Vet. Immunol. Immunopathol. 54: 373-384.

MURTHY, A.K., J.P. CHAMBERS, P.A. MEIER, G. ZHONG, B.P. ARULANANDAM, 2007: Intranasal vaccination with a secreted chlamydial protein enhances resolution of genital Chlamydia muridarum infection, protects against oviduct pathology, and is highly dependent upon endogenous gamma interferon production. Infection and Immunity, 75 (2): 666-676.

REID, G.G., and P.J. BLACKALL, 1987: Comparison of adjuvants for an inactivated infectious coryza vaccine. Avian Dis. 31: 59-63.

SAS INSTITUTE, 2004: SAS/STAT User's Guide (Release 8), Cary NC, USA.

SMART N.L., HURNIK D., MACINNES J.I., 1993: An investigation of enzootic Glasser's disease in a specific-pathogen-free grower-finisher facility using restriction endonuclease analysis. Can Vet J. 34(8): 487-490.

VANDEKERCHOVE, D., P. DE HERDT, H. LAEVENS, F. PASMANS, 2004: Risk factors associated with colibacillosis outbreaks in caged layer flocks. Avian Pathology, 33(3): 337-342.

VAN OIRSCHOT, J.T. 1994: Vaccination in food animal populations. Vaccine 12: 415-418.

WAMBURA P.M., 2010: Preparation and use of autogenous vaccine from Avibacterium paragallinarum (strain Tan 1-05) in layer chickens. Trop. Anim. Health Prod. 42(3): 483-486.

WHITE, D.G., S. ZHAO, S. SIMJEE, D.D. WAGNER and P.F. MCDERMOTT, 2002: Antimicrobial resistance in foodborne pathogens. Microbes Infect. 4: 405-412.

ZANELLA, A., G.L. ALBORALI, M. BARDOTTI, P. CANDOTTI, P.F. GUADAGNINI, P.A. MARTINO

AND M. STONFER, 2000: Severe Escherichia coli O111 septicaemia and polyserositis in hens at the start of lay. Avian Pathology, 29(4): 311-317.

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