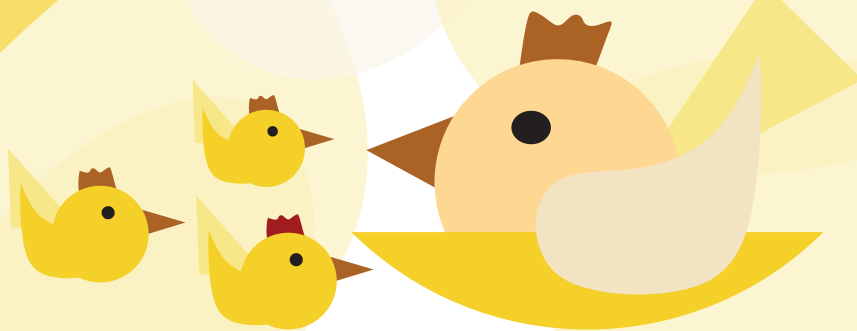




XI European Symposium on Poultry Welfare

Prague, Czech Republic, 26–29 June 2023

Proceedings



Abstracts presented at this symposium have been reviewed by members of the scientific committee and members of the working group. However, the contents in the abstracts and full papers are entirely the responsibility of the author or authors concerned and do not necessarily represent the views of the organisers of the symposium.

**XI European Symposium on Poultry Welfare
(ESPW 2023)
Proceedings**

**Prague, Czech Republic
26–29 June 2023**

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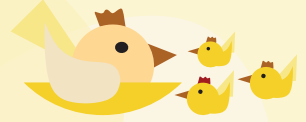


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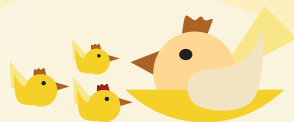
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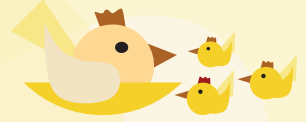
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WELCOME LETTER

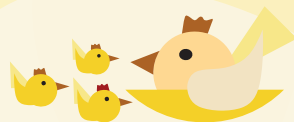
Dear colleagues,

It is our pleasure to welcome you to **XIth European Symposium on Poultry Welfare**, which take place between **26–29 June 2023 in Prague, Czech Republic**.

The symposium continues a long tradition of meetings bringing together scientists from academia and industry to present on-going research activities in the area of poultry welfare and provides an update of current knowledge for people from both industry and research institutes. The program includes invited talks on up-to-date topics in the field of poultry welfare, short communications, and poster presentation, and provides time for personal interactions between old friends and new contacts. We hope you will be delighted by both an attractive scientific program and the lovely Prague scenery, and will take home positive memories of the meeting.

Pavel Trefil

President of the Czech Branch of WPSA



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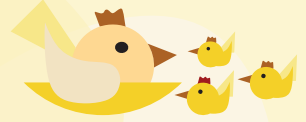
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ORAL PRESENTATIONS

Session 1 – Welfare as part of the future of poultry production

Selecting for improved animal welfare contributes to more sustainable production of eggs

Teun G. H. van de Braak

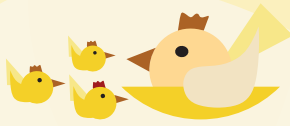
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Outline

In Europe and North America there has been a strong consumer demand to take welfare into account in animal production. Table egg consumption patterns have seen a major shift from cage to non-cage production systems because of societal pressures. This shift likely signals the future direction for the layer sector in developed countries with the acceleration of the conversion of cage to barn and aviary systems with access to wintergardens or the outdoors. Next to many advantages as the hens are much better able to express their natural behavior's, this shift can come with unintended consequences for the welfare of the hens, from bone fracture to increased disease exposure, and for the environment as cage-free housing systems have a bigger negative impact on the environment, all requiring solutions. One of the directions to improve the sustainability of the production of eggs, whilst improving the hens' welfare, is breeding for longer production cycles. As laying hens are born with the potential to produce more than one-thousand eggs, whilst this is not visible yet in the field, there is less selection pressure on selecting birds that can produce more eggs. The focus of today's breeding program is therefore on the hens' health and wellbeing. The higher their health status and their overall robustness, the better their chances to survive and remain productive. Also, from the farm economics point of view it makes sense to select for improved livability. Mortality not only comes at a big cost for egg producers, but it also has its impact on the motivation and mood of egg producers, as no single egg producer likes to collect dead hens from their barns.

With increasing numbers of laying hens kept in cage-free housing systems, (currently more than 10% of the world's laying hen population is kept cage free), the importance of successful rearing is more important than ever. The rearing period is a crucial period in the hen's life, as this is the period that the basis for a successful productive life is formed. The goal is to select pullets that have the genetic potential to grow well so they can develop a robust frame, with good feather cover, but also with sufficient appetite. Since the beginning of genetic selection hens became earlier sexually mature and have become smaller in size. This is a development that has been stopped during the past 15 years as it is acknowledged that birds that are kept for longer production cycles, without forced molting, need the time to develop as the onset of lay can be a challenging event in the lifetime of a hen. Transfer of hens is always accompanied with stress: in all housing systems the hens need to adjust to a new environment, accompanied with plenty of new faces. The goal is to select hens that can deal with these challenges, one of the most important traits to select for is their appetite. It might sound contradictory, but you want birds that can drink and eat well. This will support the hens in their development, hens continue to grow and develop till around 30-35 weeks of age,



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and they start to produce their first eggs around 20 weeks of age. Next to the energetical needs for maintenance, they require energy for both growth and production. Good appetite will allow the hens to overcome any potential deficiencies that could occur due to the onset of lay. This will prevent the hens from using their own reserves, allowing them to continue their growth till adulthood.

Injurious pecking and cannibalistic behavior are among the most common causes of mortality in laying hens. With intact beaks and/or larger group sizes it is more challenging to control any unwanted behavior in laying hens. Genetic selection towards social behavior and implementing new genetic models that include the social effects have been developed and implemented in recent decades. This has resulted in a 50% reduction of the mortality during the past 2 decades. As heritabilities for livability are getting smaller with every new round of selection it will become more difficult to achieve the same speed of genetic progress in the future. But as long as there is room for improvement, intensive selection pressure will be put on improving livability.

Selecting laying hens that maintain good condition at older age, which allows them to maintain their production of 1st quality eggs improves the environmental footprint of egg production. An increasing number of white egg layer flocks already produce till 100 weeks of age or even longer, thereby reducing the number of laying hen replacements and improving both the economics and sustainability. Differences in behavior and performance exist between different hybrid chicken breeds. The different brown and white egg laying strains are centuries apart from each other, resulting in different response to selection. When looking to the accomplishments on genetic improvement in recent decades, it is expected that the gap between brown and white laying hens in terms of behavior and performance is becoming bigger in the future. Resulting in a higher positive ranking of white egg layers breeds in terms of sustainability and animal welfare.

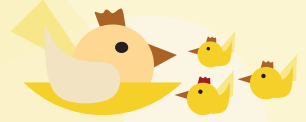
Cag-free housing systems require different capabilities of laying hens compared to those housed in (enriched) cages. The genetics of laying hen behavior in cage free housing systems is thus far poorly understood. Research collaborations, like with HenTrack (University of Bern, Switzerland), are therefor in place to gain a better understanding of the genetic involvement in the phenotypic differences that are observed in cage-free flocks. Another project is the IMAGEN project, (AnIMAl Group SENsor), which is a multi-faceted, long-term study that combines animal breeding with advances in sensing and AI technologies to help in the transition towards sustainable livestock production. By combining sensing and AI technology with animal breeding and genetics, this project aims to improve the health and welfare of livestock and reduce the ecological footprint of our food production.

A major ethical issue for the egg industry is the culling of male day-old chicks of layer breeds as the meat of the males cannot be easily marketed. Much research has and will be devoted to alternatives. Among the available solutions are in ovo sexing and dual-purpose chicken breeds. The various dual-purpose strains are less efficient and currently do not compete economically in poultry meat or egg markets. Several commercially viable in-ovo sexing techniques have become available in recent years. From a genetic point of view, research has been performed after finding a deleterious allele that could cause a “natural” death of the male embryo or on genetic engineering. So far, only the latter has been successfully developed at research institutes. But adopting and implementing the genetic engineering technology in practice comes along with an ethical debate, with strong No’s and strong Go’s.



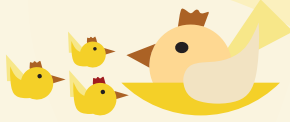
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As consumer awareness increases viable markets could emerge in the future. These priorities are the response to economic, environmental, ethical and consumer pressures that are already having a strong impact on the egg industry. They will continue to evolve in the next decade and if supported by a strong research and development effort, a more efficient and ethical egg-laying industry could emerge. The poultry breeding companies, that remain today, have placed both animal welfare and sustainability high on their research agendas. In today's world, where we are dealing with climate change, rising food insecurity and an increasing need for animal-worthy livestock farming, one cannot go without another.

Keywords: Laying hens, Animal Welfare, Sustainability, Genetic Selection



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Barriers and levers to enhance laying hen welfare in low-input and organic farming – a supply chain survey

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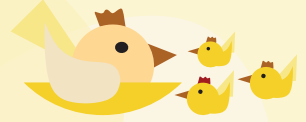
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Within low-input outdoor and organic poultry production systems, providing free range potentially favouring animal natural behaviour, but increasing risks for health, some laying hen farming practices have the goal to improve animal welfare. How supply chain actors (e.g. farmers, veterinarians and farm advisors) perceive these practices has not yet been studied, though their perspective is necessary for feasibility assessment and future decision-making. A survey was conducted to compare views of supply chain actors in nine different EU countries (Belgium, Denmark, Finland, France, Germany, Italy, the Netherlands, Romania and the UK) on management practices. Respondents were asked to assess the advantages and disadvantages of measures that aim to improve laying hen welfare, such as in-ovo sexing, the use of dual-purpose breeds, and the reduction of mutilations such as beak trimming. An exploratory factor analysis of 113 responses showed that supply chain actors were aware of the potential welfare benefits, but also of the disadvantages associated with some of these measures. Measures favoured by respondents were the provision of enrichment (85%) and shelter opportunities (86%) and the use of vaccines and antiparasitic treatments (65%). Keeping birds with intact beaks was favoured by the majority of farmers (63%), though this opinion varied between countries ($p=0.05$), thus probably reflecting local markets and regulations. Financial limitations were considered the main barrier to the implementation of welfare improving measures (70.4%).

The study was part of the PPILOW project (Poultry and Pig Low-input and Organic production systems' Welfare), receiving funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 816172.

Keywords: organic, supply chain, farmers, welfare, laying hens



Does individual performance affect animal welfare in dual-purpose chickens?

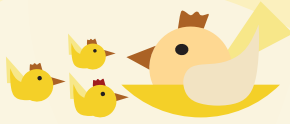
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For years it has been discussed whether higher performance has a negative impact on animal welfare. This study investigates foot pad lesions (FPL) and keel bone damages (KBD) visually/ by palpation based on the Welfare Quality® Assessment protocol in 76 hens of the British dual-purpose breed Ixworth in a free-range system regarding their individual performance. The onset of lay, egg weights (EW) and total egg count (TEC) as well as welfare indicators were recorded on an individual level. In total, 74% of the birds showed KBD at the end of the laying period (72 weeks of age (woa)), mostly alterations and dents. No significant correlation could be found between KBD and TEC ($r = 0.123$; $p = 0.288$), EW ($r = 0.058$; $p = 0.616$) and the onset of lay ($r = -0.156$; $p = 0.179$) based on the individual data assessed. Furthermore, it was investigated whether the individual EW of the hens in the first 35 woa (until ossification of the keel bone) is correlated with the KBDs. A medium positive correlation was found, indicating that the hens with bigger eggs in week 30 ($r = 0.239$; $p = 0.049$) and 31 ($r = 0.377$; $p = 0.002$) show a higher prevalence of KBD. All other ages showed no significant correlation. Considering FPL, individual data revealed no correlation with the onset of lay ($r = -0.108$; $p = 0.351$) and the average EW ($r = -0.088$; $p = 0.451$), but there was a medium positive correlation to TEC ($r = 0.302$; $p = 0.008$). These results show individuals laying more eggs have a higher prevalence of FPL. Furthermore, the combination of age and corresponding egg sizes, seems to have an influence on KBDs. In further research into welfare issues, Precision Livestock Farming such as animal-specific data adds significant value.

Keywords: Dual-purpose chicken, animal welfare, keel bone, animal-specific data



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Welfare as part of Sustainability of Poultry Meat Production

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Abstract

Poultry meat is lean, healthy and affordable with a low environmental impact. Animal breeding has contributed to these attributes and to the improvement of welfare at the same time. Breeding is at the start of the food chain and is a long-term exercise. Breeding outcomes are permanent and cumulative and disseminated widely through the supply chain. Breeding for welfare and sustainability requires simultaneously improving and optimising environmental impact, productivity, robustness and welfare. Over recent decades, meat poultry breeding programmes have implemented balanced approach by broadening breeding goals and managing trait antagonisms. Continued investments in all parts of the breeding programme, together with feedback from all stakeholders across the production chain, ensure the direction of progress is in line with the requirements and needs of the customer base and society as a whole. Robust outcome based key welfare indicators at the production level are important tools to monitor and improve welfare outcomes in the various production systems applied.

Introduction

At Aviagen, chicken and turkey welfare is a cornerstone of sustainability. Animal welfare provides a crucial contribution to sustainable meat production: healthy animals are more resistant to disease, their liveability is stronger, and they perform better. These are also positive for the environment and economic sustainability of farmers, meaning welfare and sustainability is essentially good business. This is possible because antagonistic biological outcomes like those between production and welfare and health can be and have been improved at the same time leading to a choice of crossbreeds with improvements in a broad range of characteristics.

Breeder commitment to improving sustainability

Within Aviagen a set of 5 commitments to breeding for welfare and sustainability have been established alongside supplying global food demands, have been established (Aviagen, 2018; Aviagen Turkeys, 2017). The 1st Commitment is around health and food security, securing a safe and secure supply of healthy birds to help feed a growing global population. The 2nd commitment focuses on genetic diversity through maintaining broad gene pools, the major asset of a breeding company. The 3rd commitment relates to implementation of a balanced breeding programme ensuring harmonised progress across all traits in the breeding goal. The 4th Commitment is around the importance of management and stockmanship of the improved populations which allows the expression of genetic potential across all production systems around the world. The 5th Commitment is covering transparency, communication and engagement. These commitments are in line with the Code of Good Practice for Sustainable and Responsible Animal Breeding, Code-EFABAR (EFFAB, 2020; Besbes & Neeteson-van Nieuwenhoven, 2006), and aligned to the global meat sector



sustainability commitments (Aviagen, 2021). The International Poultry Council (IPC), the global meat poultry association, has established where the meat poultry sector can make a difference for sustainability, in terms of the three pillars of environment, economy and society, and of the United Nations Sustainable Development Goals, SDGs. These are in particular SDGs 2 No Hunger, 3 Good Health and Well Being, 4 Quality Education, 9 Industry, Innovation and Infrastructure from and with good health and welfare, and 13 the Climate: where poultry impacts positively on our global footprint. This alignment with the UN SDGs was confirmed in a joint Food and Agriculture Organisation (FAO) – IPC declaration, signed in 2019 in São Paulo, Brazil (IPC, 2019).

The Aviagen breeding programmes

Since the 1970's Aviagen has demonstrated an ever-increasing focus on animal welfare and sustainability traits within its broiler and turkey breeding programme. Aviagen keeps large gene pools consisting of a wide range of pedigree chicken and turkey strains with a very wide range of biological features and attributes. These populations give rise to the great-grandparent and grandparent generations which in turn originate the parent stock and then the commercial broiler or turkey crossbreds. It takes around four years from pedigree to the broiler/turkey generation – resulting in performance and welfare-improved birds, with at the same time better health and environmental impact. These improvements are continuous and cumulative over time. It is crucial that at all times trends and requirements from customers and society are considered when setting the breeding direction. In addition, a key component of sustainable breeding is continuous investment in research and development to follow the latest scientific insights and technological advancements.

Selection of antagonistic traits

It is not taken for granted that all traits are improved at the same time, as when only production traits are improved then health, welfare, robustness and reproduction can be impacted negatively. This antagonism of traits can be overcome as long as the genetic correlation (GC) between the traits is not extreme. Figure 1 shows the GC between a number of health and welfare traits with body weight and yield in broilers (Avendaño *et al.* (2017)).

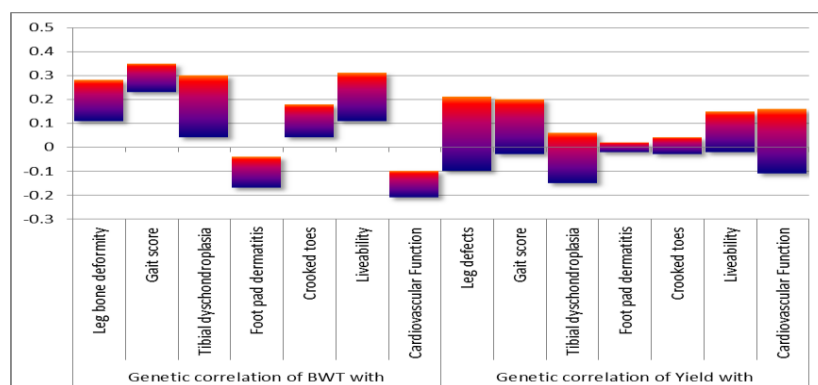
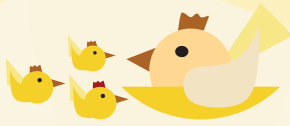


Figure 1. Ranges of genetic correlations between live weight (BWT) and breast yield (%) with leg bone deformities (%), gait score, Tibial Dyschondroplasia (%), Footpad Dermatitis (%), crooked toes (%), liveability (%) and oxygen saturation levels in blood (%; cardiovascular function). [From: Avendaño *et al.*, 2017]



The antagonism between traits can be managed if accurate phenotypes of each trait is recorded and both traits involved in the antagonism are included in the breeding goal. The more unfavourable the relation between traits, the more efforts (being more precise, measuring more animals etc.) must be made to reach improvements (Hocking, 2014).

To illustrate this, Avendaño *et al.* (2017) showed two antagonistic traits in broiler breeding: feed conversion rate (FCR) and hatchability (Figure 2). In this example, the GC between hatchability and FCR is 0.27, a typical antagonism between meat bird and breeder performance traits. The trend line indicates that moving to the left the birds have better FCR but worse hatchability while to the right the birds have improved hatchability but poorer FCR. In this example FCR would deteriorate by 0.012 (i.e., 12 g of feed per kg of live weight) per 1% improvement of hatchability. The antagonism is overcome by including both traits in the breeding goal and selecting birds that have both good FCR and hatchability. These birds are in the bottom-right box. Following this approach, all traits in the breeding goal can be improved simultaneously.

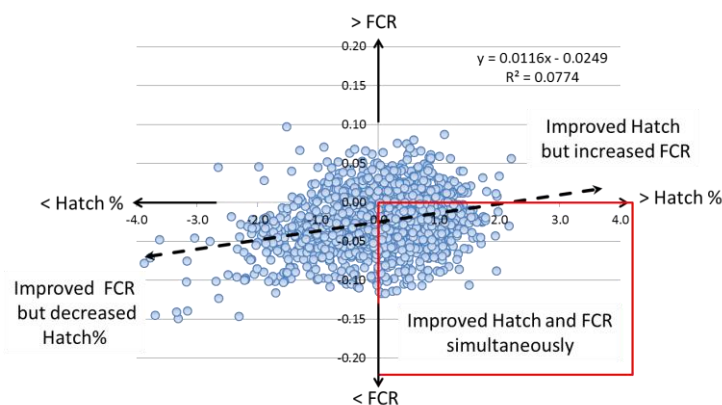
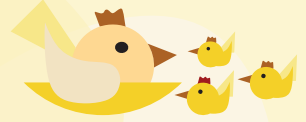


Figure 2. Estimated Breeding Values (EBVs) for 1385 pedigree birds for FCR (Feed Conversion Rate; vertical axis) and hatchability% (horizontal axis) are shown as deviations from the population mean. [From: Avendaño *et al.*, 2017]

In broiler and turkey breeding programmes with tens of traits each, these antagonistic as well as positive relationships between the different traits are considered to ensure a holistic improvement of all traits over time. At the same time differentiation and specialisation between lines for different roles in the crossbreed product is maintained allowing a different breeds to be offered to the market (Ralph, 2017a).

Improvements in welfare

Improvements in welfare are realised by managing trade-offs between performance traits (e.g., body weight, breast yield%, FCR) and welfare related traits (e.g., leg health, contact dermatitis). Kapell *et al.* (2012a, b) confirmed that leg health (e.g., valgus / varus) has effectively been improved for decades, and showed how contact dermatitis (e.g., footpad health) can be improved simultaneously in high and low hygiene environments by managing the information from pedigree birds and their siblings in different environments in an intelligent breeding programme design. This illustrated that “continued genetic improvement for production efficiency and minimisation of demand on resources is feasible without sacrificing



animal health and welfare in a multi-trait selection set up including both fitness and production traits” (Hill, 2016).

The simultaneous welfare and production improvements between 1996 and 2022 of the pedigree lines comprising the Ross 308® are illustrated in Figure 3. Although the relationships between body weight and the health and welfare traits are unfavourable within each year, there is a clear favourable direction for each trait because of simultaneous selection, that is, as bodyweight increases, cardiovascular function, leg strength and liveability increase while crooked toes decreases.

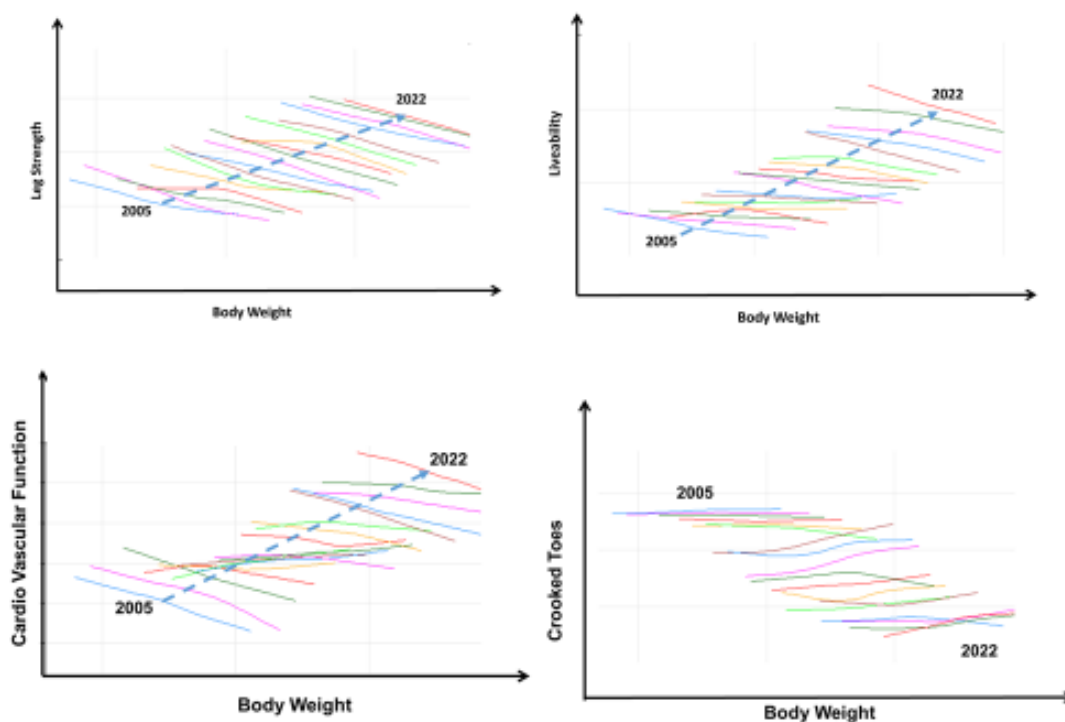
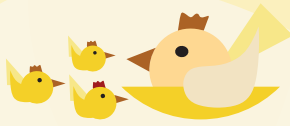


Figure 3. Long term relationships between live body weight and leg strength (%), liveability (%), oxygen saturation in the blood and crooked toes (%). Each coloured line represents the relationship between breeding values for each trait within a year. The broken arrow represents the joint direction of the average breeding value for each trait involved in the trade-off.

Similar means to the improvement of health and welfare and achieved advances in turkeys have been explained and illustrated in walking ability, footpad health, valgus / varus and tibial dyschondroplasia (Maine *et al.* 2006, 2007; Swalander, 2012; Kapell, 2016; Kapell *et al.*, 2017; Ralph, 2017b; Burnside & Ralph, 2023).

Public information from the Canadian Food Inspection Agency (CFIA, 2023; Figure 4) show that the results in the breeding programme are also reflected at the industry level – ascites, reflective of heart and lung health, and leg health have demonstrably improved between the 1990s and 2022 in broilers and turkeys.



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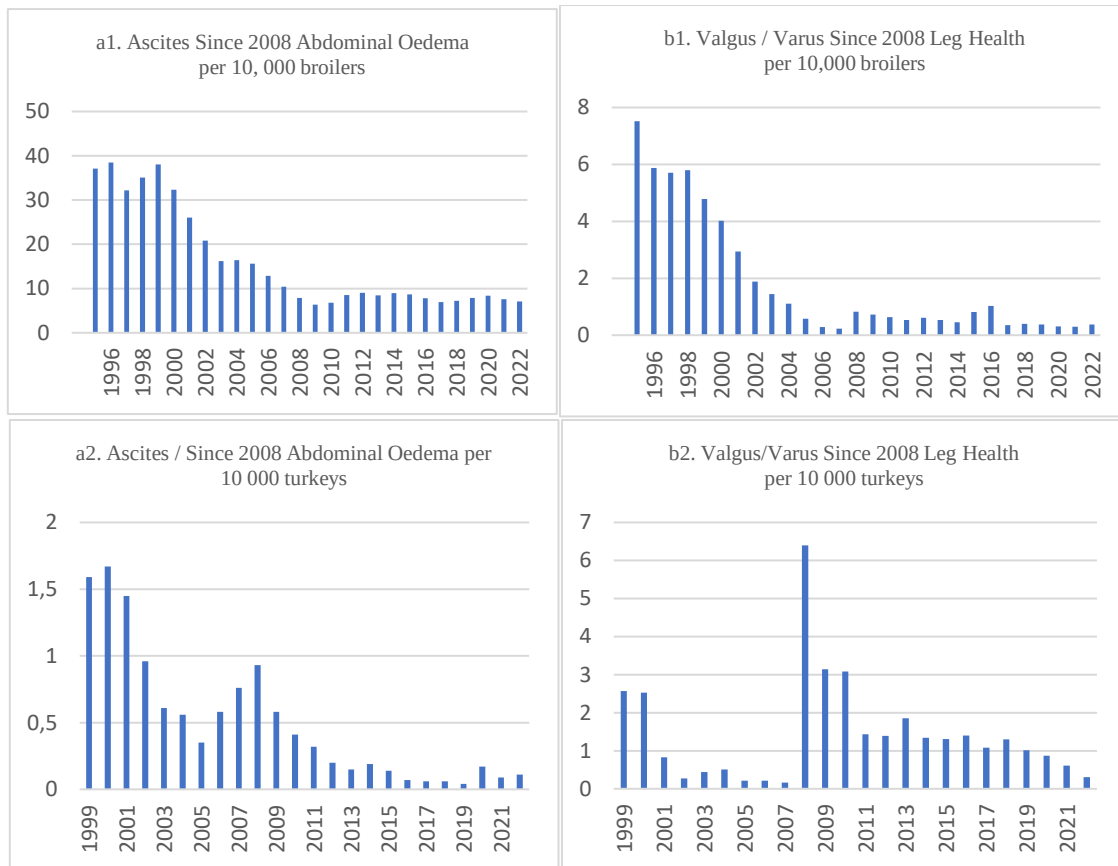
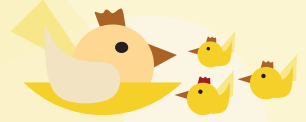


Figure 4. Ascites (*as* from 2008 abdominal oedema) (a1 and a2) and leg health (until 2007 valgus/varus) (b1 and b2) related condemnation rates in broilers/turkeys per 10,000 (1. Chickens 1995–2022; 2. Turkeys 1999–2022). Canadian Food Inspection Agency (CFIA), 2023)

Improvement of robustness

How robust meat chickens and turkeys are globally is very relevant as the birds are farmed across the world in a wide range of production regimes. The diversity of environments can be characterized by varying management practices, feed quality (form and density) and gut and immune challenges. In these environments substantial genotype $\times$ environment (G $\times$ E) interactions will exist (Hill *et al.*, 2016).

Whereas pedigree populations are kept at high standards of management, health and bio-security, it is crucial to include elements of differential production to the pedigree programme to minimise the G $\times$ E interaction and ensure the expression of the genetic potential in commercial environments. One way to do this is to combine records from high hygiene pedigree environments with those taken in low biosecurity, low input environments to represent the wide range of commercial production environments in selection decisions, e.g. via commercial sibling testing (CST; Kapell *et al.*, 2012a). Using CST improves robustness because siblings will express a range of traits related to robustness and environmental adaptability like gut health, digestive, and immune function along with liveability, growth, and uniformity. The result will be



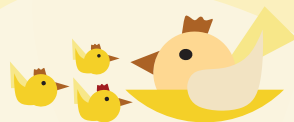
improved productivity in both environments, increased animal adaptability to the wider range of management circumstances they may encounter in the field, and more robust populations with higher liveability and uniformity (Ralph 2016).

Improvements in environmental footprint

Population growth and prosperity is fuelling demand for global meat production and increasing the importance of its environmental impact. Poultry production is in a favourable position in terms of environmental impact with 3.7–6 kg CO₂ per kg edible carcass which is much lower than the corresponding ranges for other meats (20–60 for ruminants and 7–20 for pork) (Poore and Nemecek, 2018; Ritchie and Roser, 2020). Poultry is expected to have a pre-eminent role in satisfying the increasing global demand for meat products while at the same time reducing the environmental impact of meat production globally. Recent predictions suggest that Green House Gas emissions of the meat sector are projected to rise by 9% by 2031 which is less than the 15% increase in total meat production due to the shift towards poultry production (OECD-FAO, 2022).

With a Life Cycle Assessment (LCA) tool developed by Cranfield and Newcastle University (Poultry LCA Model Version 1.0) (Williams *et al.*, 2006; Leinonen *et al.*, 2012) Aviagen recalculates the environmental impact of the broiler crossbreeds in the portfolio on a regular basis (e.g. Avendaño *et al.*, 2017). The ‘cradle to farm gate’ LCA approach accounts for all inputs and outputs of a production system as described by Leinonen *et al.* (2012). Ross 308® and 708® are globally established commercial broiler crossbreeds; Rowan Range® broiler types are slower-growing crossbreeds (eu.aviagen.com/brands/rowan-range). The Rowan Range genotypes fall under the requirements of accreditation schemes like Global Animal Partnership (GAP)’s Better Chicken in North America (GAP, 2023), ‘Kip van Morgen’ (ACM, 2015) and ‘Beter Leven’ (Dierenbescherming, 2023) in the Netherlands, ‘RSPCA Assured’ (Royal Society for the Prevention of Cruelty to Animals (RSPCA), 2023) and Red Tractor Enhanced Welfare (Red Tractor, 2023) in the UK, and ‘Für mehr Tierschutz’ (Deutscher Tierschutzbund, 2023) in Germany.

More biologically efficient crossbreeds have the lowest environmental impact in terms of pollutant emissions and other outputs in LCA (Figure 5). As average daily gain and breast yields increase, global warming potential (GWP) decreases linearly – the opposite trend is seen for feed conversion rate (FCR). These results are consistent with the findings by Leinonen *et al.* (2012): a free range and an organic production system had a predicted higher GWP of 16% and 28%, respectively over a standard production system. These findings are in line with Herrero *et al.* (2013) which conclude that feed efficiency is a key driver of productivity, resource use and greenhouse gas emissions.



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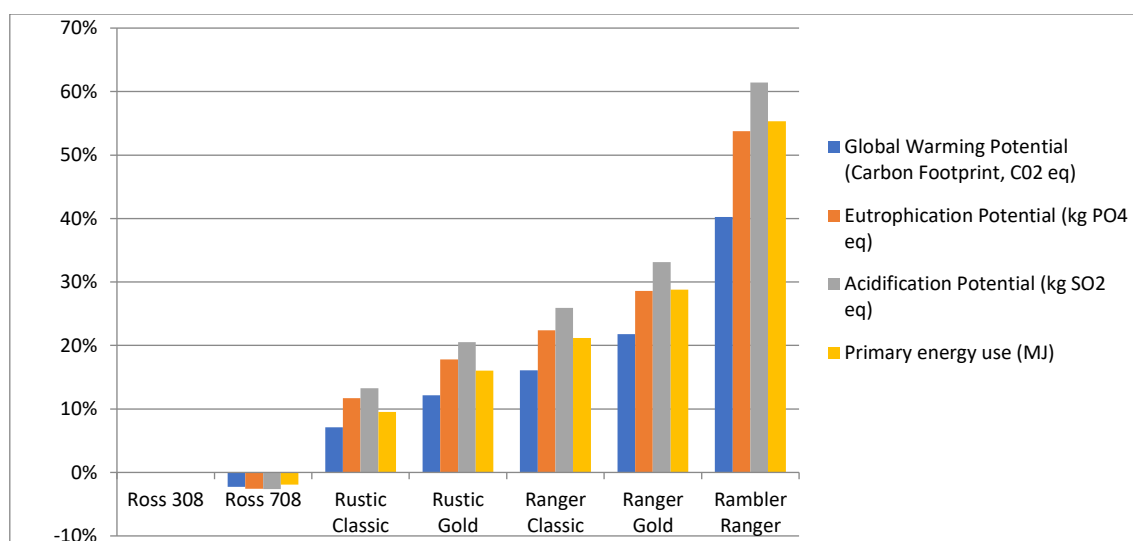


Figure 5. Relative global warming potential (GWP), acidification potential (AP), eutrophication potential (EP) and primary energy use (PEU) levels for seven Aviagen crossbreeds to 2.5 kg bodyweight (Ross 308 is the base for comparison at 1.0)

The overall conclusion is that the environmental impact of crossbreeds with lower biological efficiency on resource utilisation and environmental burdens is 30–40% higher compared to conventional genotypes. FCR differences were also the main driver for environmental impact differences in LCA research in turkeys (Kremer *et al.*, 2014, Ralph 2020) and in a study comparing male and female turkeys grown in two ventilation systems (Leinonen *et al.*, 2014a, b; 2016).

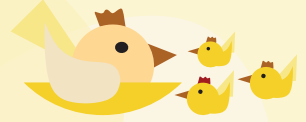
On the other hand, more broadly, the suitability of a crossbreed to a production system or market segment will depend not only on biological performance but also on consumer preference, product price and other product attributes including the perceived importance between performance and welfare (Avendaño *et al.*, 2017).

Discussion

Poultry breeding takes place at the start of the food production chain, and its improvements are permanent (in the genes of the animals), cumulative (the changes from one generation is added to the improvements of previous generations) and are disseminated to the wider industry

Poultry meat is an increasing part of animal protein in human diets and its role feeding humans continues to gain importance. A critical look at poultry production is, therefore, understandable and for the poultry sector to embrace and work diligently on continuous improvements. Data to show the improvements in productivity, health and welfare, production and environmental impact across a broad spectrum of cross-breeds will illustrate the achievements that have been made. Ideally the improvements will be reflected in the opinion about poultry production in the wider society.

An example of criticism about breeding broilers can be found in the EFSA (2010a, b) scientific opinions, which stated amongst others that “*The major welfare concerns...have a genetic basis and may interact with*



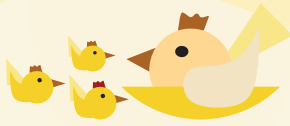
management factors to lead to poor welfare include skeletal disorders, contact dermatitis, ascites and sudden death syndrome. Most of these are linked with fast growth rates.” Indeed, with unbalanced breeding this could be the case. Later, the Farm Animal Welfare Council (FAWC) Breeding report (FAWC, 2012), and the European Commission report by Hiemstra and Ten Napel of 2013 did confirm that breeding goals in meat poultry have for decades included traits on skeletal, leg and foot health, heart function, liveability. FAWC (2012) stated that with regard to the concerns ‘summarised in 2004’ (FAWC, 2004) they were ‘encouraged that many breeding goals now include aspects of animal welfare’, and that ‘as long ago as the 1970s some breeding companies recognised the benefits of including welfare (e.g. leg health) in tandem with production traits in their selection programmes.’ For Aviagen, this highlights the importance of our commitment to transparency, communication and engagement.

In addition, information about the ways to deal with antagonism, and improvements achieved have come available from peer-reviewed literature (e.g. Kapell et al., 2012a, b; 2017, Kapell 2016)), and are demonstrated in public information like the Canadian Food Inspection Agency data (CFIA, 2023), articles in poultry magazines, and in more public friendly form from livestock sector actors like the European Livestock Voice (ELV, 2022), the Animal Agricultural Alliance (AAA, 2023), European Forum of Farm Animal Breeders (EFFAB, 2022, 2023), and the company and association websites (e.g. Aviagen 2021, 2023; Aviagen Turkeys, 2023), social media messages, webinars and continuous dialogue with the wider stakeholders including governmental bodies.

Next to IPC with their commitment to the UN sustainability pillars and SDGs, globally and at more regional levels, the poultry sector have developed welfare and sustainability initiatives. In addition to the voluntary auditable standards like National Chicken Council (USA; NCC, 2020, 2022), National Turkey Federation (USA; NTF, 2023), Red Tractor (UK; Red Tractor, 2023), Qualität und Sicherheit (Germany; QS, 2023), the US poultry sector have developed a Sustainability Framework (US-RSPE, 2023), the European meat poultry sector the Sustainability Charter (avec, 2023), and the International Poultry Welfare Alliance (IPWA) Key Welfare Indicators (KWIs) (IPWA, 2023). These are all instruments aimed to show how the poultry sector works with sustainability and welfare.

Aviagen has a long standing commitment to contributing to food security, whilst improving animal welfare as well as better health, robustness, and environmental sustainability and product base for options from affordable protein to the diversity of production systems for both conventional and slower-growing birds. At least one-third of the selection focus is on health and welfare traits. Ongoing innovation is at the heart of the company – with innovative technology, current traits will be improved and new areas explored and where feasible implemented. Examples are in gut health, behaviour, and computed tomography scanning to assess both meat yield and welfare in live birds.

In addition, from the 1st Commitment we continue to work for a safe and secure supply of healthy birds, free from diseases like Avian Influenza, Newcastle Disease, Leucosis, all *Salmonellae* and all *Mycoplasmas*, with breeding programmes on two continents and high-generation operations spread around the globe. The 2nd Commitment, the diversity of poultry lines as the basis we breed from, covers our responsibility to ensure there is poultry available for the future, and to provide a choice of cross-breeds. With the outcome of the 3rd Commitment, the balanced breeding programme, the bird populations change on an ongoing



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basis, and the way to manage them will change gradually over time, as will the conditions poultry farmers will encounter due to technological and societal changes. At the flock level a large part of the health, welfare and performance are influenced by management factors. The Greenwell project (De Jong and Te Beest, 2020; De Jong *et al.*, 2022) illustrates that with the fast and slow-growing breeds used in the Netherlands the farming system was the major factor impacting outcome-based bird welfare. Management was the key factor explaining the differences between the conventional system (fast-growing birds, standard conditions), Kip van Morgen (broilers with maximum 50-gram growth per day, farming conditions close to Better Chicken Commitment) and Beter Leven 1 star (maximum growth per day of 45 grams, additional management requirements like lower stocking density, environmental enrichment, early feeding of day old chicks). The 4th Commitment represents our investments in customer support like management materials, technical specialists, and training schools. With management gaining importance for animal welfare, there is a need for robust outcome-based KWIs to provide poultry science-based, repeatable assessment opportunities to monitor continuous improvement in welfare. For instance, the IPWA KWI guides (IPWA, 2023) are practical reference guides, developed to complement the use of other applicable standards and/or guidelines. They provide guidance on how to consistently measure and monitor KWIs in each phase of production, with the aim to help drive continuous improvement in poultry welfare outcomes. Last but not least, the 5th Commitment, engagement, transparency and communication, supports our efforts to be transparent about our breeding work through publishing articles for peer-reviewed scientific journals, trade publications, workshops/schools, and our website. This includes actively engaging at various levels via customers and associations with the wider society. It is important we enter the dialogue, explain what we do and show how we are committed to breeding welfare and sustainability which is also good business.

Conclusion

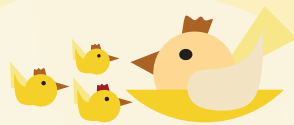
Broiler and turkey breeding will continue to focus on improving overall welfare, health, productivity and environmental impact using broad balanced breeding programmes. The portfolio of commercial broiler and turkey crossbreeds will keep expanding following the requirements of the poultry industry and consumers ensuring the sustainability of poultry breeding.

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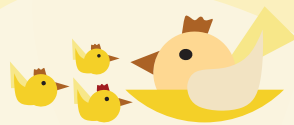
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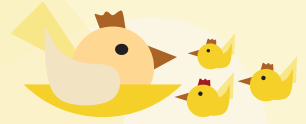
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Impact of the Better Chicken Commitment and Adoption of Slower-Growing Breeds on the Welfare of Broiler Chickens

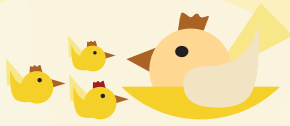
Cynthia Schuck-Paim¹, Wladimir J. Alonso¹, Kate Hartcher², Kaitlin Wurtz^{1,3}, Lauren C. Dawson⁴, Daniel O'Hagan⁵

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We assessed the impact of the Better Chicken Commitment (BCC) – a set of recommendations that includes better housing, stocking density and growth rates – on the welfare of broiler chickens. Specifically, we examined concerns that use of slower-growing breeds may increase broiler suffering by extending their lifespan for the production of the same amount of meat. To this end, we used the Cumulative Pain Framework, an analytical approach aimed at quantifying the cumulative load of negative affective experiences endured over any period and production context using a meaningful metric: time spent in pain (shorthand for ‘any negative affective state’) of four intensities (Annoying, Hurtful, Disabling, Excruciating). We compared two scenarios: conventional, represented by use of fast-growing breeds slaughtered at 2.5 kg at 42 days, and reformed, where slower-growing strains reach this weight in 56 days. In each scenario, Cumulative Pain was assessed as a result of gait-impairing conditions, cardiorespiratory insufficiency, heat stress, inability to perform potentially motivated behaviors and chronic hunger in breeders. Estimates of pain intensity were informed by clinical, neurophysiological, behavioral and evolutionary evidence. Our results indicate that adoption of slower-growing strains has a net positive effect on welfare. Because the incidence of most welfare offenses is reduced and their onset delayed, slower-growing birds experience shorter, not longer, times in pain before slaughter. Overall, for every bird the reformed scenario prevented 33 [13–53] hours of Disabling pain, 79 [–99–260] hours of Hurtful pain and 25 [5–45] seconds of Excruciating pain, corresponding to a reduction of, respectively, 66%, 24% and 78% in the time experienced in these categories of pain intensity. These estimates represent the minimum time in pain averted with implementation of the BCC. Although beneficial, adoption of better housing and management was shown to be limited if the negative welfare effects of fast-growth are not addressed.

Keywords: Better Chicken Commitment, broiler, Cumulative Pain Framework, Slower-growing breeds, welfare assessment



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Session 2 – Welfare assessment and precise livestock farming

A framework for a comprehensive poultry welfare label: scientific, logistic and ethical challenges

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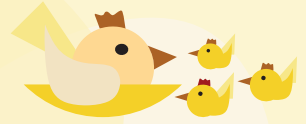
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How a comprehensive poultry welfare label forms part of an ethical society

Nine of the 27 EU member states have food labelling schemes with animal welfare claims and there's a growing plethora of private animal welfare labels (AWL), e.g. as part of the marketing strategy of retail companies (Lundmark et al 2018, Rowe et al 2021, EC 2022, Stygar et al 2022). In line with other types of food labelling systems, the impact of these labels is generally considered disappointing (Ben-Shahar 2016, Osman & Jenkins 2021, but see Mullan et al 2016, 2022). The multitude of labels often confuse the consumer, and there seems to be a clear need for raising awareness and simplifying the existing state of play of these labelling schemes (EC 2022). Both the European Commission and some national authorities, such as the Flemish Government, are therefore considering options for animal welfare labelling to better transmit value through the food chain. This paper wishes to contribute to these developments by proposing a framework for a comprehensive AWL for poultry products (mainly meat and eggs) that can contribute toward greater sustainability transparency and ethical purchasing.

There are essentially two, non-mutually exclusive, roles of labelling food with ethically relevant information: providing information only or using labels to drive behaviour change. For the AWL framework that we propose, we wish the effectiveness of the label to be judged by the extent that consumption (and hence production) patterns have shifted to the benefit of the birds. This contrasts with labels that – at least in our opinion – highlight less morally relevant features, and sometimes promote hidden agendas of animal welfare labelling schemes such as sheer profit.

In order to achieve this goal, the AWL should meet some important criteria: 1) reflect animal welfare status correctly and comprehensively, including all major aspects of animal welfare, all relevant parts of the production chain and the entire lifetime of the birds, 2) be cost-efficient, and 3) align with consumer expectations. Indeed, lack of transparency and trust, as well as costs being too high or not being aligned with expectations (value for money) have been identified as barriers for consumers to purchasing animal-friendlier products even if they state in surveys that they would prefer to do so (Ingenbleek & Krampe, 2022).



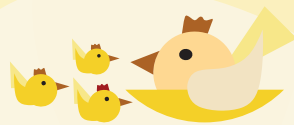
A novel framework for a comprehensive poultry welfare label

We propose a novel framework for a comprehensive AWL for poultry that incorporates morally relevant attributes (animal welfare status) with a fair reward and penalisation system favouring high *potential* and *actual* welfare status. Our poultry AWL consists of 4 tiers of increasingly better welfare that combines the best of input (resource-based) and output (animal-based) measures of poultry welfare. Each tier is labelled, for example with 1, 2 or 3 stars, whereas the lowest tier has no star.

In first instance, the highest animal welfare tier that can be claimed by a farmer, catching team, transporter or slaughterer is determined by Method of Production (MoP) requirements for the respective stages of the birds' life. These input requirements concern the conditions in which the animals are kept and how they are managed from hatching until slaughter. The degree to which these requirements provide opportunities for experiencing positive welfare and preventing negative welfare determine the maximum number of stars that can be achieved. The "Good Life Framework" developed by Edgar et al (2013) for laying hens and by Rowe & Mullan (2022) for broilers (as well as beef cattle and pigs) is an example of a potential starting point for this.

As MoP requirements determine the potential rather than actual welfare status and as there are few stimuli to strive for optimal welfare once the housing and management requirements for a certain tier are met, the birds are also screened for key animal-based measures (ABM). Whilst suitable ABMs could theoretically be monitored across all the life settings of the birds, we propose in the first instance to do this screening at the slaughterhouse for various reasons that relate to feasibility, comprehensiveness and reliability: 1) it allows monitoring of ABMs that relate to the various stages of the birds' live (production – catching – transport – slaughter), 2) it is cost & time efficient as all birds from the multiple farms eventually end up at the slaughterhouse, 3) there are few bio-security risks, 4) it is the most feasible place to automate the monitoring of ABM (using various sensor technologies and artificial intelligence) and to centralise data collection & processing, 5) conditions in which ABM are recorded are highly standardized reducing the likelihood of biases and noise due to variations in e.g. the age or weight range of the birds, or the visibility of the birds during scoring, 6) animal welfare officers are already present at slaughterhouses and existing meat quality inspections can be expanded by animal welfare assessments.

Protocols for (automated) at-slaughter monitoring of ABM have been or are being developed for both spent laying hens (e.g. www.legmoni.be; Van Noten et al 2022) as well as broiler chickens (e.g. www.awish-project.eu; Maselyne et al 2022). These protocols also provide automated personalized feedback to the person responsible for each production stage (farmer, catching team, transporter, slaughterer) in the form of benchmarking and evolutions in time. At present it isn't fully known whether such feedback in itself suffices for people to take actions to improve the welfare of the birds during that stage of production that they are responsible for. We expect the potential impact of such feedback to be greater if the assessments are used to determine the number of welfare stars that are granted on the AWL as well. The at-slaughter monitoring can perhaps be complemented with relevant and routinely available flock records (such as on mortality, performance, use of antibiotics and other veterinary drugs or treatments), as has been explored mainly for dairy cattle welfare assessments (Sandgren et al 2009, Nyman et al 2011, de Vries et al 2014, Lutz et al



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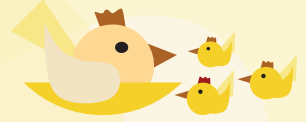
2021). In time, it can also be complemented with automated sensor-based monitoring of poultry welfare on-farm and during transport (Rowe et al 2019, Van Veen et al 2023).

Conclusion

By combining resource and animal-based welfare measures, we have developed the framework for a comprehensive and graded poultry welfare label with the primary goal of improving the quality of life of poultry in the egg and meat industry as effectively as possible. Exact criteria for MoP requirements and ABM monitoring still need to be determined, as well as the optimal feedback mechanism through the supply chain, and consumer-facing information, to drive change towards higher welfare production.

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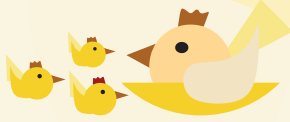
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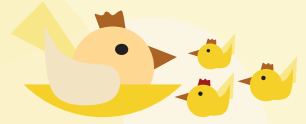
Automated activity tracking in broilers and applications of the resulting individual-level data

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Broilers are often kept in large groups, are similar in appearance and are difficult to monitor individually. However, individual-level data on activity can be informative for health, welfare and performance, and for breeding programs. Therefore, automated approaches for tracking broiler activity are required. In earlier work, a radio frequency identification system was implemented to track broiler activity throughout life, in a 4.7 m² pen, in five production rounds (~80 birds/round). Broiler activity levels were determined using a grid of antennas underneath the pen and tags fitted to the broilers' legs, based on recorded positions over time. With the resulting individual-level activity data (average distances moved per hour for each day), the relationship between activity in the first two weeks after hatching and average daily weight gain (ADG) was examined and genetic parameters for activity were estimated. For the examination of early life activity and ADG, data from 318 birds with daily activity records were used. We observed no statistically significant relationships between ADG and the mean, skewness, autocorrelation or entropy of (deviations in) activity. However, there was a negative relationship between ADG and the root mean square error of activity (estimate = -1.08, p = 0.011), indicating that birds that are more variable in their activity levels early in life show a lower ADG across the full production period. Potentially, this variation in activity is linked to variation in feeder visits and subsequent feeding, but this requires further investigation. A heritability of activity across the full production period of 0.31 ($\pm$ 0.11) was observed, based on data for 387 broilers, indicating that there is potential to select for activity in broilers. Current work focusses on scaling up the activity tracking to larger groups through combination with video recordings, to allow for fast and objective phenotyping of broilers throughout life.



Aviary Transect – A practical welfare assessment tool to improve the management of loose-housed laying hens

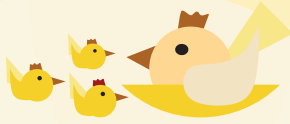
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The Aviary Transect (AT) is a method for assessing welfare in cage-free laying hen flocks. The AT comprises standardized walks along each aisle screening the whole flock for selected welfare indicators: feather loss (FL) on head, back, breast, and tail, wounds on head, back, tail, and feet, dirty birds, enlarged crop, sick birds, and dead birds. The method is quick (20 min for a flock of 7,500 hens), has good inter-observer agreement and shows positive correlations with individual bird sampling methods. However, there is a knowledge gap concerning whether AT can detect differences in flock welfare related to housing conditions. The aim of this study was to evaluate how AT findings relate to selected environmental and management inputs. The study was conducted on 33 commercial non-beak-trimmed white-feathered layer flocks of similar age (70–76 weeks) and flock size, kept in multi-tiered aviaries in Norway. The most prevalent findings were feather loss on the back (mean 0.97 %) and breast (0.94%) followed by feather loss on the head (0.45 %) and tail (0.36 %). Addition of fresh litter during the production cycle was associated with reduced feather loss on the head ($p < 0.05$) and tail ($p < 0.001$), whereas increased time spent by the farmer in the animal room was associated with increased feather loss on the back ($p < 0.01$) and tail ($p < 0.01$). Earlier access to the floor area underneath the aviary was associated with fewer birds with wounds ($p < 0.001$). Higher dust levels were linked with increased feather loss on the head ($p < 0.05$), back ($p < 0.01$), breast ($p < 0.01$) and tail ($p < 0.05$), and more sick birds ($p < 0.001$). Improved litter quality was associated with reduced feather loss on the head ($p < 0.05$) and fewer dirty birds ($p < 0.05$). In conclusion, outcomes from AT varied with environmental factors, supporting the validity of AT as a relevant welfare assessment method.

Keywords: Animal welfare, Commercial egg production, Management tool, Welfare assessment



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Do chicks vocalise more or vocalise differently under thermal stress conditions? Towards automated chicks' stress monitoring based on their vocalisations

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Poultry meat is expected to be the most demanded animal protein in the future. However, the increasing demand for more sustainable and welfare-friendly animal protein challenges poultry supply chains to be more efficient. The climate during chick hatching, holding and transport may compromise the wellbeing and health of the birds during grow out. The detrimental effects of heat stress on broilers range from reduced growth and egg production to decreased poultry and egg quality and safety.

Audio signal processing is emerging as a tool for PLF due different appealing characteristics (non-invasive, no direct line of sight, group monitoring, and relatively cheap). While the application of this tool to broilers' stress monitoring is not new, only few studies have applied audio processing to chicks vocalisation detection. Those that are available borrow techniques and features from the mature field of human speech processing, with risk for misinterpretation. For example, recent research in the field has promoted the spectral entropy as useful feature to predict welfare, linking the stress to the number of chick calls.

In this work, we show the preliminary results comparing spectral entropy to a different time-frequency audio feature when applied on 6 hours recordings of 25-day-old chicks that were exposed to different temperature conditions. Results show stronger correlations to the proposed feature going beyond linking the stress perception to the number of calls. Additionally, a subset of the recordings was manually labelled for each 15 seconds snippets covering different thermal conditions and their analysis showed a low correlation to the calls count. Further research is crucial to translate the proposed features to large-scale use-cases.

Keywords: Thermal stress, Day-old chicks, Acoustic features, Chick vocalisations



Session 3 – Best practices for poultry housing and management

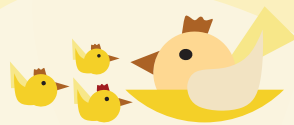
Precision poultry farming and welfare

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These days European poultry farmers are under extreme pressure to remain economically viable in the face of many challenges like farm succession, high input costs, negative environmental impact, and ever stricter animal health, animal welfare and biosecurity restrictions. There is a growing need for better technologies to support farmers in managing environmental emissions, animal feeding, health and welfare. Consequently, Precision Farming (PF) is a burgeoning field where the aim is to exploit Information and Communications Technologies (ICTs) to facilitate high resolution planning and management of farming operations. PF has seen great success in the crop production sectors, where it can be used to maximize the spatial-temporal productivity of the crop within the certain environmental constraints. However, the application of precision farming to livestock production, i.e. precision livestock farming (PLF), has been more challenging given that sentient beings, whose behaviors are not always predictable or monitorable, are at the center of the process. As a result while there are various PLF technologies under research for poultry health, welfare and productivity monitoring and management, the translation to functioning robust farm technologies that capture and act on information is still taking time. Part of the problem is the difficulty in demonstrating the value for the farmer and the animal alike. This requires an answer to how PLF technology can help give the animal a life worth living while at the same time farmers have a livelihood worth pursuing. One way to seek this answer is through a multi-stakeholder approach to technology development. My presentation will look to the opportunities provided by PLF for the poultry sector, the recent state of the art as well as some pitfalls to consider when translating research into functioning solutions.



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Best Practice Hens: an EU pilot project to support the transition to cage-free housing systems in the European Union

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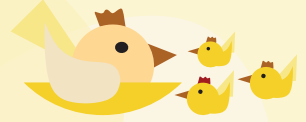
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Since 2012, conventional cages for laying hens have been banned in the EU and all laying hens should be housed in either furnished cages or cage-free systems. However, even in furnished cages, laying hens have limited space and opportunities for natural behaviour. There is considerable variation in the uptake of cage-free systems among EU countries. The Best Practice Hens project aims to help EU egg producers by providing practical guidelines on how to transition to cage-free systems. The Best Practice Hens project combines input from experts from countries that already have a high percentage of cage-free systems (DK, NL, DE, FR; cage-free countries) and experts from countries that are still transitioning (ES, PT, PL, BE; target countries). Best practices for keeping pullets and laying hens in cage-free systems were developed based on the review of scientific literature, management guides and other technical information available. For the scientific review, 39 papers for pullets and 167 papers for laying hens were included after screening and were described in detail. In addition, 59 sources of technical information were found and semi-structured interviews with the egg industry were organized in the cage-free countries. This information was used to formulate 68 best practices, 24 for pullets and 44 for laying hens, some of which are general while others apply to specific housing systems. This detailed set of 68 best practices was further reorganized into 31 general best practices (15 for pullets and 16 for laying hens), which were discussed with stakeholders at EU level and from the involved countries, and finalized based on their input. This final set of 31 best practices is the basis for our dissemination activities, consisting of publication of practice abstracts, social media contributions, our website (www.bestpracticehens.eu) and dissemination meetings in the target countries and a large dissemination event in Brussels.

Keywords: cage-free, laying hen, pullet, housing, best practice



Cage-free housing systems – Laying hens between freedoms and capabilities

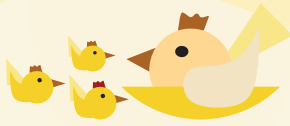
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The capability approach to animal welfare distinguishes between the theoretical freedom to perform a behavior with available resources as well as whether the individual is able to perform the behavior if it wishes to (*capability*) and if it actually does so (*functioning*). In contrast, a resource-based measure approach is often used to judge whether behavioral needs of farm animals are met. The aim of the study was to apply the capability approach to individual laying hens in an aviary system. We tested whether the *functionings* of hens changed when they were victims of toe pecking. At 18 weeks of age (WOA), 1115 Dekalb White laying hens were introduced into 5 pens of a Bolegg Terrace aviary system. All hens were tracked individually with a passive RFID system across 5 zones: wintergarden, litter, and three stacked tiers with different resources. At 40 and 60 WOA toe injuries were rated on a continuous visual analogue scale. The change in use of the zones between these 2 ages in reference to the change in toe injuries was analyzed by general(ized) linear models with pen as a random variable. The more severe the increase in toe injury, the less frequent the hens switched between the zones ($F_{1,1053} = 61.5$, $P < 0.0001$) and the more they preferred higher tiers than they did before the toe injury ($F_{1,242} = 11.5$, $P = 0.0008$). Birds with injuries visited the litter ($F_{1,951} = 28.1$, $P < 0.0001$) and the wintergarden ($F_{1,999} = 12.5$, $P = 0.0004$) less often per day and the wintergarden on less days ($F_{1,859} = 8.2$, $P = 0.004$) than those without. In conclusion, despite being victims of toe pecking, birds were still capable of accessing all zones in an aviary but changed the pattern of use (pattern of *functioning*).

Keywords: laying hen, aviary, toe pecking, behaviour



XI European Symposium on Poultry Welfare

Prague, Czech Republic, 26–29 June 2023



Environmental complexity during rearing and laying: effects on stress responsivity and fearfulness in laying hens

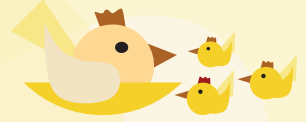
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Laying hens are often housed in environments varying greatly in degree of complexity (cage vs aviary systems). The environmental complexity, both during early and adult life, contributes to shaping individuals' fearfulness and stress responsivity. We aimed at testing whether aviary-reared hens were less fearful and sensitive to an acute stressor than hens reared in cages, and whether provision of additional enrichment during the laying phase could reduce fearfulness and stress responsivity. We tested White Leghorn laying hens reared in a cage (N=128 selected from the same cage) or in an aviary (N=128 selected from the same aviary) in the same room at the rearing farm. They were then housed in furnished cages from 18 weeks of age, with or without the provision of additional enrichment (N=16 cages per group). Between 52 and 54 weeks of age, two hens per cage were tested in a restraint stress test (one control and one stress hen per cage). Blood was collected at two time points (baseline at the start of the test and after 12 min) to measure plasma corticosterone levels. At 60 weeks of age, we tested the hens in a novel object test in their cages and recorded the latency to approach the novel object. Data was analysed with Linear (Mixed-Effects) Models. Birds housed in enriched cages tended to have lower plasma corticosterone concentration ($F_{1,51}=3.35$; $p=0.07$) and were significantly faster to approach the novel object ($F_{1,61}=19.02$; $p<0.01$) than birds housed in standard furnished cages. No significant effects of the rearing environment were found. These results suggest that the environmental complexity during rearing had no long-term effects on fearfulness and stress responsivity. However, they suggest that provision of additional environmental enrichment during adulthood can have positive effects on fearfulness and stress responsivity.

Keywords: stress, fearfulness, laying hens, adult enrichment, early-life



Male percentage: Less is more

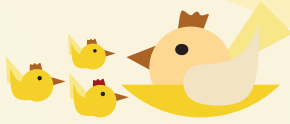
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A low male percentage at onset of lay is a promising method to prevent excessive mating, feather and skin damage of the females and decreased fertility. However, hardly any information is available on different male percentages in broiler breeder production. Therefore, an experiment was carried out to study the effect of a low male percentage at 20 weeks of age (WOA). In a commercial breeder house with the Veranda system, 8 groups with each 660 Ross 308 females were housed. The groups were equipped with separate sex feeding, nipple-drinkers, LED lighting, nest boxes and scratching areas. Four groups with 26 males (3.9%; EXP) were compared to four groups with 49 males (7.4%; CON). Male percentage in the EXP groups was increased to 5%, 6%, and 7% at 30, 40, and 50 WOA, respectively. In the CON groups male percentage was slowly decreased to 6.2% at 60 WOA. At 29, 34, 39, 44, 49, 54 and 59 WOA general female behaviour was observed via scan sampling, mating behaviour of males via focal sampling, feather cover of females was scored, and incubation traits were studied. Data were analysed via ANOVA. No differences were found between treatments on general behaviour, courtship behaviour and successful matings. However, in the EXP groups, more voluntary matings (30.2% vs. 14.2%; $P=0.029$) were observed. Furthermore, feather cover was better between 29 and 54 WOA in the EXP groups. No differences in incubation traits were observed between 29 and 44 WOA, however, true fertility (including embryonic mortality) was on average 2.6% ($P=0.064$) higher for the EXP groups between 49 and 59 WOA. In conclusion, starting with a low male percentage results in improved mating behaviour, feather cover and true fertility at the end of the laying phase.

Keywords: broiler breeders, male percentage, mating behaviour, feather cover, fertility



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Innovative multifunctional platforms for broiler chickens: effects on litter quality and animal welfare

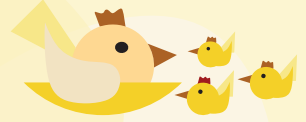
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Chickens are highly motivated to access elevated structures for perching and roosting. For fast growing broilers elevated platforms better accommodate this behavioral need as they find it difficult to access, and maintain balance on, narrow perches. In order to increase the animal welfare benefits and commercial uptake, we've designed prototype platforms with additional functionalities: local cooling for thermoregulation via the footpads, a dark brooder underneath the platform, and manure collection trays (to prevent excreta from falling on the litter). A study was conducted to test the effects of these multifunctional platforms on broiler performance and welfare. Here we report specifically on the effect of the manure collection functionality on litter quality and associated broiler welfare indicators. In each of the 4 rounds, 560 broiler chicks (Ross 308) were equally and randomly distributed over 4 pens, with a stocking density of 3,8 birds/m². Two pens were enriched with the multifunctional platforms (n=3), whereas in the other 2 pens no platforms were installed (control group). Manure on the collection trays was collected ca. weekly or more frequently according to the accumulation of excreta. When the chickens were 39d old, litter samples (4 per pen) were assessed for dry matter (DM) content, pH, and quality. Furthermore, 15 birds from each pen per round were assessed for plumage cleanliness, footpad dermatitis and hock burns. Results of the first round indicate an improved litter quality with better litter score (1.08 vs 1.16), higher DM (72.80% vs 69.15%) and lower moisture content (27.19% vs 30.84%), and a trend for lower hock burn scores (p=0.072) in the pens with the platforms. These preliminary and promising outcomes about the effect of collecting manure underneath the platforms will be complemented by data from the 3 subsequent rounds.

Keywords: Broiler chickens, enrichment, elevated platforms, animal welfare, litter quality



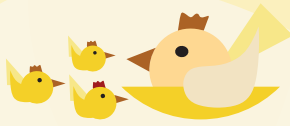
Platforms make better elevated structures for commercial fattening turkeys than perches made of planks

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Providing elevated structures for fattening turkeys is important as resting and sleeping in an elevated position is part of their species-specific behavioural repertoire. As fattening turkeys are intensively bred for weight gain, ability to access elevated structures may be impaired thus considering how to provide structures is relevant. In this experiment, three different elevated structures were evaluated on a commercial turkey farm in three consecutive flocks within the same barn. Birds were kept separately by sex until 12 weeks of age (woa) after which females were slaughtered, and the males had the whole barn at their disposal until slaughter at 18 woa. Structures consisted of two commercially available plastic platforms differing in surface area and grid gap width as well as a structure made of five narrow wooden planks arranged at two different heights. All three structures were placed per pen ($n = 3$ replicates per sex and structure). Data on behaviour (i.e., counts of birds using structures as well as percentage of successful transitions between structures and litter) was collected using video observations on one day per week from 2 to 18 woa. Females used the elevated structures more than males and both sexes preferred the platforms over the planks ($\chi^2 = 45.12$, $df = 2$, $p < 0.001$). Use of the platforms decreased with increasing age whether the use of the planks stayed relatively stable over time ($\chi^2 = 157.56$, $df = 2$, $p < 0.001$). Transitions between planks and litter were assessed as being more problematic including balance problems and falls compared to transitions between platforms and litter ($\chi^2 = 9.32$, $df = 2$, $p = 0.009$). Platforms seemed more suitable as an elevated structure for turkeys as birds made greater use of them and had fewer problems moving on and off them as compared to planks.



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Session 4 – Bone quality

Effect of environmental enrichments on leg health and behaviour of fast-growing broiler chickens reared at two different stocking densities

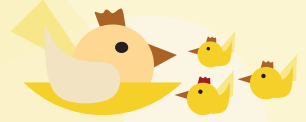
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In conventional rearing systems, fast-growing broiler chickens reared at high stocking densities commonly experience welfare issues, in particular leg problems such as contact dermatitis or walking impairment. Enriching their environment may be a way to reduce the occurrence of these issues, thus improving their welfare. The objective of this study was to evaluate the benefits of elevated platforms and straw bales on the welfare of fast-growing broiler chickens reared at two different stocking densities. A total of 14,994 Ross 308 broilers were housed in 12 pens according to 4 treatments: 31 kg/m² with or without enrichments and 41 kg/m² with or without enrichments. The broilers' walking ability was evaluated at 26 and 32 days of age. Footpad dermatitis (FPD) and hock burns (HB) were assessed at 25 days of age and at slaughterhouse in post-mortem observation. Stocking density had a negative effect on FPD and HB, whereas enrichments reduced the occurrence of FPD and HB at both densities. There was a positive enrichment effect and a negative density effect on walking ability. Preliminary work was also done on broilers' interactions with the elevated platform and straw bales at both stocking densities. Direct observations of the use of the enrichments were done at 4, 7, 12, 15, 18, 21, 26, 28 and 32 days of age and, videos of animal behaviour undisturbed by human presence were obtained from camera recording from 0 to 16 days of age at six time points of each day, 5 am, 7 am, 12 am, 2 pm, 5 pm and 7 pm. For both types of observations, the use of enrichment by the broilers was recorded: perching on the platform or ramps, clustering around the straw bales and standing on top of straw bales.

Keywords: broiler, enrichment, footpad dermatitis, hock burn, stocking density



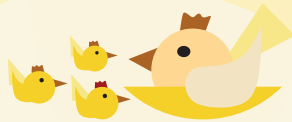
New insight to keel bone fracture morphology and the ossification process based on radiographic imaging of Norwegian layers

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With its high prevalence and the likely associated pain, keel bone fractures (KBF) are an important health and welfare concern in laying hens. Investigation of fracture morphology is an important step towards an improved understanding of the etiology. In this repeated cross-sectional study, one commercial layer farm housing 7500 Dekalb White layers in a multi-tiered aviary system, was visited 11 times from 17–57 weeks of age. At each visit, 30 layers were radiographed to assess their keel bone using a portable digital x-ray system. Each layer was held carefully in an inverted position by the legs, and images were shot in a latero-lateral view. The number of KBF, fracture type, anatomical location, callus material, displacement, angulation, and ossification were recorded. Prevalence of KBF at each visit was calculated. One incomplete fracture of the caudodorsal aspect of the keel bone was seen at 20 weeks of age. Complete fractures appeared at 25 weeks. The greatest increase in both prevalence and the number of fractures per hen occurred between 28 and 32 weeks, although both metrics increased throughout the study. Most fractures occurred in the caudal third of the bone. Fractures of the cranial third were also observed and appeared later in the production cycle. Incomplete fractures at the cranial third of the bone occurred only on the ventral surface, and incomplete fractures of the caudal third only on the dorsal surface suggesting a different pathogenesis for the two locations. Many fractures showed little callus formation, in particular for the fractures of the cranial third. For many of the fractures with poor callus formation, appearance was similar to folding fractures seen in other species due to disorders of calcium metabolism, suggesting that bone pathology such as calcium depletion may affect KBF pathogenesis, in addition to external and internal forces to the bone.

Keywords: Keel bone fractures, laying hens, layers, poultry, radiography



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Effects of perch type and genotype on resting behaviour and keel bone damage in laying hens

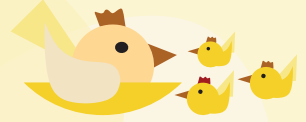
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Deviations and fractures of the keel bone occur frequently in commercial laying hens, although the causes are not always clear. The objective of our study was to investigate the effects of laying hen genotype and perch type on keel bone damage. At 12 weeks of age, a total of 357 laying hens of Slovenian provenance Prelux (120 brown, 117 black, and 120 barred) were housed in 36 enriched cages in a three-tier battery. Half of the hens of each genotype were housed in cages with metal round perch, the other half in cages of the same type, with the metal perch covered with rubber. Twelve-day data on egg production, feed consumption, body weight, and keel bone damage were collected at each of the five age stages (25.5, 29.5, 33.5, 37.5, and 41.5 weeks). Keel bone damage was assessed by palpation by the same person using a four-grade scoring system. The duration of hens' resting while sitting or standing on perches or wire floors was recorded for 24 hours at 29.5, 33.5 and 37.5 weeks of age. At 37.5 weeks of age, keel bone damage was noted in all hens. Perch type had a statistically significant effect on keel bone damage and forms of resting, while these two traits were not significantly affected by genotype. Hens housed in cages with rubber-covered perches had significantly ($p = 0.02$) higher score of keel bone damage, but only for the tip of the keel, not for the top and middle part. These hens spent significantly higher percentage of time standing ($p < 0.0001$) and sitting ($p = 0.02$) on rubber-covered perches and lower percentage of time standing on the wire floor ($p < 0.0001$). Rubber on perch increased grip, but softer materials should be considered to reduce pressure on the keel bone during resting.

Keywords: laying hens, keel bone damage, enriched cages, rubber-covered perches, genotype



Computer vision derived pose features are associated with lameness in broilers

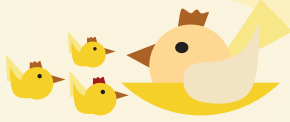
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Lameness is a prevalent disorder among broilers, with negative welfare consequences ranging from behavioural changes and pain to culling and mortality. Selective breeding offers potential to improve walking ability, but it requires individual phenotypic records on a large scale. The current practice of gait scoring by human experts could be further improved by using precision phenotyping tools, which may assess gait more objectively and with higher throughput. We studied the relationship of gait with seven computer vision derived pose features in 83 broilers at 33 days of age. Individually tagged male broilers were filmed from behind while walking through a corridor one by one. Gait was scored on a scale from 0 (no impairment) to 5 (severe impairment) based on the videos, and broilers were classified into good gait (score 0–2, n = 53) or suboptimal gait (scores 3–5, n = 30) categories. Individual body weight was recorded, and the analyses accounted for the differences in body weight. Suboptimal gait was associated with sharper hock joint lateral angles (-1.9%, 95% CI: -3.4 – -0.5%, P=0.009), sharper medial angles between the shank and the floor (-2.8%, 95% CI: -5.1 – -0.5%, P=0.017), and lower hock-feet distance ratio (i.e. the ratio between the horizontal distance of hocks and that of the feet; -3.6%, 95% CI: -6.6 – -0.6%, P=0.021) during the double support phase of walking. During steps, maximum leg lift was 14.0% lower (95% CI: -21.8 – -6.3%, P<0.001), on average, in broilers with suboptimal gait. These insights confirm that computer vision can be used to phenotype and monitor broiler gait, and our results can be used to build and understand more sophisticated gait prediction models in the future.

Keywords: broiler, lameness, gait, computer vision, DeepLabCut



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Validation of a refined assessment protocol for keel bone damages in local chicken breeds

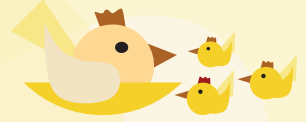
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Keel bone damages are a current topic in the animal welfare discussion in poultry, for which causes and solutions are urgently sought. To collect on-farm prevalence of keel bone data, the Welfare Quality® Assessment (WQA) Protocol for Poultry, for example, provides a scoring system. Based on this protocol, the prevalence of keel bone damages in dual-purpose breeds and hybrids was assessed. For example, in 25 Malines (project: RegioHuhn) the prevalence of the score 0 was 84% and score 1 was 16% according to WQA, while score 2 was not given. For a more detailed insight into the problem, the keel bones were dissected and pulped *post mortem* by dermestid beetles. In the following examination, keel bones were categorized based on callus formations after a previous fracture, dents, deviations from the straight axis and other alterations. At the same time, the location of the previous mentioned damages, was quantified. The prevalence of callus formation was 32%, of vertical dents 44% and of deviations 48%. Based on the dissected keel bones, the proportions for WQA scores would have been 36% for score 0, 32% for score 1 and 32% for score 2. These more detailed findings were compared to those data, which were scored on live animals based on WQA. The data sets showed a moderate agreement based on Kendall's coefficient of concordance ($w = 0.49$; $p \leq 0.001$). Based on our findings, we present a refined protocol that could be applied to live animals on farm and whose outcome would contribute to a differentiated understanding of keel bone fractures aiming to improve animal welfare.

Keywords: keel bone, animal welfare, assessment protocol, local chicken breed



Genetic evaluation of tibial *in vivo* X-ray density measurement in a White Leghorn pure line population

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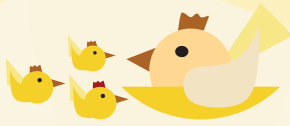
Bone quality, particularly keel bone fractures, is becoming increasingly important in laying hens with the advent of longer production cycles and the number of birds housed in cage-free environments. Skeletal damage is discussed as a possible animal welfare issue. Genetics has a major impact on bone quality and fractures. The aim of this study was to investigate the genetic influence on bone strength by estimating the heritability of X-ray bone density measurement in live adult hens. Furthermore, genetic correlations were estimated to assess the impact that breeding for better bone strength might have on other performance traits.

The tibiotarsus of 1000 White Leghorn pure line hens were measured *in vivo* using a portable digital X-ray at 25, 40, 72 and 98 weeks of age. To quantify the tibial density, the image analysis software FIJI (ImageJ) was used. Heritability of the bone density “gray value” was in a range of $h^2 = 0.28$ to 0.62 , depending on age. The measurements at different ages were highly correlated ($r_g = 0.79$ to 0.88). The genetic correlations between bone density and egg quality traits were moderate, being negative for egg breaking strength ($r_g = -0.08$ to -0.21) and positive for egg weight ($r_g = 0.15$ to 0.18). The strongest genetic correlation was for tibia density and the onset of lay or age at first egg ($r_g = 0.23$ to 0.39).

In conclusion, the *in vivo* tibia density measurement has a moderate heritability and could be integrated into a breeding program to improve the bone density of laying hens. The genetic correlations suggest that birds with early sexual maturity could be more prone to fractures resulting from lower bone density. This should be considered in the selection process to avoid any negative effects.

Funded by the Foundation for Food and Agricultural Research, grant 550396.

Keywords: Laying hen, Bone strength, Keel bone, X- ray



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Session 5 – Welfare challenges

Ethical aspects of embryo-sexing in chickens

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After the disappearance of dual-purpose chickens in modern poultry industry, chickens are for meat-only production or egg production. The males of laying hen strains are not economically profitable nor ecologically sustainable, because of high feed conversion rate or low feed efficiency, and higher CO₂-output per kilogram meat produced compared to meat strain chicks.

Therefore these males are eliminated which is seen as a proportional benefit. If not, elimination of males of layer strains is not acceptable, and if so, the question is why embryo elimination should be acceptable and day-old chicks not. Is it only a matter of public acceptance? A day-old chick is cute, an egg is not and hardly seen as a living animal by a broad public. Until now, routine killing of male chicks of laying strains remains common overall practice, but more and more countries are planning a ban of this practice or already legally implemented this.

The ban on chick killing also regulates the use of sex determination methods in chicken eggs. Such methods are used to determine the sex of chicken embryos during incubation. In the case of brood eggs designated as “male”, incubation is interrupted and the hatching of male chicks thus prevented.

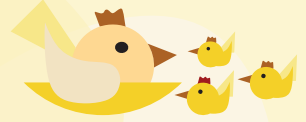
The legal ban on chick/embryo killing stipulates that such an approach is only permitted if it takes place before a certain day of development (to be determined) of a total of 21 incubation days. (e.g. the German Federal Ministry of Food and Agriculture states that it has to be before the 7th day, as this day is determined as decisive, because from this day the “beginning” of the pain sensation of the chicken embryo cannot be excluded)

Commercially applied methods for embryo-sexing:

- 1) liquid-based methods: they have in common that the sex of the chicken embryos is determined from the embryonic urinary (allantoic) fluid, from which samples have to be taken, from 9th day onwards.
- 2) determination of sex of embryos by plumage colour (by hyperspectral analysis) and for brown strains of layers only, or magnetic resonance imaging (of genital organs of embryos) are only possible from 12–13 days of incubation onwards.

Other spectroscopic or genetic engineering methods that claim earlier determination of the sex of embryos or brooding eggs are still in experimental phase and not sure if they could be successfully applicable.

This means that sex determination methods on embryos are currently only applicable on day 9, 12 to 13 of incubation, and methods for earlier sex determination are not yet available, or methods to prevent the hatching of male chicks which are not based on sex determination during incubation are not yet foreseeable for application in practice.



If any legal ban or embryo/chick killing after day 7 of incubation would be implemented or suggested, the question arises what the basis is for such a ban? Has it a biological or ethical basis? Is pain or suffering the background for such ethical basis? Or is it merely on lobbying of certain pressure groups or companies? Should sex-determining methods adapt to legal regulations or is it the other way around, and legal regulation be based on physiological and neuro-physiological observation? In this context, a discussion on pain and suffering, stress and welfare of chicken embryos becomes relevant

Pain and suffering, stress and welfare

Nociceptive processes and reactions do not necessarily indicate pain, are often biologically useful and do not provide a basis for empathy or compassion.

On the other hand, awareness of pain, its unpleasantness and its unbearable aspects (suffering) does provide a basis for empathy or compassion.

Pain in mammals and birds has different components:

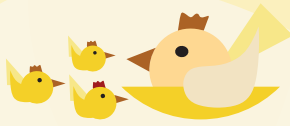
- a sensoric component (place, degree, duration)
- a motoric component (fight or flight)
- a vegetative component (unvoluntary reactions)
- an effective component (unpleasantness that can be increased or decreased as a function of earlier experience, mental state etc. etc.)

The nociceptive info reaches via afferent ways the thalamus in which the message is processed, balancing with other external and internal information, tuned by reciprocal fibers to and from the cortex. The motoric reaction via efferent ways is also going through and influenced by these higher brain centers. Therefore, pain can give rise to suffering, also psychic suffering, remembering this in a conscious way etc.

Pain is not to be confounded by stress of an organism although there are links between both and pain can be stressful and vice-versa. The ability to suffer is associated with the typically (human) form of consciousness (or self-awareness)

Consciousness is always connected with the thalamo-cortical parts of brain, hence with the functional connectivity of different parts of the brain. It is an emerging characteristic of interactive association fields (lateral fronto-parietal network for mediating consciousness of environment, and medial fronto-parietal network for mediating internal consciousness).

It allows a better coupling of exteroceptor mediated perception with the interoceptor mediated needs, hence allows a better “grip” on the environment and increased survival chance. Therefore this may be the evolutionary drive for the more complex neocortical possibilities of the brain in birds and mammals. Consciousness is also not an all or none phenomenon, but shows non-linear, gradual differences between man and animals.



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Waking-like brain function in embryos

The 3 states of brain, wake, NREM (non-rapid eye movement), REM (rapid eye movement), differ in brain metabolic and gene expression patterns, as well as in the organism's responsiveness to environmental perturbations.

Behavioural or EEG-changes (electro-encephalogram records) in response to environmental stimulation first appear late in embryonic development, both in sheep and chicken embryos (80-90% of embryonic development). Waking-like brain function is present in a latent but inducible state during the final 20% of embryonic life in the chick (by PET& CT scan) (Balaban et al., 2012)

Changes in higher-brain activation follows a developmental pattern in chick embryos, and combining brain and behavioural information, 4 time periods of interest can be discerned (after Balaban et al., 2012):

- embryo day 15.5–16.5: high rates of movement with no higher brain metabolic activity
- embryo day: 16.5–17.5: first appearance of higher brain activation with decreasing movement rates as development proceeded.
- embryo day 17.5–18.00: all embryos had higher-brain activation
- embryo day 18.0–21.00: different brain patterns or states.

Higher-brain activation is stimulus-selective and chicken sound exposure developmentally advances higher-brain activation. The pontine and mesencephalic reticular formation importantly regulate postnatal brain state (in mammals and birds), also in embryos at 70–80% of embryonic development.

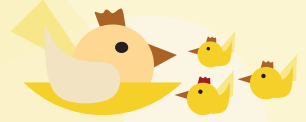
Additionally, in many developing neural circuits, GABA (gamma-amino-butyric-acid), the major inhibitory neurotransmitter in the adult brain, is initially excitatory. GABA becomes inhibitory beginning at day 14–15 of incubation in chick embryos, and this paves the way for increasing control of the brainstem and spinal cord circuits by the higher brain centers.

Conclusion

To conclude, as for our current knowledge about embryo neurophysiology, there is no biological basis regarding pain and suffering, for putting a ban on embryo elimination before half of the incubation of chicken eggs, or even before day 13–14.

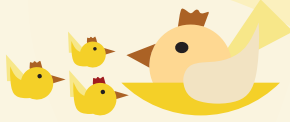
Therefore, the question if sex-determining methods should adapt to (premature?) legal regulations or the other way around, should legal regulations be based on neuro-physiological observations, is relevant.

However, in this discussion the broader framework for acceptability of these and other techniques should also be taken into account; e.g. the question if it is ethical to make instrumental use of animals is appropriate. There is no objective scientific answer to such questions however, as they involve as all ethical questions, a broader and philosophical (or religious) worldview of people.



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Diet coarseness and stress during rearing affects toe pecking in laying hens

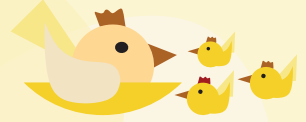
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Toe pecking (TP) has a negative impact on laying hen welfare. It causes wounds, pain, fear, infections and/or loss of toes. Two types of TP can be distinguished: auto TP (own toes) and allo TP (conspecific toes). Although TP is limitedly studied, the incidence is rising in countries where beak trimming is banned/not applied. The causation of TP is multifactorial; experiences during rearing and diet form can be important factors. Therefore, a study was performed to test a coarse and fine mash together with stress during specific periods in rearing on the occurrence and severity of TP. In total, 1560 one-day-old pullets were divided over 60 identical pens. Treatments were arranged according to a 5x2 factorial design with stress imposed on four different age intervals (wk 1–3, 3–5, 5–7, 7–9) or no stress, and two diet forms (finely or coarsely ground mash) throughout the experiment. The imposed stressor consisted of removal of litter material, perches and pecking blocks and housing on solely slats. Severe wounds that needed treatment were found during the egg production phase from 24 weeks of age (WOA). In total, 91 hens (5%) were treated for wounds on their toes. Around 2/3 of these victims were fed the coarse diet. At both 10 and 24 WOA, hens fed the coarse diet had a higher probability to perform allo TP (+4.96 %point and +24.80 %point) than those fed the fine diet (+0.04 %point and +0.20 %point; $P < 0.001$). These probability differences on allo TP between diets were largest when the stressor was applied from 7–9 WOA. On the other hand, the imposed stressor as such had its smallest and largest effect on probabilities for allo TP in the periods 7–9 WOA and 3–5 WOA, respectively. This study showed that both factors could affect the occurrence of TP in laying hens.

Keywords: Laying hens, Toe pecking, Rearing, Diet form, Wounds



Assessment of hunger in broiler breeder pullets fed high-fibre diets or roughage

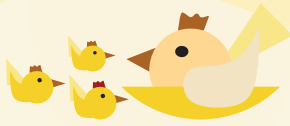
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This study aimed to investigate effects of four dietary treatments, differing in fibre types and content, on behaviour and hunger of broiler breeder females during rearing. The treatments were: 1) standard feed (Control), 2) standard feed diluted with insoluble fibres (Insoluble), 3) standard feed diluted with both insoluble and soluble fibres (Mixed) and 4) standard feed supplemented with maize silage (Roughage). The energy content of all treatments was similar to obtain similar growth curves. 1200 female Ross 308 breeder chicks were housed in 24 pens from day-old to 19 woa, six pens of 50 birds/treatment. Behavioural time-budgets were determined based on 6 observations of focal birds for 2 min in the following periods of the day relative to feeding time: 1h before, 1h after and 5h after. Frustration during thwarted feeding, and motivation to gain access to fresh litter were also measured. Compared to the Control, Mixed birds were less likely to forage ($P=0.001$) and walk ($P<0.008$) but more likely to drink ($P<0.0001$). Control birds did not differ from Roughage birds but performed more behavioural transitions than Insoluble and Mixed birds ($P=0.004$), indicating more frustration and higher feeding motivation. Mixed birds were more motivated to gain access to fresh litter with more and faster crossings into the litter compartment ($P\leq 0.01$) and spent more time performing comfort behaviour ($P=0.02$) and resting ($P<0.0001$) than Control birds, suggesting a high motivation for increased comfort. Litter quality was best in the Roughage and worse in the Mixed pens ($P=0.0001$). In conclusion, the fibre-rich diets had limited effects on behaviour of broiler breeders compared to the standard feed, although the Mixed and Insoluble diets may, to some extent, reduce feeding motivation. However, the Mixed diet induced negative effects such as poor litter quality, which likely contributed to the increased motivation for access to fresh litter.

Keywords: broiler breeders, qualitative feeding restriction, hunger, welfare, behaviour



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Modelling broiler welfare – approaching space allowance by the combination of planimetric and behavioural data

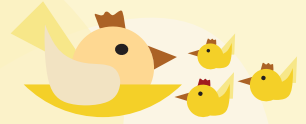
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Space allowance is one of the most serious factors affecting animal welfare. In addition to the density of animals per square metre itself and the social stress potentially associated with it, there are secondary effects such as limited possibilities for exploration, locomotion and undisturbed resting, reduced litter quality, and others. Using scan sampling data sets of fast-growing broilers from scientific literature, we present a model based on planimetric and behavioural data in order to calculate welfare-supporting stocking densities i.e., the space necessary for each broiler to perform its behavioural needs. In this model, the area covered by an individual animal expressing a certain behaviour and the average inter-individual distance between two animals during this behaviour are used to derive an area per animal that promotes active behaviour and thus improves animal welfare. The planimetric data were obtained from published data sets and these were converted into a circular model to represent the physical space occupied by the bird. From the literature, inter-individual distance divided by 2 was added to this circular model of a broiler. Walking, foraging (including scratching), dustbathing, preening, wing/leg stretching and wing flapping were defined as active behaviours to be promoted whereas standing, sitting/resting and eating/drinking were categorised as stationary behaviours. Based on active and stationary behaviours, an average broiler profile was derived representing the frequency of those behaviours that a fast-growing broiler would show under optimal conditions. Taken together, the model suggests stocking densities of about 11 kg/m² or lower to allow the realisation of the behavioural needs. The model outcome is far below the stocking densities applied in practice. Therefore, the stocking density of fast-growing broilers should be significantly reduced to enhance animal welfare.

Keywords: Space allowance, Stocking density, Broiler, Behaviour, Modelling



Elicitation of expert knowledge of the quantitative relation between stocking density in fast-growing broiler chickens and two animal-based measures: Foot pad dermatitis score and percent time spent walking

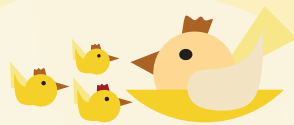
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In the framework of the Farm-to-Fork Strategy, the European Commission requested the European Food Safety Authority (EFSA) to assess the welfare risks associated with stocking density (SD) in broiler chickens. EFSA conducted two expert knowledge elicitations (EKEs) to quantitatively assess the impact of SD on Footpad dermatitis (FPD) score and percentage time walking (TW) that are animal-based measures (ABMs) for restriction of movement and soft tissue and integument damage. The values for the two ABMs at a low SD (3 kg/m²), intermediate SD (20 kg/m²), high SD (36 kg/m²) were elicited as well as the maximum SD at which the FPD and TW remain the same as with low SD. A linear relationship between SD and the ABMs FPD and TW was assumed. It was assumed that at a low SD the FPD score is 0. Both EKEs yielded similar results on the SD that will prevent FPD: 10 kg/m² (90% Certainty Interval (CI) 5.2–17.3 kg/m²) and that would maximize the % time walking: 12 kg/m² (90% CI: 8–17 kg/m²). At the low SD, a bird is expected to walk on average 8.5% of the 17h light period (90% CI 2.4–20%). At the high SD, the average TW was elicited at 4.5% (90% CI 1.3–9.7%) and the average FPD score was at 0.64 (90% CI 0.2–1.05). At the intermediate SD, the average TW was elicited at 6.3% (90% CI 1.8–14.1%) and the average FPD was 0.28 (90% CI 0.06–0.64). This is the first time that the previously established relation between SD, FPD and TW in broilers was supported by a quantitative model. In conclusion, both EKEs showed that a considerable reduction in the currently applied stocking densities of 36–42 kg/m², down to 10–12 kg/m², would be needed to prevent the appearance of FPD and restriction of TW.

Keywords: Broiler chickens, Expert Knowledge Elicitation, Stocking density, Footpad dermatitis, Percentage time walking



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CRISPR/CAS9-mediated gene knock-out and gene editing as a biotechnological approach to chicken resistance to viral diseases

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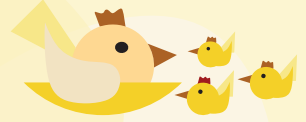
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Avian leukosis virus (ALV) diversified into six phylogenetically relative subgroups (A, B, C, D, E, and J) present as either exogenous or endogenous viruses in domestic chicken. These subgroups are unequivocally classified by the subgroup-specific receptor usage. ALV subgroups enter the cell through Tva, a member of the family of low-density lipoprotein receptors, Tvb, a tumor necrosis factor receptor-related protein, Tvc, a butyrophilin family protein with two immunoglobulin-like domains, or Tvj identified as the chicken Na⁺/H⁺ exchanger type 1 with twelve transmembrane segments and prominent extracellular loop 1. The physiological function of Tva receptor was revealed recently as an uptake of cobalamin (vitamin B₁₂) into cells.

For all ALV receptors, virus-resistant alleles exist, mostly due to the frame shift mutations or amino-acid substitutions. For example, single C40W substitution makes the Tva receptor molecule resistant to virus entry. Some of ALV-resistant receptor alleles segregate in domestic chicken and can be used for breeding the ALV-resistant lines.

Using the CRISPR/Cas9 gene editing tools, we introduced frame shift indel mutations into the endogenous *tva* receptor gene in the chicken primordial germ cells. Ultimately, we prepared a chicken line with frame-shift knock out of *tva* gene and demonstrated that this chicken line is resistant to ALV-A infection *in vivo*. We also found that B₁₂ depletion manifests as an accumulation of methylmalonic acid, the most sensitive member of B₁₂-driven metabolism. The new techniques of gene knock-out and gene editing in chicken employed in this work will be described. Our original methods of orthotopic transplantation of primordial germinal cells into adult recipients might become the useful biotechnological tool increasing the importance of chicken in virology, immunology and developmental science. The consequences of *tva* knock out for the chicken health, nutrition, and welfare will be discussed.

Keywords: gene editing in chicken, avian leukosis virus, cobalamin – vitamin B₁₂, virus receptor



Session 6 – Welfare of day-old chicks, including early nutrition and on-farm hatching

Are alternative hatching systems beneficial for chicken welfare?

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Summary

Conventional hatching of chicks in the hatchery includes several stressors that may have a negative impact on chicken welfare. Chicks are feed and water deprived during the first day(s) until placement on the farm, kept in darkness until pulling, exposed to high noise levels and concentrations of dust in the hatcher as well as subjected to handling (sorting, vaccination, sexing) and transport to the farm. To overcome (some of) these drawbacks of conventional hatching, alternative hatching systems have been developed. These include on-farm hatching, where eggs are transported to the farm on day 18 of incubation, or systems where chicks are provided with feed and water in the hatchery before transport to the farm. These systems are currently applied in practice, but still not very commonly, and are mainly used for broiler chicks. In combination with in-ovo sexing the alternative hatching systems are used for layers as well, although still on a very small scale.

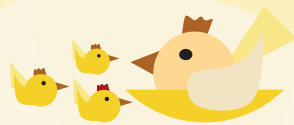
Research has mainly focused on the effects of on-farm hatching on broiler welfare, including the effect on chick quality, performance, mortality, behaviour and welfare indicators like contact dermatitis. In this presentation, we summarise the results of these studies. Although there are differences between individual studies, on-farm hatching generally has beneficial effects on body weight in the first week and results in less footpad dermatitis in fast-growing broilers. Although day-old chick quality may be negatively affected, this does not result in higher mortality. Beneficial effects on fearfulness have been found in some, but not all studies. Effects on behaviour seem to be short-term or minor, including increased resting and feeding during early life.

Overall, alternative hatching systems have beneficial effects on chicken welfare. Importance of climate control during hatching, grading and humane culling of unhatched eggs will be discussed, in addition to areas that deserve further research.

Keywords: day-old chicks, early feeding, on-farm hatching, performance, welfare

Introduction

Knowledge of the influence of conventional hatching on the welfare of chicks has increased considerable during the last decade (e.g., Hedlund et al., 2019; Giersberg et al., 2020a). Several stressors have been identified that potentially may have a negative impact on chicken welfare. This includes the feed and water



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deprivation of chicks from hatch until they are placed on the farm. Other stressors associated with conventional hatching of chicks in the hatchery are exposure to high noise levels (Tong et al., 2014), continuous darkness (Archer and Mench, 2014) and high concentrations of dust (de Gouw et al., 2017) while the chicks are in the hatcher. When pulled from the hatcher, the chicks are subjected to handling during the hatchery procedures, including sorting, vaccination, sexing, counting and crating, which usually involves an automated system with rollers and high-speed conveyor belts in addition to some manual handling (Knowles et al., 2004). Then, while being in the boxes, the final stages before placement in the barn involve a holding period, loading on the truck, transport to the farm and unloading from the truck. During the stages from pulling to being placed in the barn there is an increased risk of either heat stress (e.g., when chicks are densely packed in boxes) or cold stress (e.g., during loading and unloading).

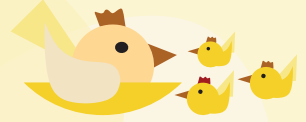
To overcome (some of) these drawbacks of conventional hatching, alternative hatching systems have been developed. These include on-farm hatching, where eggs are transported to the farm on day 18 of incubation, or systems where chicks are provided with feed and water or moist feed in the hatchery before transport to the farm. Multiple systems have been developed for on-farm hatching, ranging from a very simple system, where eggs are placed directly in the litter, to a quite complex system that involve a fully equipped multi-tier system where broiler chickens are kept from hatch to slaughter (for a review see EFSA AHAW Panel et al. (2023)). Similarly, different systems exist that supply the chicks with moist feed or feed and water in the hatcher at the hatchery.

On-farm hatching systems and hatchery systems allowing access to feed and water and exposure to a light/dark schedule are currently to some extent applied in practice in countries like Denmark and the Netherlands, but overall, the practice is still not very commonly used in EU or globally. Furthermore, the alternative hatching systems are mainly used for broiler chicks. The introduction of in-ovo sexing of layer chicks has opened the possibility of applying on-farm hatching for layer chicks as well, although this is still only practised on a very small scale (e.g., in the Netherlands). In line with practise, research has until now mainly focused on the effects of on-farm hatching on broiler welfare, although a few studies have addressed the effects of alternative hatching systems on layers. In this paper, we summarise the knowledge from available research on alternative hatching systems in relation to animal welfare.

Impact of alternative hatching systems on performance

For alternative hatching systems to be successful, hatchability has to be maintained at a high level. Studies have found no differences in hatchability of eggs placed either on farm or in the traditional hatchers at hatcheries, neither when counting second grade chicks as unhatched (standard practice at hatcheries; (de Jong et al., 2019)) or when counting second grade chicks as hatched (Jessen et al., 2021b; Souza da Silva et al., 2021). One study even found hatchability to be higher on-farm than in hatcheries when counting second grade chicks as unhatched (Jessen et al., 2021b).

The quality of the resulting chicks has been reported to be reduced in on-farm hatched broiler chicks due to issues with unhealed navels and red hocks (van de Ven et al., 2012; de Jong et al., 2019; de Jong et al., 2020; Jessen et al., 2021b; Souza da Silva et al., 2021), with the exception of one study which found no difference in occurrence of unhealed navels (Molenaar et al., 2023). Too high temperatures, particularly during the last week of incubation, are known to increase the risk of poorly absorbed yolk sacs (du Preez,



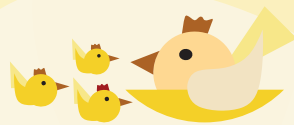
2007; Hamidu et al., 2018; van der Wagt et al., 2020), i.e., an increased occurrence of unhealed navels in chicks hatched on-farm may reflect difficulties in delicately controlling the microclimate around the eggs when kept in the large barns as compared to the hatcher. This would imply that the occurrence of second-grade chicks can be higher when hatching on farm, but in fact it has been reported to be lower in on-farm hatched broiler chicks than in hatchery-hatched chicks, which may, however, be an artefact of less handling and suboptimal conditions for identifying second grade chicks on farm (Jessen et al., 2021b).

With the potential risk of reduced chick quality and overlooking second grade chicks a higher first week mortality may be expected, but this is not the case. In contrast, lower first week mortality, as well as total mortality, has repeatedly been found in both fast-growing and slower-growing broiler chickens hatched on-farm compared to hatchery-hatched chickens (van de Ven et al., 2009; Hollemans et al., 2018; de Jong et al., 2020; Jessen et al., 2021b). Furthermore, broilers hatched on farm have been reported in most studies to have better growth performance during the first week than hatchery-hatched chicks (de Jong et al., 2020; Jessen et al., 2021a; Souza da Silva et al., 2021; Molenaar et al., 2023), though one study found no effect (de Jong et al., 2019). In line with this, Dieryck et al. (2022) found that the diamine oxidase serum level was higher in chicks hatched on-farm, suggesting that the intestinal development took place earlier than in hatchery-hatched chicks. However, the difference in body weight has typically disappeared at slaughter age (Hollemans et al., 2018; de Jong et al., 2019), implying that the hatchery-hatched broilers, although starting off more slowly during the first week, are able to compensate later in life. Few studies reported the on-farm hatched broilers from young parent stock to remain heavier than hatchery-hatched broilers even at slaughter age (Souza da Silva et al., 2021; Molenaar et al., 2023), and it was suggested that especially chicks from young parent stock, often being smaller and more vulnerable, could benefit from on-farm hatching with respect to performance. In layers hatched on farm, a tendency of higher body weight has been found up until 11 weeks of age (Witjes et al., 2022).

Impact of alternative hatching systems on behaviour

The calmer start of life of on-farm hatched chicks is reflected in early age behaviour, fearfulness and stress reactivity. During the first 23 h after placement in the barn of hatchery-hatched broiler chicks, the on-farm hatched chicks rested more, whereas no difference was found at 35 and 47 h or on day 7 of age (Jessen et al., 2021a). Feeding was more frequently observed in on-farm hatched chicks than hatchery-hatched chicks at 11 h and 35 h, whereas no difference was found at 5, 23 and 47 h or on day 7 of age (Jessen et al., 2021a). Generally, more hatchery-hatched chicks than on-farm hatched chicks were drinking (Jessen et al., 2021a). In layer chicks, more resting, less drinking and less foraging was observed in on-farm hatched chicks during the first 5–10 h after placement of hatchery-hatched chicks, whereas the opposite pattern was found at 15–20 h (Witjes et al., 2022). On-farm hatched chicks showed more wing-assisted running at 0–5 h and 15–20 h than hatchery-hatched chicks, which may be interpreted as play behaviour (Rayner et al., 2020), whereas no difference was found at 5–10 h and 10–15 h (Witjes et al., 2022).

In contrast to the effects of hatching systems on behaviour at an early age, no differences were found in general behaviour later in the rearing period between on-farm hatched and hatchery-hatched chickens (Giersberg et al., 2020b). With automated activity recording, Giersberg et al. (2023) observed an earlier



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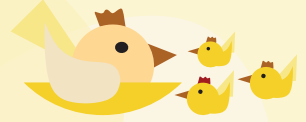


peak in activity for on-farm hatched (d18) than for hatchery-hatched and hatchery fed chicks (d19). Comparing weekly averages indicated a higher activity index at weeks 1, 4 and 5 in hatchery-hatched and hatchery-fed chickens than in on-farm hatched chickens. In week 3, higher activity indexes were found in hatchery-hatched compared to hatchery-fed and in hatchery-fed compared to on-farm hatched chickens. Overall, on-farm hatched chickens had the lowest activity-index and hatchery-hatched the highest, with hatchery-fed in between (Giersberg et al., 2023).

Elevated corticosterone levels have been reported in hatchery-hatched layer chicks after pulling, after completion of the hatchery procedures, post-transport and 20 h after placement in the barn as compared to on-farm hatched chicks (Hedlund et al., 2019; Witjes et al., 2022). Studies have shown that the fear-level is reduced in broiler and layer chicks hatched on-farm or under similar conditions as compared to hatchery-hatched chicks, with the effect being more pronounced at younger ages (Hedlund et al., 2019; Jessen et al., 2021a). Contradicting this, Giersberg et al. (2020b) found on-farm hatched broiler chicks to be more fearful than hatchery-hatched chicks. Hollemans et al. (2018) found early access to feed and water to increase fearfulness at 3 days of age in transported broiler chicks when compared to delayed-fed and transported chicks. Interestingly, the opposite result was found at 30 days of age. The authors explained this as a result of early nutrition stimulating brain development, including fearfulness at 3 days of age, while at the same time providing long-term advantages for the broilers' ability to cope with stress later in life, such as the fear test performed at 30 days of age. Nevertheless, despite these effects of hatching systems on stress reactivity and affective states, no differences in cognitive abilities have been demonstrated (Witjes et al., 2022).

Impact of alternative hatching systems on clinical welfare indicators

Whereas no studies have found any influence of hatching system on walking ability (de Jong et al., 2019; de Jong et al., 2020; Giersberg et al., 2021), then conflicting results have been found regarding occurrence of contact dermatitis (de Jong et al., 2019; de Jong et al., 2020; Giersberg et al., 2021; Molenaar et al., 2023). In fast-growing broilers, some studies have found on-farm hatching to result in less footpad dermatitis (de Jong et al., 2019; de Jong et al., 2020; Giersberg et al., 2021), whereas others found no difference (Molenaar et al., 2023). Hock burns are usually found not to be affected by hatching system (de Jong et al., 2019; de Jong et al., 2020; Giersberg et al., 2021), although Molenaar et al. (2023) found a higher frequency of hock burns in hatchery-fed broiler. In slower-growing broilers, litter quality and occurrence of footpad dermatitis did not differ between on-farm hatched or hatchery-hatched broilers (Jessen et al., 2021b). In the studies where footpad dermatitis was found at a lower frequency in on-farm hatched broilers compared to hatchery-hatched broilers, the litter quality was often reported to be improved (de Jong et al., 2019; de Jong et al., 2020). It is yet unclear whether this is related to improved gut health, although there are suggestions that particularly early feeding may be beneficial (Wijnen et al., 2021). In terms of resilience, Molenaar et al. (2023) did not find evidence for improved resilience of alternatively hatched broiler chickens in an IB challenge model whereas other studies suggest that early feeding may improve resilience (Wijnen et al., 2021; Wijnen et al., 2022).



Conclusion

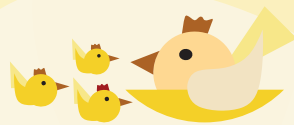
Overall, the current scientific evidence shows that alternative hatching systems have beneficial effects on chicken welfare. The different welfare indicators investigated generally either show no difference or indicate improved welfare in on-farm hatched or hatchery-fed chicks compared to traditional hatchery-hatched chicks. The exception is the risk of reduced chick quality and second grade chicks, but neither the results on growth nor mortality support that this is a real issue. The effect of alternative hatching systems on growth is generally only short-term as hatchery-hatched chickens are able to compensate later during rearing.

To ensure good welfare when practising on-farm hatching, farmers need to be prepared to invest the extra time needed for management during the hatching period, i.e., tight monitoring of the microclimate around the hatching eggs and proper grading of the chicks. Proper handling of second grade chicks is required, whatever that be sick pens with increased attention or culling. Furthermore, disposal of the remains after ended hatching involves proper culling of unhatched live chicks in the remaining eggs to avoid unnecessary suffering.

Many gaps of knowledge remain as alternative hatching systems are still a relatively new topic, and even in the available literature, contradicting results exist for some topics, warranting more investigations. Nordquist et al. (2022) showed that the stress level increases during transport of 18 days incubated eggs and pointed out that it is important to figure out how to refine transport of the eggs for hatching to minimise exposure to stress. Combining in-ovo sexing with hatching on-farm also has great potential for layer chicks, where it is generally acknowledged that reduced stress during early life, minimises the risk of developing injurious pecking later in life (De Haas et al., 2021). Moreover, only few studies compared hatchery-fed with on-farm hatched chickens, and research on hatchery-feeding in layers or slower-growing broilers is absent. Hatchery-feeding involves transport of fed chicks, which could require other transport conditions, but this has not been investigated yet. Finally, it remains unclear whether alternative hatching systems improve chicken resilience.

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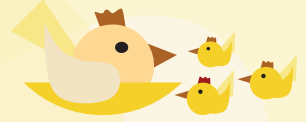


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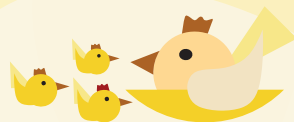
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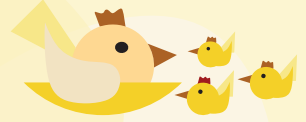


On-farm hatching can improve welfare, chicks quality, breast yield and daily body weight gain

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On-farm chick hatching is significantly growing in many European countries. Three days before hatching, eggs are transferred from a hatchery to the farm, allowing chicks to access feed and water while avoiding the perturbations generated by sorting, sexing, vaccination and transportation. Despite real benefits of this practice in terms of welfare, its long-term impacts on zootechnical criteria and meat quality when different types of barn heaters are used. This study proposes a multi-criteria evaluation of Ross 308 broiler production with two different heating systems (radiant heating system or gas cannon heater) compared with conventional hatching and two pre-incubation storage durations for eggs (5d vs. 15d). Chick quality, as evaluated based on Tona score, was improved for chicks hatched on-farm compared to chicks from the experimental hatchery. Behavioural and health indicators were collected by observation of the animals (adapted EBENE®) method). No impact of heating system or pre-incubation storage duration on the occurrence or severity of pododermatitis, on the number of lame, dirty and nervous broilers, or on pecking, grooming, exploring and resting behaviour. Regarding zootechnical values, average daily body weight gain was higher (+3 g/d) for on-farm hatched group resulting in a difference of 100–120g/broiler at slaughter age compared to controls. Breast yield followed this trend by showing +0.4 points on average. Feed conversion ratio as well as meat quality indicators (fat percentage, colour, breast defects) remained unchanged. Eggs stored for 15 days pre-incubation were negatively impacted on embryo mortality, hatching rate, mortality on farm, duration of the hatching window (37h vs. 29h with short storage) independently of the hatching practice. This study demonstrates that on-farm hatching, although improving broiler welfare, can also show zootechnical and economical benefits. However, results reveal that hatching temperature must be well controlled, to avoid temporal and spatial fluctuation, especially for long-stored eggs.



On-farm hatching and contact with adult hen posthatch induce sex-dependent effects on performance and health in broiler chickens

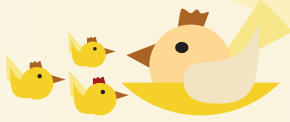
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To improve the early perinatal conditions of chicks, alternative hatching systems have been developed. On-farm hatching (OFH) with an enriched microbial and stimulating environment by the presence of an adult hen is a promising solution. Effects of OFH, conventional hatcher (CH), treated or not with antibiotics (CH + AB), with an adult hen (OFH + H, CH + H) or not were analyzed on hatchability, chick quality, performance, health and robustness. The OFH chick BW was significantly greater than that of CH chicks at hatching. Whereas there was no effect of hatching conditions, the presence of hens decreased the hatchability rate, the quality score of OFH chicks and increased mortality at hatching. Treatment of CH chicks with antibiotics temporarily decreased chicken BW at D19 but the feed conversion ratio (FCR) was not modified. At D19, OFH chicks had the best BW compared to the other groups and the presence of hens had negative impacts on both chicken BW and FCR regardless of the hatching condition. An interaction between the effect of hatching condition and chicken sex was observed later. In males, the OFH chicken were the heaviest compared to the other groups at D34 but not anymore at D56, the presence of hens had eventually a negative impact on chicken BW at D56. In females, there was no effect of hatching condition on the BW at D34 and D56 and the presence of hens had eventually a positive impact on OFH chicken BW. In conclusion, the OFH system is beneficial for the performance of the chicks throughout the rearing period compared to the CH system. The effects of the presence of hens at hatching and during the chick start-up phase is dependent on hen behaviour, which interacts with the hatching condition and the sex of the chickens.

Keywords: On-farm hatching, Hatching with hen, Broiler chicken, Performance, Chick quality



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Effects of hatching system factors on laying hen chicks in a step detour test

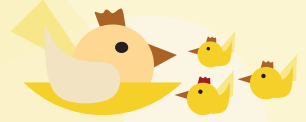
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On-farm hatching (OFH) will become feasible for laying hens due to increased accessibility of *in-ovo* sexing, though the importance of environmental hatching factors on development of brain-lateralization are poorly understood. A pilot (E1) and subsequent experiment (E2) sought to investigate the influence of relevant factors during OFH on laterality. Hatching eggs (39–41/treatment pen) were exposed to one of five treatments from 18 days of development to <24 hours post-hatch E1: light (L), litter shavings (LIT), feed/water (FW), as well as all factors missing (CON) or present (ALL) (3 pens/treatment). E2 followed a 2x2-factorial design with L and FW as factors (4 pens/treatment). Focal chicks (15-16/treatment) were selected to assess treatment effects during a step detour test at 5 and 7 weeks of age (WOA). Behavioral measures were: latency to leave the start-box (LAT), side (left/right) and strength of preferences for foot usage, and detouring. Data were analyzed using linear mixed models with treatment as a fixed effect and chick-ID nested in pen as a random factor. No treatment effects were found for LAT in either experiment ($p > 0.05$). During E1, LAT decreased with age ($p < 0.0001$). E2 showed a trend with FW+/L- being faster than FW-/L- at 5WOA ($p = 0.07$). For E1, CON showed a stronger foot preference compared to ALL and LIT ($p < 0.05$) and detour preference was left-biased for CON and right-biased for LIT ($p < 0.05$). For other treatments, no significant effect could be found ($p > 0.05$). For E2, an interaction effect between foot preference and WOA was found ($p < 0.005$), and FW-/L+ showed a trend for a stronger foot preference compared to FW+/L- at 5 WOA ($p = 0.08$). At 5WOA, detour preference was stronger than at 7WOA ($p < 0.05$). In conclusion, decrease in LAT is influenced by age and possibly habituation. Laterality findings might indicate that side preferences are not fixed and potentially influenced by perinatal stimuli.

Keywords: on-farm hatching, side preference, laterality, cognition



How do strain and maternal omega-3 fatty acids affect the reaction of layer offspring to a novel object?

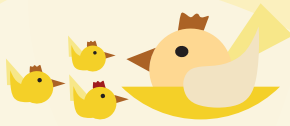
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Commercial chicken breeder diets are deficient in omega-3 fatty acids (n-3 FA). This study aimed to increase the currently limited and conflicting research on maternal fed n-3 FAs and offspring fearfulness. ISA Brown and Shaver White layer breeders were fed a control or flaxseed diet. Layer offspring (860 females) were housed by treatment and strain in furnished floor pens (12 pens per strain/maternal diet). Novel object (NO) tests were conducted at 13 days of age (DoA, orange traffic cone) and 23 weeks of age (WoA, striped cylinder). Cameras recorded all 12 pens per room simultaneously. NOs were placed in the middle of each pen by a single experimenter who then vacated the room. NO tests were recorded for 10 minutes, and videos were sampled at 30-second intervals. The percentage of chickens in each zone (Zone 1 within one, and Zone 2 within two body lengths of the NO) and use of furnishings (i.e., platform, perch) at each time interval were recorded. Data were analysed in R (v.4.1.2) using linear mixed effects models. There was no effect of maternal diet or strain on the percentage of chicks in Zone 1 and 2 at 13 DoA. A higher percentage of brown chicks were observed on top of ($\chi^2=14.409$, $p<0.001$) and white chicks underneath ($\chi^2=24.591$, $p<0.001$) the ramped platform. At 23 WoA there was no effect of maternal diet on the percentage of hens observed in Zone 1, though there tended to be more white hens ($\chi^2=3.092$, $p=0.079$). More white hens were observed in Zone 2 ($\chi^2=35.978$, $p=0.015$), whilst brown hens were observed on elevated furnishings ($\chi^2=70.919$, $p<0.001$) and these tended to be from the flaxseed maternal diet ($\chi^2=2.879$, $p=0.090$). We found little evidence to suggest that maternal n-3 FA affected offspring fearfulness. However, we found strain dependent reactions to the NO.

Keywords: Fearfulness, Maternal diet, Omega-3 fatty acids, Strain differences



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Session 7 – Fear and stress in poultry – identifying causes, preventive and mitigative strategies

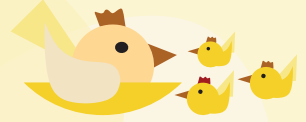
White hens are from Venus, Brown hens are from Mars – a review and meta-analysis of strain differences in fear response

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Fear is a negative affective state that occurs in response to perception of a threat and can result in numerous physiological and behavioural responses. Fearfulness and fear-provoking events are common and major threats to the welfare of laying hens. Thus, a variety of ‘fear tests’ are used to assess welfare and to evaluate management practices that reduce fearfulness (Forkman et al., 2007). While it has long been observed that white- and brown-feathered strains have different temperaments (Murphy, 1977; Jones, 1987), strain comparisons reported in the literature often paint an unclear or contradictory picture. Recent research in our lab has identified several consistent differences between strains that indicate either a difference in the perception of threatening stimuli or, more importantly, a different type of response to a fear-provoking event. Brown birds appear to respond more strongly to novel objects and novel places, whereas white birds respond more strongly to humans or predatory stimuli. In response to novelty, brown birds tend to be “freezers” and white birds are “fleers” (Pusch et al., 2018; Peixoto et al., 2021; Rentsch, 2023) although in social isolation tests brown chicks produce more distress vocalizations (Peixoto et al., 2020; Whittle, 2023) and white chicks freeze more (Whittle, 2023). When subjected to a rapid startling stimulus (i.e., flash of light), brown birds show a pronounced startle reflex (i.e., involuntary muscular response) (Ross et al., 2019; Rentsch, 2023) and white birds do not (Rentsch, 2023). Additionally, in comparison to brown strains, HPA activation in response to restraint (i.e., corticosterone) is greater in white strains (Fraise and Cochram, 2006; Pusch et al., 2018; Peixoto et al., 2021), whereas heterophil:lymphocyte ratios are lower (Pusch et al., 2018; Chew et al. 2021).

To get a better understanding of the differences in fear response between strains, we conducted a meta-analysis comparing two measures of fearfulness: duration of tonic immobility (TI) and novel object (NO) approach rate (Rentsch et al., 2023). We included 16 studies reporting TI durations and 11 studies reporting NO approach rates in brown and white egg layers (pre- and in-lay) published around the 1980s, the 2000s, and the 2020s. White hens in the 1980s had longer TI durations (709.4 ± 143.88 sec) than brown hens in the 1980s (282.9 ± 59.70 sec) and brown hens (208.8 ± 50.82 sec) or white hens (204.9 ± 49.60 sec) in the 2020s, suggesting a decrease in fearfulness during manual restraint in white layers in the last five decades. However, the phylogenetic difference in the 1980s was no longer detectable after enforcing an upper limit on TI durations (10 min), as became common practice in later studies. White hens had a higher NO approach rate (0.7 ± 0.07), indicating lower levels of fearfulness than browns (0.5 ± 0.11 , $P \leq 0.05$) and older birds (in-lay) a higher approach rate (0.8 ± 0.07) than younger birds (pre-lay) (0.4 ± 0.12 , $P < 0.05$). The NO approach rate reported in papers published in the 2010s (0.8 ± 0.09) was higher than in the 2020s (0.2 ± 0.12 , $P < 0.05$),



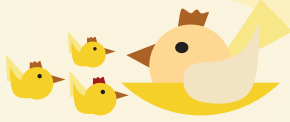
suggesting an increase in fearfulness of novelty in the last decade. Furthermore, the meta-analysis indicated that both fear tests are highly sensitive to changes in the methodology.

Our findings indicate that different phylogenetic groups may not only have very different perceptions of what types of stimuli are threatening but also how they respond to them. Additionally, these differences may be changing over time as both genetics and housing systems evolve. This raises some very important questions about ‘standardised’ methodology for assessing fearfulness, potential changes in fearfulness between strains over time, and the interpretation of fear tests for predicting welfare consequences in commercial egg production.

Keywords: laying hens, fearfulness, fear tests, review, meta-analysis

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Brown and white layer pullet hybrids show different fear responses towards humans, but what role does light during incubation play in that?

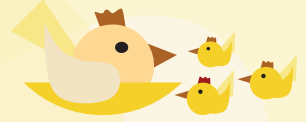
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Early-life conditions are increasingly recognized as essential to animal welfare later in life. Light during incubation is being investigated as a method to improve welfare in later life in poultry. It approximates more closely chicken natural environment before hatching, compared to the current conventional incubation conditions in darkness. Previous studies were promising: lighted incubation resulted in more species-relevant behavior and lower stress reactions to various stressors after hatching. Most research, however, has been conducted on broilers, and little information on lighted incubation is available for laying hens. In addition, white hybrids have been shown to be flightier than brown hybrids. In the current experiment, Dekalb White and ISA Brown eggs were incubated in complete darkness or in a cycle of green 12L:12D throughout incubation. Five different human fear tests were performed on 387 chickens during the rearing phase. We expected dark-incubated chickens to show more fear and higher corticosterone levels than light-incubated chickens. The voluntary approach test matched this hypothesis, as the dark-incubated chickens froze more than the light-incubated chickens ($p < 0.05$). The four other tests, though, showed no significant difference between the incubation treatments. In addition, we expected white chickens to show more fear and higher corticosterone levels than brown chickens. Four of the five tests ($p \leq 0.002$) confirmed that hypothesis. In the tonic immobility test, though, brown chickens showed more fear than white chickens. Finally, we expected light during incubation to have stronger effects on white chickens than on brown chickens, because of the better transmission of light through white eggshells. However, the interaction between hybrid and incubation was never significant ($p \geq 0.18$). To conclude, this experiment did not show clear evidence that light during incubation could improve laying hen welfare. Hybrids show differences in fear-related tests, and that should be taken into account in research and in laying hen management.

Keywords: chicken, prenatal, behaviour, corticosterone, welfare



Could the live black soldier fly larvae supplementation affect the welfare of medium-growing chickens?

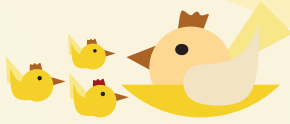
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Investigations on welfare of medium-growing chickens administered with live black soldier fly larvae (BSFL) are not available. This research aims at evaluating the effects of live BSFL supplementation as environmental enrichment on the Label Naked Neck (LNN) hybrid. A total of 240 LNN birds were reared from 21d to 82d of age, allotted in 4 experimental groups (6 replicate/treatment, 10 birds/pen) according to gender and live BSFL inclusion in diet or not (control males-CM; control females-CF; larvae males-LM; larvae females-LF; 10% larvae inclusion of the expected daily feed intake). Video recordings were performed on 4 replicates/treatment before (9am), during (11am), after (4pm) the live BSFL provision, counting the number of birds exploiting specific behaviors at 25, 40, 75d of age. Tonic immobility (TI) and avoidance distance test were executed at 26, 39, 60, 76 and 27, 41, 62, 76d of age, respectively, while skin, leg and feather conditions were assessed at 35, 49, 63, 77d of age. The fecal corticosterone content was determined at 26, 39, 76d of age and blood samples were collected at 82d of age to calculate the heterophile/lymphocyte (H/L) ratio. A greater number of supplemented (L) birds performed wing flapping at 9am than control (C) ones ($P<0.001$). Moreover, the stretching frequency was always higher in L groups than C ones ($P<0.05$). The L chickens displayed higher walking than C ones both at 9am ($P<0.01$) and 11am ($P<0.05$). The shaking and scratching frequencies were greater for L birds than C ones at 11am and 4pm, respectively ($P<0.001$ and $P<0.05$). A greater percentage of C birds remained immobile at the first TI attempt than L ones ($P<0.05$). Finally, the H/L ratio resulted lower in C than L groups ($P=0.05$). In conclusion, despite no benefits were observed in stress reduction (H/L ratio and fecal corticosterone content), live BSFL provision could be successfully used as environmental enrichment, positively stimulating the bird physical activity.

Keywords: medium-growing chickens, welfare, live larvae, poultry behavior



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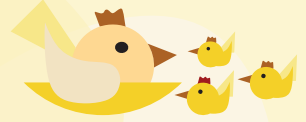
Duration of scotoperiod has minimal effects on fearfulness responses of turkey hens

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Turkeys are proposed to be reactive during periods of complete darkness, but broilers and layers are less fearful when provided with longer scotoperiods. The relationship between the duration of scotoperiod and fearfulness has not been formally studied in turkeys. Our objective was to assess fearfulness using a novel object test (NOT) and wing flapping test (WFT; modified inversion test) in turkey hens reared under one of four dark periods. Nicholas Select hens were provided with 24h light for the first 3 d post-placement, then scotoperiod treatments were applied until 12 wk of age (0h, 2h, 4h, 8h; n=4 rooms/treatment, 55 hens/room). At 4 and 10 wk, a NOT was conducted following the Welfare Quality® assessment protocol for poultry. The object was placed on the floor in each room for 15 min. Video recordings were used to determine the latency to approach, latency to touch (i.e. pecking or stepping on the object), and the average number of hens within one body length, two body lengths, and touching the object. A WFT was also performed at 4 wk where individual hens were held for 30 sec to record the number of vocalizations and wing flapping intensity (number of wing flaps/duration of wing flapping; 10 hens/room). Data were analyzed with linear mixed effect and generalized linear mixed effect models in R software. Across ages, hens reared with 4h and 8h scotoperiods were faster to touch the novel object than 0h and 2h hens ($p = 0.019$). No other metrics scored in the NOT were affected by treatment, age, or their interaction. In the WFT, none of the variables were influenced by treatment ($p > 0.05$). The length of scotoperiod had minimal effect on fearfulness in the two tests, suggesting that fearfulness may not be a useful justification for determining lighting duration.

Keywords: scotoperiod, novel object test, fearfulness, turkey, welfare



Heat stress increases fluctuation asymmetry in broiler chickens

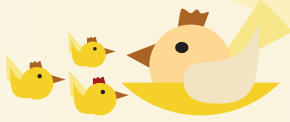
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Fluctuation asymmetry (FA) is a measure of developmental instability. FA is considered a potential indicator of animal welfare since it reflects the ability to cope with the challenges that may affect growth during the lifetime. This lack of buffering capacity may result in morphological differences in the two sides of bilateral traits (right and left). This study aimed to test whether FA is sensitive to heat stress in broiler chickens. A total of 560 broiler chickens (Ross 308) were randomly assigned to two treatment groups, with two pens of 140 broilers per group. The experiment will be repeated four times. One treatment consisted of the temperature as recommended by the Ross manual (control), and the other one consisted of 6 hours of heat stress for 2x5 weekdays when the birds were 29 to 43 days old. At 43 days, 20 broilers from each treatment were euthanized. The left and right sides of 11 bilateral traits were measured twice from the animal's intact carcasses by a single researcher: wattle width, eye length, first secondary feather length, beak length, radius length, tarsometatarsus length and width, and middle, outer, and back toe length. Measurements were taken with a digital caliper (precision 0.01 mm), apart from the first secondary feather length, which was measured using the software Image J. Means were compared using variance analysis followed by Tukey test, considering differences at 5%. Preliminary results of the first production round indicate differences between treatments on middle toe ($P=0.025$) and outer toe ($P=0.017$), where FA was lower in the control group. Eye length FA also tended to be lower for the control versus heat stress treatment ($P=0.083$). These results are preliminary, but the finding indicates that FA in some morphological traits may be used as an indicator of heat stress in broilers.

Keywords: developmental instability, animal welfare, poultry



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Influence of stocking density on measures of fear in conventional broiler chickens

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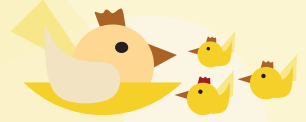
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Previous studies have found contradicting results on the relationship between stocking density and broiler fear levels. This study aimed to examine the influence of stocking density on measures of fearfulness in Ross 708 birds. Birds were initially kept in pens with an expected stocking density at slaughter of 30.4 kg/m². On day 27 of age, half of the pens were doubled in size providing an expected stocking density at slaughter of 15.2 kg/m² (reduced-density), while the other half of the pens continued as before (control). Two tests were used to measure the birds' fearfulness, the Stationary Person Test (SPT) and the Novel Object Test (NOT), on days 21, 27 (before the reduction in stocking density), 29, and 37 of age. Statistical analyses were performed in R with the formulation of Generalized Additive Models. An effect of the interaction between age and treatment was found for the measurements of both the SPT (P=0.020) and the NOT (P<0.001). However, post hoc analysis for the SPT revealed that the difference between the two treatments was not significant on any of the test days. The post hoc analysis for the NOT revealed that birds assigned to the reduced-density treatment were less fearful than the control birds (P<0.001) at 21 days of age, i.e. before the increase in space allowance. However, after the provision of extra space in the reduced-density pens, the birds tended to be more fearful than the control birds at 29 days of age (P=0.077), whereas no difference was found between treatments at 27 and 37 days of age (P<0.601). We conclude that the influence of stocking density on fear of humans was minimal, whereas the fear of novel objects, at least temporarily, increased as a response to the increased space allowance.

Keywords: Poultry, Stocking density, Fear, Welfare Assessments



Session 8 – Open session

Transport of broilers during high ambient temperatures – A dose-response relationship

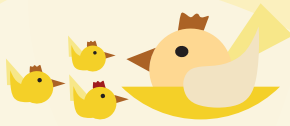
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The past summers in the Netherlands have shown heat waves with temperatures over 30°C and even over 35°C. During these hot periods, animal transport to the slaughterhouses poses a potential animal welfare risk. Therefore, to determine this risk an animal welfare risk assessment was performed, based on the EFSA (European Food Safety Authority) methodology. The hazards identified were temperature, humidity, ventilation, loading density in the truck, journey duration and other contributing factors related to the animal or the situation at the slaughterhouse. Heat stress was characterized as the welfare consequence, with varying impacts on the animals, from mild (panting) to very severe (death). To quantify the impact on broiler welfare during transport, the percentage dead-on-arrival (DOA%) was related to temperature and the temperature-humidity-index (THI). Results showed that from 27.3°C a rise in temperature with 1°C has a greater effect on the DOA% compared to lower temperatures. From a THI of 75.5, a higher THI value has a greater effect on the DOA% compared to lower values. Temperature and THI were derived from national weather data. The exposed population of broilers was derived from in house data. The estimated animal welfare risk for the transported broilers was assessed to be medium to high. Panting was identified to be the best animal indicator to establish heat stress, although no thresholds were found in the literature. A combination of animal and resource-based indicators is considered to measure the impact on animal welfare caused by exposure to the hazards. Further research should elucidate the best combination of indicators to be used to establish animal welfare risks caused by transport during thermoregulatory challenges.

Keywords: Transport, Broiler, Heat stress, High temperatures, Animal welfare risk assessment



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Influence of container type, husbandry system/fattening method, conveyor belt speed and season on loading-related injuries in broilers

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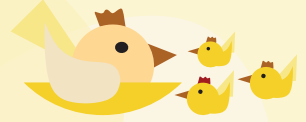
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The aim of the presented project was to optimize the mechanical loading of broiler chickens. Data of 32 loadings by a catching machine were used for analysis. The broilers were examined considering loading-related injuries after loading on farm. The examination groups studied differed in conveyor belt speed, husbandry type/fattening method, container type and season. Husbandry systems were grouped according to the specifications of a retail trade's label into "Indoor Plus" and "Outdoor Climate." These classifications include a fast growing genetic and environmental enrichment in an indoor housing system (indoor plus) or a slow-growing genetic and environmental enrichment (outdoor climate). Classifications of fattening methods followed the specifications of the "Standard", "Standard Premium", and "Premium" labels, which differ, amongst others in stocking density (max. 35 kg/m² (Standard), 30 kg/m² (Standard Premium), 25 kg/m² (Premium)), dark period (min. 6 hrs (Standard), 8 hrs (Standard Premium and Premium)) and access to a wintergarden (Standard Premium and Premium).

Animals of eight containers per group were examined. The most common type of injuries after loading were hematomas on the wing (6.55%), followed by hematomas on the wing tip (6.17%), abrasions on the body (4.92%), abrasions on the wing tip (4.25%), severe wing injuries (1.13%), and hematomas on the wing proximal to the wing tip (0.38%). An influence on the injury rate was observed for all four parameters studied. Lower belt speed was associated with a reduction in injuries. A significantly lower risk of serious injuries, total abrasions, and wing tip abrasions was observed when loading broilers of husbandry type 3 compared with animals of husbandry type 2. In the comparison of the individual fattening methods, it was observed that animals of the fattening method "Standard" had a significantly higher risk for serious injuries than those of the fattening method "Standard Premium" and the fattening method "Premium". It seems that not only genetics, which is one of the major differences between husbandry type 2 and 3, influences the injury rate. Other differences in the fattening procedures, such as a longer dark period, a lower stocking density at housing, more enrichment and access to the winter garden are rather potential influencing factors.

Keywords: loading, mechanical loading, broilers, injuries, influencing factors



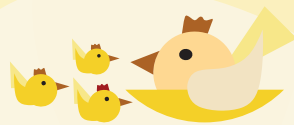
Alternative gas mixtures to carbon dioxide in two phases in broiler chickens

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The aim of this study was to evaluate the time to onset of unconsciousness and death and the aversive behaviours that may occur during the induction to unconsciousness when using different gas mixtures in broiler chickens. For this, 39-day-old mixed-sex Ross 308 broiler chickens were submitted to a pre-filled gas stunning system. First, broilers were submitted to atmospheric air serving as control. Then, birds were exposed to one of the three experimental treatments. Treatments were 40C90C (CO₂ in two phases: <40% CO₂, 2 min followed by >90% CO₂, 2 min), 40C60N (40% CO₂, 60% N₂, ≤2% O₂, 4 min) and 20C80N (20% CO₂, 80% N₂, ≤2% O₂, 4 min). Behavioural and neurological responses (through EEG) to gas mixture treatments were assessed. EEG was recorded and analysed before and during gas exposure to assess the time to onset of unconsciousness and death. Behavioural assessment included prevalence and sequence of onset to aversive behaviours, onset to loss of posture and onset to motionless. All birds were dead before the end of the stunning process. However, the exposure to birds to any of these treatments did not induce immediate unconsciousness. However, the exposure to 40C60N and 20C80N induced unconsciousness faster but also with less variability between individuals compared to 40C90C (30s, 29s, 60s respectively, $P>0.001$) and took less time to die (64 s, 70 s and 169 s, respectively; $P>0.001$). Regardless of the gas mixture tested, all broiler chickens experienced several aversive behaviours during the induction to unconsciousness. Nevertheless, the sequence of these behaviours revealed that birds in 40C90C showed wing flapping as an escape attempt while 40C60N and 20C80N exhibited wing flapping immediately before the onset of unconsciousness indicating an attempt to maintain posture before losing consciousness. Results suggest that either 40C60N or 20C80N are recommended over 40C90C on welfare grounds.

Keywords: broiler chickens, gas stunning, slaughter, aversion, welfare



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Welfare-related causes of mortality identified in a longitudinal study in Danish commercial layers

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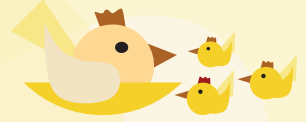
Mortality is considered a welfare indicator, but little is known about the reasons behind mortality in commercial layers in general and in non-cage housing systems in particular. Necropsies can aid in explaining causes of mortality in layers and thus guide intervention strategies. The objective of this study was to investigate the causes of mortality and risk periods for particular welfare challenges in commercial layers housed in barn/aviary and organic production in Denmark. We followed seven commercial layer flocks (three organics, four barns) on six farms from placement to slaughter. The first ten dead hens (culled or found dead) every second week were sampled throughout the production phase. Two experienced poultry pathologists performed *lege artis* necropsies of all carcasses.

A total of 1,801 layers were necropsied. At least one possible cause of mortality was determined for 1,758 layers (97.6%).

The ten most frequent causes of mortality were: salpingitis-peritonitis (24.8%), cannibalism (18.3%), egg bound (13.3%), pecking (11.7%), uraemia/nephropathy (8.8%), chronic salpingitis (6.3%), septicaemia (5.6%), internal haemorrhage (5.3%), gastrointestinal disorders (5.1%), trauma (3.4%). We observed differences in the prevalence of causes of mortality between housing systems and between flocks. The largest differences between causes of mortality in layers housed in organic systems compared to barn/aviary systems were observed for pecking (3.9% versus 17.3%, hereof toe pecking (0% versus 13.8%)), cannibalism (12.7% versus 22.2%) and gastrointestinal disorders (10.3% versus 1.4%). Besides classical welfare-related conditions (e.g. pecking, cannibalism), disorders like (chronic) salpingitis, uraemia and egg bound have significant impacts on layer welfare.

The study highlights a range of welfare-related conditions of acute and chronic nature as causes of mortality in commercial layers. Future studies should investigate how these results, combined with production data and practical experiences of egg producers, can be integrated to support management decisions to improve health and welfare in commercial layers.

Keywords: mortality, cannibalism, pecking, risk periods



Impact of increased activity on woody breast prevalence in a fast and slow-growing broiler strain

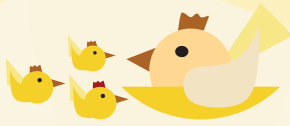
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Woody breast (WB), a muscular myopathy characterized by hardening of the *Pectoralis major* muscle, has been associated with fast growth, high body weight and breast yield, and impaired mobility. These factors are, in turn, associated with lower activity levels. We hypothesized that increased the birds' activity reduces the occurrence and severity of WB in broiler flocks, with larger impacts in a fast- than a slow-growing strain. A total of 188 Ross 708 (fast-growing) and 213 Ranger Gold (slow-growing) broilers were raised to their target ages of 42 and 56 days. Chickens were raised in wood shavings lined 3.05 x 3.05 m² pens in groups of 23–24 (fast-growing) or 26–27 (slow growing; 8 pens/strain). The birds in the four control pens/strain were raised in a routine manner. The remaining birds underwent an exercise regimen, which was implemented over 6hr/day on 5 days/week, and aimed to increase the frequency with which the broilers stood up, and their overall walking and standing activity. Gait and WB prevalence and severity were evaluated for every individual at slaughter age. WB data was analyzed using the one-tailed Fisher's Exact Test. Exercise reduced the overall prevalence of WB in fast-growing ($p=0.013$; 77.5 vs 90.5% control) and slow-growing ($p=0.005$, 57.9 vs 76.4% control) broilers. The occurrence of severe WB was also reduced in the fast- ($p=0.02$; 12.9 vs 24.2% control) and slow-growing ($p=0.01$; 4.7 vs 0.02% control) strains. Of the few birds that exhibited moderate to severe gait problems (fast-growing: 7.5% treatment vs 11.6% control; slow-growing: 5.6% treatment vs 0.9% control) strains, all displayed some degree of WB. Increasing broiler activity was sufficient in reducing WB occurrence and severity in both a fast- and slow-growing strain in this proof-of-concept study. Commercial scale studies are needed to confirm the relationships between WB, broiler welfare indicators, activity and bird strain.

Keywords: broiler, woody breast



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Prague, Czech Republic, 26–29 June 2023



Session 9 – Poultry behaviour and environmental enrichment

Effects of qualitative feed restriction on learning ability and judgement bias in broiler breeder pullets

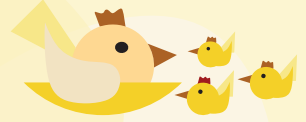
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Restrictive feeding, a common practice during rearing of broiler breeders, may result in prolonged hunger, and thus compromised welfare. This study employed a spatial judgement bias task to assess learning speed and affective states of 24 Ross 308 breeder females assigned to one of three treatments: 1) CON: feed-restricted standard commercial diet, 2) EXP: feed-restricted fibre-rich diet to increase satiety, 3) ADL: EXP or CON diet fed ad libitum for a week before and throughout training and testing. At 9 weeks of age, birds were trained on a spatial discrimination task (approach and flip a lid to reveal a food reward or no reward, at opposite sides of the test arena). Once birds met the training criteria, individual judgement bias testing was performed over 3 days. Two EXP and three CON birds failed to meet the criteria and were excluded from testing. In addition to the rewarded and un-rewarded locations, birds were presented with lids to flip in three intermediate (ambiguous) locations (near-rewarded, center, and near-unrewarded); these locations were never rewarded. ADL birds met the training criteria sooner than EXP birds ($P = 0.03$), perhaps due to reduced hunger that may improve cognitive ability. However, no differences were observed between CON and ADL or EXP and CON treatments. CON and EXP birds had a 3.45 ($P=0.002$) and 2.49 ($P=0.027$) -fold higher rate to reach the center location and a 4.75 ($P<0.001$) and 2.20 ($P=0.050$) -fold higher rate to reach the rewarded location than ADL birds, respectively. This optimistic judgement bias indicates a difference in affective state in feed-restricted individuals who may experience greater motivation to search for and obtain food rewards. Overall, results indicate that both quantitative and qualitative feed-restricted diets may impair birds' acquisition of cognitive tasks and their judgement of ambiguous information, likely due to high food motivation.

Keywords: broiler breeders, feed restriction, motivation, learning, judgement bias



Does unpredictable chronic mild stress induce pessimistic judgment bias in laying hens?

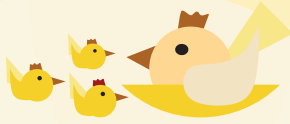
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The seminal study of Harding et al. (2004) demonstrated negative judgement bias in rats subjected to unpredictable chronic mild stress. In our previous studies, we did not prove the judgment bias in laying hens housed under different housing conditions. We supposed that a larger contrast between treatments is needed to induce judgment bias. We tested the hypothesis that unpredictable chronic mild stress will induce negative ('pessimistic') judgment bias in laying hens. Dominant Leghorn hens (n=22) housed in deep litter pens were trained in a touchscreen operant chamber to discriminate between positive (white circle; rewarded with mealworm) and negative (80% grey circle; punished with white noise) stimuli. For half of the hens, the colours of stimuli were reversed. Fourteen laying hens that mastered the discrimination training were divided into two groups. Eight of them were exposed to unpredictable chronic mild stress (mixing social groups, placing groups into a crate, thwarting access to water and food, removing perches and nestboxes) for four weeks, and the remaining six served as control. Then the hens were tested in judgment bias tests in which, in addition to the positive and negative stimuli, 3 ambiguous stimuli (20, 40 and 60% grey) were presented. At the end of the experiment, the blood samples for the plasma corticosterone assay were collected from the wing vein. The results of the behavioural tests were analysed using GLIMMIX procedure (SAS) and plasma corticosterone levels using a t-test. The treatment did not affect either visual discrimination ($F_{1,252}=1.43$; $p=0.234$), or the proportion of responses in the judgment bias test ($F_{1,153}=0.08$; $p=0.773$), nor did it affect the plasma corticosterone levels. Unpredictable chronic mild stress, or more specifically the combination of stressors in the protocol used here, did not induce the pessimistic judgment bias in laying hens.

Keywords: judgment bias, laying hens, unpredictable chronic mild stress



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Does complexity of aviary design affect hippocampal plasticity in laying hens?

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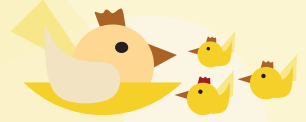
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There are many different cage-free housing systems in use with laying hens, which each present different welfare challenges. This study compares two free-range housing designs: flat-deck (a large flat floor with one raised area containing perches) and multi-tier (with 5 tiers that birds are free to move between). We hypothesised that birds housed in the lower complexity flat-deck system would experience higher levels of chronic stress than those housed in the more complex multi-tier aviaries, in which they had greater opportunity to explore and therefore higher cognitive stimulation.

Pullets (n=12) were sampled from a rearing site with a flat-deck design at 14 weeks of age. When the remaining birds reached 16 weeks of age, some were moved into flat-deck adult housing, and some were moved into multi-tier housing. The birds were allowed to settle in their new housing for 8 weeks, then 12 average birds were randomly selected from each group. Brains were collected from all 3 groups (pullets, flat-deck, multi-tier) and one hemisphere from each brain was fixed and cut to 50µm coronal sections. These were immunohistochemically stained to visualize doublecortin (DCX), a marker of neural plasticity. We counted stained cells in the caudal and rostral hippocampal formation (HF) in order to quantify DCX⁺ cell density, which has previously been found to decrease in the avian caudal HF in response to a variety of chronic stressors. We expected that DCX⁺ cell density in the rostral HF, which has a role in spatial memory, would be significantly higher in the multi-tier group because of greater cognitive stimulation.

There were no significant differences in DCX⁺ cell density among the housing groups. The difference in complexity between the multi-tier and flat-deck aviaries was therefore not great enough to have a detectable effect on hippocampal plasticity and any welfare differences would be minimal.



Use of artificial light cues improve early life ramp use behaviour of laying hens in rearing aviaries

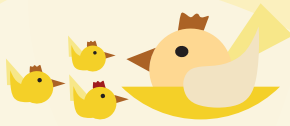
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Providing ramps in rearing aviaries has been shown to facilitate inter-tier transitions and earlier use of elevated structures. To maximize the welfare benefits associated with early life ramp use, we investigated if an artificial light cue that simulated the movement of a small moving particle, (i) can increase early life ramp use of laying hens and (ii) subsequently improve bone biomechanical properties at a later age in commercial rearing aviaries. We used 4800 Dekalb white birds housed in eight pens (600 birds/pen) from one until 119 days of age (DOA) in a semi-commercial rearing aviary. Aviaries were fitted with ramps in all pens and in half of the pens the ramps were fitted with LED strips (LED) which provided light cues intermittently throughout the day, while the other four served as control pens with no light cues (Control). We counted the number of inter-tier transitions using ramps and the number of active use (e.g., walk/run, wing assisted inclined running) of the ramps that did not result in a transition by scan sampling recorded videos for three time points each when the cue was on vs. off at 8, 11, 16, 23, 30, 37, 44 and 56 DOA. Bone biomechanical properties were assessed for tibiae and humeri collected from 160 birds (20 per pen) at 105 DOA. Linear mixed models with parametric bootstrapping analysis revealed that birds in the LED group performed more transitions ($p < 0.001$) as well as more active use of ramps ($p < 0.001$) when the cues were on compared to when the cues were off. No difference in bone biomechanical properties were found between the Control and LED groups. Our results show that the artificial light cues offer to be a promising tool to encourage vertical locomotion in the early life of laying hen chicks.

Keywords: early life, laying hens, skeletal properties, innate predispositions



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Light intensity preference of layer pullets is affected by behaviour at a young age

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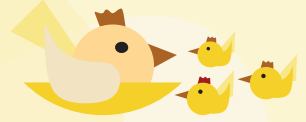
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While the rearing period is essential for welfare and performance of laying hens, there is little knowledge on light intensity preferences of layer pullets. Therefore, we determined preferences of white and brown pullets for light intensities, taking into account their age and behaviour.

Day-old chicks from a white (Lohmann White Classic) and brown (Lohmann Brown Classic) layer breed were housed till 10 weeks of age in groups of 24 per pen (8m²) with 8 pens per breed. Each pen was divided into 4 equal areas with one light intensity per area that switched daily (5, 20, 50 and 100 lux). Areas were separated from each other using black plastic, with a central opening to all areas. Cameras were installed for continuous recordings and an algorithm was developed to automatically count the number of pullets per area. Behaviour observations were performed via scan sampling at 6 ages. Behaviours were analysed as percentage of the total number of pullets in a specific area and using generalized linear mixed models with a binomial distribution and fixed factors intensity, breed, age and two-way interactions and random factors pen and area within pen.

Count data needs to be analysed, but both brown and white pullets were most present in 100 lux at all ages, although there were always pullets present at each intensity. Pullets showed less ingestion, standing and locomotion, and more inactive behaviour in 5 and 20 lux compared to 50 and 100 lux at a young age ($P < 0.05$), with no differences at older ages. Furthermore, pullets showed more foraging with increasing intensity ($P < 0.01$), while comfort behaviour did not differ between light intensities. Thus, breeds did not seem to differ in light intensity preference with age or behaviour. Yet, light intensities can stimulate or inhibit behaviours, especially at a young age.

Keywords: layer pullets, light intensity, breeds, behaviour, preference



Something to stay for? Does an automatic enrichment device increase the attractiveness of a winter garden for laying hens?

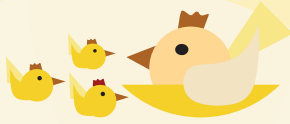
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The offer of a winter garden (WG) provides additional space for hens to exercise normal behaviour. To encourage the use and foraging behaviour, the area should contain further stimuli in addition to litter. In this study, the influence of an automatic enrichment device dosing grain on pecking plates (PP) installed in the WG of an organic laying hen farm was observed. Around 12,000 laying hens (Lohmann Brown Lite), divided in 4 equally sized groups, were housed in the same henhouse. While one group served as a control and had no PP (CG), two groups were offered one plate for 500 hens (SEG) and one group a plate for 250 hens (DEG). Behavioural observations via photo and video recordings were performed to detect the number of hens over the whole day and the duration of stay during two hours in the afternoon in a 19 m² area. Data was collected during three phases over the whole production period. The results showed that the automatic enrichment device positively influenced the animals' use of the WG. Over the whole husbandry period (60 weeks), on average, 1.48 hens/m² were detected in the CG, and 2.27 hens/m² in the enriched groups (SEG and DEG). Furthermore, the offer of PPs had a significant influence on the duration of stay in the WG area. Hens in the DEG stayed significantly longer (mean: 129 sec, SD: 126 sec) than in the CG (mean: 79 sec, SD: 91 sec; $p < 0.05$) and SEG (mean: 75 sec, SD: 83 sec; $p < 0.005$). Furthermore, at the end of observation significantly less hens used the WG ($p < 0.05$) and stays tended to be longer than at the beginning. This work was financially supported by the BMEL based on a decision of the Parliament of FRG, granted by the BLE.

Keywords: laying hens, enrichment, behaviour



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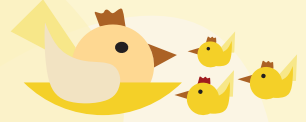
How providing dried black soldier fly larvae (*Hermetia illucens*) affects broiler activity

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Broilers are challenged with various welfare issues, such as rapid growth and poor leg health, which impairs their activity. Impaired activity, in turn, negatively affects broiler leg health. This study aimed to assess whether broiler activity can be stimulated by replacing 8% of their diet (based on expected dry matter intake) by dried Black Soldier Fly Larvae (*Hermetia illucens*, BSFL). In addition, the use of pixel changes as a proxy for broiler activity was explored. Broilers were housed at semi-commercial densities (<33kg/m²) in groups of 28 (n=4 pens in total). Dried BSFL were scattered through the pen in four equal portions a day at 08:00, 11:00, 14:00, and 17:00 h to the treatment group. The control group did not receive BSFL. Locomotor activity (movement) was assessed both manually using 30-sec scan sampling, and as percent pixel changes using the grey scaling method in EthoVision XT 10 (Noldus Information Technology, Wageningen, the Netherlands) for 15 minutes pre- and 15 minutes post- BSFL provisioning, at 14, 21 and 28 days of age. Pearson correlations between activity (average levels per min) as measured by both methods were calculated. Providing dried BSFL strongly increased broiler activity as was measured by manual scoring the behaviour ($p<0.001$) and by assessing pixel changes ($p=0.006$). Activity levels scored using pixel alterations and manual scans were positively correlated ($r_s=0.64$, 95%CI [0.49, 0.74], $p<0.001$). Assessing the percentage of pixel changes in Ethovision XT seems to be a promising and timesaving tool to score active behaviour in broilers. More research is needed to validate these findings in various settings.

Keywords: Broiler Welfare, Black Soldier Fly Larvae, *Hermetia illucens*, Activity, Environmental enrichment



Tree-ranging broiler chickens: how silvopasture impacts ranging behavior

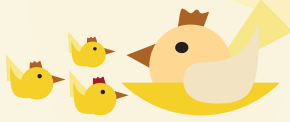
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Free-range access for broiler chickens can benefit bird welfare by providing opportunities to express highly-motivated normal behaviors, yet chickens' range use can be limited. Providing access to silvopasture, a range with trees that serve as a source of income for the producer, may benefit ranging behavior. The objective was to determine the impact of pasture type (silvopasture; SP or open grass pasture; OP) on range use in fast-growing broiler chickens. Day-old mixed-sex Ross 708 chicks (n=648) were housed indoors until day 23 of age and then moved to coops within six 125m² SP plots ($\bar{x}$ =32% canopy cover) or six OP plots (without canopy). Birds had access to the range between 8:00–17:00h. Plot-level range use (% of the flock outside) was observed on days 29, 30, 34, 35, 40, and 41 of age. Data were analyzed with mixed models in JMP Pro with treatment, age (week; wk), and their interactions as predictors, tested by time of day (morning, midday, afternoon), with plot ID as random factor. Data are presented as LSmeans $\pm$ SEM. More birds ranged in SP compared to OP (14.6 $\pm$ 1.1% versus 9.1 $\pm$ 1.1%; $F_{1,10}$ =13.21, P =0.005). In the morning, more birds ranged in SP (29.0 $\pm$ 1.6%) compared to OP (21.5 $\pm$ 1.6%; $F_{1,10}$ =11.44, P =0.007), and more birds ranged in wk5 (30.0 $\pm$ 2.2%) and 6 (31.7 $\pm$ 2.2%) compared to wk4 (14.1 $\pm$ 2.2%; $F_{2,272}$ =17.20, P <0.001). At midday, more birds ranged in SP in wk5 (4.0 $\pm$ 0.9%) compared to OP in wk5–6 (0.3% and 0.1 $\pm$ 0.9%) and compared to SP in wk6 (0.9 $\pm$ 0.9%; $F_{2,200}$ =3.62, P =0.029). In the afternoon, more birds were ranging in SP in wk5 (11.4 $\pm$ 1.6%) compared to OP in wk4–6, and to birds in SP in wk6 (4.4 $\pm$ 1.5%; $F_{2,200}$ =7.57, P <0.001). This study confirms broilers' preference for pastures with natural overhead cover over open grassland and illustrates the importance of natural overhead vegetation in the range for broiler chicken welfare.

Keywords: Agroforestry, Animal welfare, Behavior, Outdoor access, Silvopasture



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Laying hens display resilience to sleep disruption

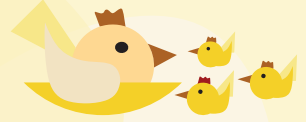
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Sleep behaviour may be an important indicator of animal welfare, however, sleep in birds is poorly understood. This study measured sleep states (slow-wave sleep, SWS; and rapid-eye-movement sleep, REM) and wakefulness in laying hens during disturbed and undisturbed dark periods and undisturbed light periods. Twelve individually-housed hens (56–77 weeks old) were used across four batches. Electroencephalograph (EEG) devices were surgically implanted to collect brain wave data to identify sleep states. Behavioural data was collected using infrared cameras. Following a week of surgical recovery, hens were exposed to disturbed, undisturbed and recovery nights to establish a baseline for sleep data and observe effects of mild disturbances (including daytime recovery sleep). Disturbances were: noise (90 dB), wind, and light (20 lux), applied for 5 min every 30 min between 21:00–03:00 (lights off: 19:00–05:00). Sleep states were scored with Slipanalysis software (4 sec epochs/min) using electrophysiological and behavioural data from which the proportion of time spent in each state was calculated. Data were grouped into time intervals (2 h intervals during lights off, 3.5 h during lights on) and intervals per bird with $\leq 25\%$ artefact (electronic noise) and/or missing data were analysed using linear mixed models in R. Birds spent 0.15–0.39 (± 0.04) in wake, 0.42–0.67 (± 0.05) in SWS, and 0.10–0.21 (± 0.04) in REM over the different types of nights. Overall, time interval during lights off was significant ($P < 0.001$) for all three states, but disturbance treatments were not ($P \geq 0.05$). Interaction between time interval and type of night was significant ($P < 0.05$) during lights off for wakefulness and SWS only. Overall, sleep in laying hens showed expected signs of rhythmicity: SWS was higher during early night and was steadily replaced by REM as night progressed. Hens seem to show resilience towards mild disturbances and appear to be able to return to sleep easily.

Keywords: Sleep, Behaviour, EEG, Laying Hens, Welfare



Fat and furious: Dominant hens gain more weight over time in aviaries

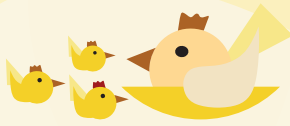
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In cage-free laying hens, social dominance could have negative implications for low-ranking individuals, such as denied access to resources, resulting in decreased welfare. Conversely, low-ranking hens may adapt over time by avoiding crowded areas and dominant individuals. To investigate the relation between dominance rank, hen movement, and health, we housed 1350 hens (Dekalb White) across 6 pens in a semi-commercial aviary system. We selected 36 focal birds, for which we recorded their movements between tiers of the aviary via a tracking system for 164 days, from 22–55 weeks of age (WoA), and calculated daily movement similarities (DMS) between and within birds. We observed social interactions via video for 2h/day on 3 days for each focal bird to compute a relative dominance index. Every 5–6 weeks we weighed the animals, scored their plumage and the severity of keel-bone fractures (KBF). For the analysis we used mixed effects models with WoA and dominance index as independent variables. We found that birds with a higher dominance index gained more weight over time than birds with a low index ($p = 0.046$), whereas no effect was found for plumage or KBF. Hens with a lower dominance index had higher within-individual DMS with increasing age than hens with a high index ($p < 0.001$). The between-individual DMS decreased more over time the larger the difference in dominance index between hens ($p < 0.001$), although the explained variance was small (marginal $R^2 = 0.2\%$). One explanation for our detected body mass difference over time might be that dominant hens were reported to have priority feed access. The decrease in between-individual DMS indicated lower-ranking hens adapted to avoid higher-ranking individuals, however it is questionable how biologically relevant the effect was. Adaptation was possibly hampered due to unpredictability of higher-ranking hens, as indicated by their lower within-individual DMS.

Keywords: Social dominance, Laying hens, Tracking data



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POSTER PRESENTATIONS

Session 1 – Welfare as part of the future of poultry production

P-01-01

Global perspective on broiler animal welfare & sustainability

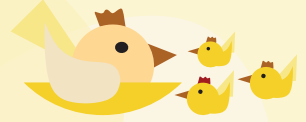
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Regardless of species, animal welfare is critically important for all veterinarians and animal care providers. For food animals, especially poultry, the intersection of animal welfare and sustainability is a key factor for achieving the 2050 global targets of increasing food production and enhancing sustainable agriculture practices. As a result, there is interest in more transparency for welfare and sustainability in intensive farming systems and verifiable incremental progress. This demand for transparency has also resulted in increasing oversight, evolution of requirements for welfare standards and audits, unique supply chain collaborations, and expanding expectations from many external stakeholder groups. There is no doubt that societal perceptions for animal care, housing and production outcomes are evolving, and this public perception is impacting how, where, and what animals are raised for human consumption. Specifically for broiler chickens, which are the most produced food animal in the world, the interest in improving welfare and sustainability outcomes has become a dominant focal point in developed countries and the topics are also escalating in developing countries. In this presentation, a global outlook on broiler animal welfare and sustainability will be provided. This will include a summary about how genetic selection has advanced sustainable broiler production, which input (resource) related metrics and output (animal or environmental) related metrics are commonly used for broilers, and how fascinating changes in science-based research are changing our understanding of what broilers want and need. Finally, a perspective on the challenges of balancing welfare with sustainability targets, food security expectations, and environmental impacts will also be included to provide the audience with a holistic and comprehensive global viewpoint for the future of broiler production.

Keywords: Broiler welfare, Poultry sustainability, Broiler breeder genetics, Sustainable agriculture



P-01-02

SELEGGT process to end chick culling

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Introduction: Every year, around 330 million one-day-old male chicks are culled in the egg industry in Europe after hatching due to their inability to lay eggs and because their grow-out is not economically profitable compared to broilers. The pioneer country to end this practice is Germany.

SELEGGT process: It is a liquid-based process that determines the embryo gender by analyzing the allantoic fluid

via endocrinological analyses on the 9th day of incubation. The technical data of the process are:

- 99% accuracy
- Able to analyze eggs of all colors and genetics
- Detection of gender as early as possible is available on the market today
- High-performance pipetting leads to a sampling time of one hatching egg per second
- Non-invasive method

Debate on pain perception: There is a great debate in Germany about the possibility of the embryos' pain perception during gender identification in the hatching egg. Scientific studies have confirmed that brain activity only takes place from the 12th day of incubation onwards. This makes it clear that no emotional feeling of pain is possible at least in the first half of the incubation period.

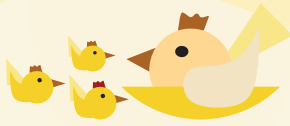
Legislation in Europe: Germany, France, Italy and Switzerland have already formulated legal conditions to end chick culling. Other European countries like Netherlands and Belgium have converted some supply chains to "Free of Chick Culling". There are very big differences in the requirements of different governments to end chick culling.

Conclusions: Gender identification in the hatching eggs is becoming the most desired solution because it is the most cost-effective and sustainable solution to end chick culling in Europe.

The SELEGGT process is the earliest process used in daily operations to end chick culling.

A European Union regulation banning chick culling would be the best solution for the industry and European fair competition.

Keywords: SELEGGT, respeggt, animal welfare, chick culling, in-ovo sexing



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P-01-03

Good enough? Animal welfare on organic laying hen farms in Sweden

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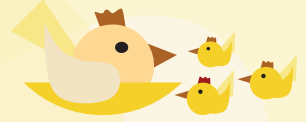
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Organic animal production must comply with high animal welfare standards. To increase our knowledge of animal welfare in commercial production, on-farm studies are important. Eleven organic laying hen farms in Sweden (11% of all Swedish organic laying hen farms) were visited in 2020. The aim was to describe the present welfare situation in terms of housing, free-ranging, bird health and behaviour. The median (min–max) flock size was 5750 (1118–17373) and the farm size was 18000 (3000–120000). The median flock age was 74 (73–78) weeks. Data collection included farmer interviews, assessments of the indoor and outdoor environment, behavioural observations and clinical examinations (n=550).

The median flock prevalence of moderate-severe plumage damage on the breast and belly, tail, and wings was 96 (84–100) %, 96 (72–100) % and 98 (94–100) %, respectively. The median flock prevalence of keel bone deviations, breast skin lesions and hyperkeratosis were 67 (32–84) %, 57 (10–74) % and 70 (38–100) %, respectively. The prevalence of severe hyperkeratosis was lower ($p=0.003$) in flocks housed on a thick layer of litter, in which more dust bathing events were also observed ($p=0.007$). The majority (95%) of the avoidance distance test trials (n=231) were unsuccessful (i.e. the birds distanced themselves from the observer before the test could be completed). On seven farms the free-ranges consisted mainly of pasture ($\geq 80\%$). Six of these had no or very little vegetation cover ($\leq 5\%$). Artificial shelter was provided on five of the latter, however the area offering protection was limited. The hens were mainly observed ranging close to the house (≤ 20 m).

The results show that important issues remain also in higher welfare systems such as organic production and that, in order to increase poultry welfare in practice and not only in theory, the content of organic standards must transfer all the way to commercial farms.



P-01-04

Sustainability in higher welfare chicken production

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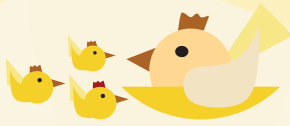
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Animal protection groups across the EU are asking corporations to sign up to the Better Chicken Commitment (BCC); 6 standards designed to tackle the most pressing welfare concerns in commercial chicken production (compliance with EU legislation, stocking density, enrichment, breed, CAS & third party auditing – see betterchickencommitment.com). Over 300 companies across the UK and Europe have now committed to incorporating these criteria into their entire chicken supply chains by the end of 2026, with some already meeting the BCC in a much shorter time frame.

The impact of the BCC on sustainability – in particular the requirements to use slower growing (SG) breeds of chicken and lower stocking densities – is cited by some companies as a barrier to adopting these minimum standards, as it increases the amount of feed and land required. We will present evidence to show that, rather than compromising sustainability objectives, the BCC can actually help to improve sustainability, aligning to the One Health approach. We will consider the impacts of bird mortality (FG = ~9.6%, SG = 5.2%), antibiotic usage (category B antibiotic usage 6.7 times higher in FG breeds) & muscle myopathies (moderate white striping up to 63.9% compared to 8.7%, and wooden breast up to 23.4% compared to <1%, in FG and SG breeds respectively) on human, bird and environmental health & present commercially relevant mitigations to further improve sustainability. We will also consider nutritional differences and associated emissions between breeds, demonstrating that higher welfare systems can have lower overall feed related emissions.

Lastly, we will showcase a current commercial example of how sustainable higher welfare supply is already being achieved by one company, reducing their total birds reared by 3m as a result of using SG breeds, with further ambitious emission reduction goals and a climate neutral slaughterhouse already in operation.

Keywords: Sustainability, Welfare, Broilers, Environment



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P-01-05

Animal welfare awareness among cage-free farmers: knowledge exchange via in-person workshops

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Egg industry stakeholders of the global North are more aware of the importance of welfare issues, both in terms of ethics and economics. While familiar with proper care and management leading to higher productivity, the significance of welfare has not yet secured a foothold in other regions of the world. To address this issue, we carried out two 2-day training workshops, where cage-free Kenyan egg farmers were sensitized about the importance of animal welfare. Participants were provided with the knowledge necessary to properly care for the animals by experienced instructors. Internationally acquired actual information on pressing welfare challenges was provided. The farmers were recruited in two counties: Murang'a (n=18) and Nakuru (n=18), in collaboration with county-level Livestock Office staff. The program consisted of multi-presenter classroom instruction with visual and hand-out aids, covering topics such as the basics of animal welfare, hen welfare specifics, good practices in cage-free farming, the importance of adequate nutrition, hen bone health, and open discussions. The program was evaluated using voluntary pre-, post-, and three-month post-surveys and farmer observations throughout the workshops. Just 41% of the participants were familiar with animal welfare despite most believing that hens can feel pain and experience suffering. Most identified high farming input costs and space availability as limitations for improving on-farm welfare but >95% were willing to improve. Hen welfare awareness and knowledge were effectively increased, with effects remaining three months after the workshops. At least 65% of participants claimed to have implemented on-farm improvements and >90% expressed willingness to continue learning. This work provides insight into real welfare gaps and discusses if in-person 2-day training workshops are appropriate means to raise farmed animal welfare awareness among cage-free farmers. The methods and outcomes shall be studied more extensively and could be used by welfare stakeholders in other regions.

Keywords: animal welfare awareness, in-person farmer training, cage-free egg farming, animal farming in Kenya



P-01-06

Perspectives of consumers towards cage-free eggs in China

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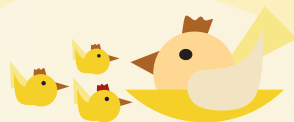
In China, farm animal welfare is only beginning to gain public awareness. Approximately 90% of eggs are produced within cage systems and research on the motivations for Chinese consumers to choose cage-free eggs over cage eggs are limited. The aim of this study was to better understand the perspectives of Chinese consumers towards cage-free eggs through exploring how and why they purchase and consume these products.

We used purposive sampling to recruit 19 participants who identified themselves as purchasers and end-users of cage-free eggs. Each participant participated in 2–4 semi-structured interviews. Participants were also asked to keep a food diary, in which they recorded purchasing, cooking, and eating experiences with eggs. This method was used to highlight the subjective experiences of consumers, build rapport, and prompt further topics for in-depth discussions. All data were analysed using reflexive thematic analysis.

All participants felt that cage-free eggs (非笼养鸡蛋) were a superior product compared to conventional or 'normal' (普通) eggs. The main perceived benefits of cage-free eggs included improved: 1) flavour and texture of eggs, 2) lived experience of the chickens, 3) health of the chickens and thus the consumers themselves, and 4) environmental impact. How participants obtained cage-free eggs reflected their trust in these products. Some participants repeatedly purchased cage-free eggs from family relatives, companies, or individuals they trusted and strongly believed in the superior quality and benefits of cage-free eggs. Participants who don't have such confidence with specific egg products often purchased cage-free eggs from multiple sources and were more flexible when choosing between cage and cage-free eggs.

Understanding purchasing behaviours and priorities for consumers may facilitate targeted strategies for marketing and education on laying hen welfare and cage-free housing systems in China.

Keywords: Cage-free eggs, China, Consumers, Purchasing behaviour, Trust



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P-01-07

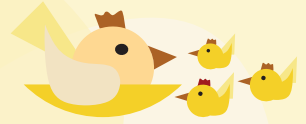
Pasture botanical composition and use frequency by two genetic lines of chickens

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Access to pasture enriches chickens' diet and improves their welfare, but range use frequency varies across individual chickens and breeds. The aim was to investigate if the frequency of exits outdoors by broiler hybrid Sasso (S) and Polish heritage chicken Green-legged Partridge (GP) is associated with the botanical composition of the ranging areas. In August 2018, 60 non-beak trimmed birds per strain were housed in groups of 10 from week 5 to 10. Each pen had access to an individual outdoor range of 30 m², video-recorded continuously to obtain frequencies of birds' range uses. The botanical composition of the sward (%) was determined based on botanical-weight analysis at the end of the study from each range used by the birds and from 6 control plots. Plant material was separated into groups of plants and individual species. The data were analysed by ANOVA model in SAS software (v 9.4). The higher share of weeds (S:8.66 (1.42) and GP:7.61 (0.59) and grasses (S:10.36 (0.96) and GP:12.10 (0.65) was at the birds' ranges as compared to control plots (1.45 (1.34) and 3.86 (2.97), respectively), in contrast to legumes (S:0.01 (0.01) and GP:0.32(0.26)) and sedges (S:0.64 (0.48) and GP: 0.09 (0.06)) most present in the control plots (10.88 (1.55)). Sasso was more frequently seen outdoors in plots with a higher proportion of *Heracleum sphondylium* (L.), *Rumex acetosa* (L.), *Festuca rubra* (L.), and *Lolium perenne* (L.), while Green-legged Partridge with *Armoracia rusticana* (L.), *Stellaria media* (L.), *Ranunculus repens* (L.), *Cirsium arvense* (L.) and *Rumex crispus* (L.). Concluding, the botanical composition of the ranging areas was affected by the presence of the chickens. It differed between breeds, but it remains unknown if birds used the pasture more frequently depending on its botanical composition or if the pasture composition depended on the frequency of its uses by the birds.



P-01-08

How to conciliate animal welfare and low price for indoor broilers? Perceptions of French stakeholders

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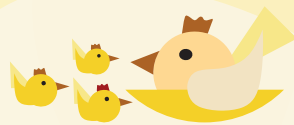
During the 20th century, broiler farming experienced a strong development, which resulted in an overall improvement of the health status, the intensification of production and the implementation of genetic optimization of animals mainly on productivity criteria. However, these improvements in productive performance have had some negative impacts on animal welfare and it seems essential today to rebalance productive capacity and animal welfare.

The topic of animal welfare holds an increasing place in citizens' concerns, and is progressively being essential in the considerations, encouraging the sector's players to take initiatives in that way. Regarding economic, environmental, animal welfare and health issues, as well as farmers' well-being, the sector is giving thoughts about tomorrow's livestock system, which is sustainable, and which best answers societal challenges.

In that context, the project COCORICO (2021–2024), coordinated by ITAVI and financed by the French Ministry of Agriculture, aims to improve the welfare of indoor broilers (through the addition of relevant enrichment, low density, slower genotypes, ...) and identify a balance between price and societal expectations. To do that, it proposes to reflect with all the sector's stakeholders on a new production standard for broilers, in order to contribute to enlightening choices and developing perspectives.

This study aims to deepen the perceptions of the sector's players on various solutions proposed to better meet the behavioral needs of broiler chickens, by identifying the obstacles to these changes and their potential levers. The qualitative study carried out, through 25 semi-structured interviews, made it possible to classify several proposals of solutions promoting broiler chickens' welfare, depending on their feasibility and their relevance according to the sector's players' perceptions.

Keywords: animal welfare, qualitative, broiler chickens, price, stakeholders



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Session 2 – Welfare assessment and precise livestock farming

P-02-01

How to set the scene to “Tag ‘n’ Track”?

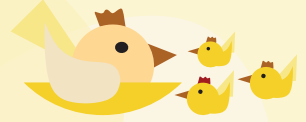
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Passive RFID (radio frequency identification) has the potential to advance poultry behaviour research as it provides automated, individual-specific, longitudinal, and non-invasive monitoring. These attributes are not always present in traditional approaches to monitoring animal behaviour. However, there is a lack of guidelines to implement and report on an RFID system for poultry research; as a result, significant insufficiencies and inconsistencies, revealed by our systematic literature review, are noted in existing reports on the validity and technical aspects of that technology deployed for poultry. Our systematic search was carried out on 19.11.2022 in Scopus and Web of Science using the following query: Topic = (RFID OR “radio frequency identification”) AND (poultry OR chick* OR bird OR hen OR broiler); search = “All Fields”. After the screening, we obtained 74 relevant papers related to the 23 different RFID systems identified. We noted that the technical aspects, such as architecture, infrastructure, operating parameters, and outcomes of installation testing of 6 of these systems, were not reported. Additionally, validation, assessable by quantifying performance indicators, such as precision, validity, accuracy, sensitivity, reliability, agreement, concordance, or other means, was not addressed for 10 of the identified systems. Furthermore, we noted, across papers, some inconsistencies that may lead to confusion: various authors calculated in the same way the difference between their gold standard and the RFID but referred to the output of that adequacy indicator differently: “delayed RFID detection time”, “identification speed”, “average differences”, “accuracy”, “time differences” or “time discrepancies”; for example. To tackle these bottlenecks undermining the potential of the technology in poultry science, we suggest a six-step roadmap for implementing the technology for such usage and a standard for reporting on its validity and technical aspects. These can be a reference for animal behaviour scientists, RFID system integrators and equipment manufacturers.

Keywords: poultry behaviour, RFID installation, automated tracking, Validation



P-02-02

Sensor technology: Detection of changes in movement caused by respiratory symptoms

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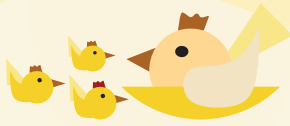
Diseased animals are considered to have poor welfare, and early detection of disease could improve welfare and health management of livestock. Technology that measures changes in activity could assist with early detection. Here, wearable technologies were used to identify changes in movement caused by a respiratory vaccine in laying hens.

Twenty previously unvaccinated hens (Hy-Line Brown, 25–35 weeks old) were used in 5 replicated experiments (4 hens/replicate). Hens were placed in groups of 4 into a 180 m² room that contained a litter floor area, feeder, drinker, perches, and nest boxes. On day 1 (D1), two wearable sensors (FitBark, FitBark Inc. and TrackLab, Tracksys Ltd.; total weight 35 g) were mounted on each hen's back using a simple string 'backpack' loop to detect changes in distance travelled (m/day) from day 1–12. On D3, saline (control) was administered to all birds by nasal and ocular drops. On D6, birds were given ILT (Infectious Laryngotracheitis) vaccine using the same method. Birds were observed until D12. Data was analysed in RStudio using linear mixed models and Tukey's post-hoc tests. The study was ethically approved at SRUC.

Mean moving distances were significantly different over days (FitBark and TrackLab, $P < 0.001$). With TrackLab, birds moved more on D3–4 than D10–12, on D5–6 than D11–12, and on D7–9 than D12. Conversely with Fitbark, birds moved more on D2–3 than D10–12, on D4–5 than D11–12, and on D6–9 than D11.

Changes in hens' moving distances caused by a live vaccine were detected using sensor technology, but while the day of changes detected were different with the two sensors, both found significantly less movement from day 10 (4 days after the vaccine was applied). These wearable sensors could be used in future to detect early signs of diseases and to monitor health conditions of laying hens.

Keywords: Animal welfare, Animal disease, Wearable technology, Moving distance



P-02-03

Use of implantable heart rate, temperature and activity loggers in broiler chickens

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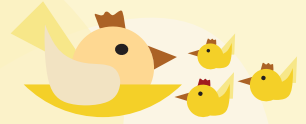
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Physiological indicators are crucial for assessing the welfare and health of broiler chickens. However, conventional measurement methods usually require handling the animals which is often laborious and stressful. Consequently, there is an increasing interest in automated methods using loggers attached to the animals. Given the rapid growth rate of broilers, the use of wearable sensors is a challenge. In this pilot study, implantable loggers were tested to remotely record broiler heart rate, temperature and acceleration.

Three two-week old broilers were anesthetized and surgically implanted with loggers (DST milli-HRT ACT, Star-Oddi, Gardabaer, Iceland): two intracoelomically and one subcutaneously in the neck. Loggers recorded heart rate, temperature and acceleration every 10 minutes until removal. The body temperature recordings were validated using intramuscular thermochips. The quality of the ECG derived heart rate data was graded by quality index (QI), an algorithmic assigned value, where QI₀ is 'great', QI₁ is 'good', and QI₂ and QI₃ indicate reduced quality.

The broiler with the neck logger was euthanized at 3 weeks of age due to surgical wound dehiscence. The other two were euthanized at 6 weeks of age, at the end of the trial. There were in total 8,707 measurements per parameter. A higher percentage of good quality heart rate data was obtained for the intracoelomic vs neck logger (62.67% vs 12.21 %). Average (+SD) temperature (°C) was slightly lower for the neck versus intracoelomic loggers (40.04±0.34 vs 41.01±0.33). Coelomic recordings corresponded better with the mean temperature recorded by the thermochips during 48 timepoints (40.94±0.40). Mean (+SD) of variance of external acceleration (mg) was 2,679±6,006 for the neck logger and 1,467±4,334 and 1,242±3,342 for intracoelomic loggers. Intracoelomic loggers for monitoring broiler heart rate, temperature and activity seem feasible and promising. Further validation studies on more animals are being conducted currently.

Keywords: bio-logging, implantable sensors, heart rate, body temperature, activity



P-02-04

Quantitative assessment of the welfare impacts of methods for the commercial slaughter of broilers

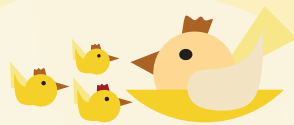
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We quantified the welfare impacts of different methods of poultry stunning prior to slaughter. To this end, we use the Cumulative Pain Framework, an analytical approach aimed at quantifying the cumulative load of negative affective experiences (referred to as ‘pain’) endured over any period using a meaningful metric: time in pain of four intensities (Annoying, Hurtful, Disabling, Excruciating). We evaluated electrical water-bath systems and multi-stage controlled atmosphere stunning with carbon dioxide (CO₂). From placement in the stunning unit until loss of consciousness, the time the average broiler is estimated to spend in intense (Excruciating and Disabling) pain is, respectively: (A) 0.02 [0.01–0.03] and 8.8 [5.4–12.2] seconds in properly managed CO₂ systems, (B) 2.18 [0.9–3.5] and 69 [23–115] seconds in stun-kill systems; (C) 6.4 [0.5–12.3] and 93 [25–161] seconds in electrical systems set to reduce carcass damage (low voltage-high frequency); and (D) 1.19 [0.6–1.8] and 71 [24–115] seconds in properly implemented (electronarcosis) electrical systems. A transition to properly implemented CO₂ systems is thus expected to reduce the time spent in intense pain compared to systems that stun-kill or are set to reduce carcass damage (B,C), in which intense pain often results from pre-stun shocks, electro-immobilization, compression of injured legs, fractures and live scalding. However, there is a trade-off between reducing intense pain with CO₂ stunning and increasing the duration of (milder) pain given the aversive nature of CO₂ prior to loss of consciousness. Also, the benefits of CO₂ stunning are lost if birds regain consciousness after leaving the stunner. In such cases, time in intense pain is longer (3.2 [0.03–6.37] and 14.5 [5.65–23.3] seconds in Excruciating and Disabling pain, respectively) than that typical of properly managed electrical systems (D).

Keywords: stunning, carbon dioxide, electrical waterbath systems, broilers, welfare assessment



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P-02-05

Vocalization of broiler chickens and laying hens as a measure of welfare

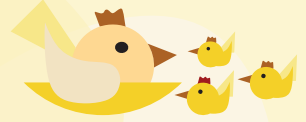
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The aim of this systematic review was to summarize, analyse, and critically compare studies that assessed vocalizations of broilers and layers in relation to their welfare status. Automated sound analysis has been used to monitor the behaviour of animals and their biological responses to external stimuli. The strategy “PICO” was applied to build the research question. The final keywords chosen for the systematic review were: *(broiler OR chicken) AND (sound OR vocalization OR “voice recognition”)*. The research was performed in four different databases: Pubmed, Scielo, Scopus, and Web of Science. A set of 44 papers was obtained. Selected papers were published from 2000 to 2022. Only 9 papers were published in the first studied decade, from which 8 were developed by European research groups. The first paper developed in a country outside Europe was published in 2005 by a Brazilian research group. China leads the number of literatures about sound analysis (18%). In total, 28 papers were related to broilers and 18 to hens. Seventeen distinct vocalisations could be identified by sound analysis in the selected papers. Of different welfare dimensions papers mostly focused on good health and appropriate behaviour (13 papers), followed by good housing (10 papers), and good feeding (8 papers). Most studies focused on the production phase of broilers (37 papers). There were no papers dealing with transport or pre-slaughter conditions. Most vocalization was recorded continuously (34 papers), and most experiments were conducted in experimental environments (29 papers). Thus, evaluating broilers` and hens` vocalization by sound analysis has been used to assess animal`s welfare, mainly in relation to good health and appropriate behaviour, which is a potential tool that allows continuous monitoring to provide useful information to the farmer.

Keywords: poultry, chicken, precision livestock farming, sound, vocalization



P-02-06

Improving laying hen welfare through computer vision

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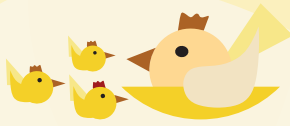
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Continuous measurements generated through computer vision of (social) behaviour in groups improve the method we use for studying the behaviour of hens. Instead of relying on limited data from direct behavioural observations by a human observer, we can use the recorded video data of all social encounters in order to improve welfare through management practices and breeding.

In the IMAGEN-project, we are developing a computer vision algorithm for tracking laying hen position and behaviour on the individual level within groups. We focus on severe feather pecking and piling behaviour as indicators for negative welfare, as well as dust bathing behaviour as an indicator for positive welfare. These measurements could be implemented in management practices and in breeding.

We aim to predict animal welfare parameters through the use of continuous position data and behaviour data on group level. We used video data from an overhead camera Dekalb White and ISA brown pullets divided over 20 groups in floor pens of maximum 10 birds (100 birds per line in total, lines housed separately). We extracted the positions of all animals on the video frames using a custom FairMOT computer vision model, with an IDF1 (correct detections) of 88.8%, MOTA (accuracy) of 86.8%, MOTP (precision) of 72.8% (Guo et al., 2022).

Activity patterns, locations and grouping behaviour were examined to gain a general overview of daily patterns of behaviour. Also, we used a subset of position data combined with behaviour data to build a prediction model for laying hen behaviour. The analysis of the data is currently ongoing. The results will be presented at the conference. Computer vision is expected to yield valuable tools, both for detailed individual tracking in small groups of hens and for more general group or flock level tracking in commercial flocks of laying hens in cage-free systems.



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Session 3 – Best practices for poultry housing and management

P-03-01

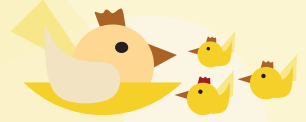
Development of vertical space use in rearing aviaries facilitated by ramps in laying hen chicks

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The welfare benefits of providing ramps in both laying and rearing aviaries are increasingly recognized. However, it is critical to understand the role of ramps in facilitating early life development of vertical space use in multi-tier aviaries to improve bird management and housing design. Therefore, we aimed to investigate the development of use of (i) middle and upper tiers and (ii) two different paths provided by ramps for inter-tier transitions in two semi-commercial rearings (Direct and Offset) systems with three tiers. We used two pens with 600 Dekalb white chicks each per rearing system. The two different paths consisted of a (i) continuous long ramp that connected the bottom and upper tier, and (ii) two pairs of discontinuous short ramps, with the bottom pair connecting the bottom to middle tier and the top pair connecting the middle and upper tier. Chicks' densities on elevated surfaces, short ramps, and bottom and top halves of long ramps were counted by scan sampling recorded videos at 13-time points per day for 8 to 16 days of age (DOA). Mixed model analysis showed a significant effect for the interaction between age and location for chicks' density on elevated surfaces (Direct: $t = 13.22$, $p < 0.001$, Offset: $\chi^2 = 4.28$, $p < 0.001$), short (Direct: $\chi^2 = 17.21$, $p < 0.001$, Offset: $\chi^2 = 13.42$, $p < 0.001$) and long ramps (Direct: $\chi^2 = 25.30$, $p < 0.001$, Offset: $t = 3.85$, $p < 0.001$). Our results show that the chicks show a gradual progression in using higher parts of the aviary such as upper tiers (chick density for 8 and 13 DOA, Direct: 5.61 and 39.66, Offset: 0.02 and 7.66), and the top half of the long ramps (chick density for 8 and 13 DOA, Direct: 0.73 and 9.14, Offset: 3.20 and 5.78).



P-03-02

Collaboration to drive improvements in commercial broiler production

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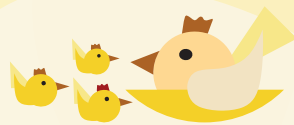
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Health and welfare problems in intensive commercial chicken production are well documented in the scientific literature. Production has been pushed to a point where basic animal welfare needs can no longer be met. In an attempt to lower food conversion ratios and shorten production times, modern fast growing broiler breeds experience significantly worse health and welfare issues than those in higher welfare systems. Fast growth leads to high levels of lameness, metabolic disorders, muscular myopathies and reduced behavioural repertoire, further exacerbated by high stocking densities and inappropriate environmental provision.

To address these and other critical welfare concerns, animal protection organisations across the UK and Europe collaborated to develop the Better Chicken Commitment; a set of 6 standards criteria to help address these most pressing welfare concerns. To date over 300 companies across the UK and Europe have committed to incorporating the BCC requirements into their supply chains by the end of 2026, some moving ahead of this deadline.

In this presentation we will share the evidence based rationale for the selected criteria (see betterchickencommitment.com). We will explore the detrimental welfare impacts of fast growth rates, stocking density, and electrical waterbath stunning, as well as the welfare improvements provided by basic environmental provisions. We will also outline BCC progress to date – in companies ranging from retail, hospitality and manufacture – and the commercial viability of incorporating these welfare standards.

Keywords: Broilers, Welfare, Standards



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P-03-03

Range use relationship with welfare and performances in four strains of organic broilers

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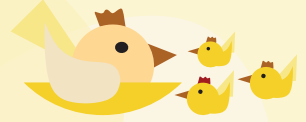
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Alternative productions with outdoor access are acclaimed by consumers as animals can better express their natural behaviours. Eating grass and longer locomotion for example can impact animal use of outdoor range. To understand range use relationship with broiler's welfare and performance, we studied four intermediate to slow-growing strains: JA757 (734 animals, rearing duration (RD): 71 days), S757N (735 animals, RD: 85 days), White Bresse (747 animals, RD: 106 days) and a dual-purpose crossbreed (771 animals, RD: 99 days). One hundred males of each strain were ranked according to their range use and divided in two extreme groups of 25 high- and low-rangers. We compared the means of our indicators regarding welfare (hock burns, pododermatitis, struggling on the slaughter line, tibial bone length and breaking-resistance) and performances (carcass weights and yields) by range use group. We did not observe any significant differences in welfare indicators collected at the slaughterhouse depending on range use in the four studied broiler strains. Only in the JA757, we found better leg health in high-rangers compared to low-rangers with 4% ($p=0.006$) shorter and 2% ($p=0.021$) stronger tibial bone which could be thanks to a higher locomotor activity in high-rangers compared to low-rangers. We reported significant reductions of carcass traits with range use in all the studied strains. We observed significantly lower carcass weights by 12% ($p=0.006$) in JA757 and by 7% ($p=0.006$) in White Bresse and significantly lower carcass yield by 2% ($p=0.006$) in S757N and by 1% ($p=0.009$) in the dual-purpose strains. These results could be due to higher locomotor activity of high-rangers compared to low-rangers. Therefore, later research could focus on how to maximize range use and its health benefices without compromising performances.

The project PPILOW has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement N°816172.

Keywords: organic broilers, range use, performance, health, blood indicators



P-03-04

Keep some distance – Stocking density during rearing has long-term effects on the immune system and behavior of laying hens

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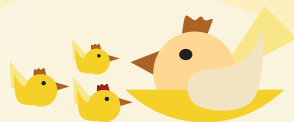
Throughout life, chickens are exposed to a variety of stressors that have the potential to modify the immune system and alter behavior with possible detrimental impacts on health and welfare. One important stressor is overcrowding. Most studies to date have focused on the effects of stocking density (SD) on broilers and layers, but not on pullets, although these effects may remain well into the laying period. Our study therefore examined possible short- and long-term consequences of high SD during rearing on the welfare of laying hens.

Pullets were reared in floor pens at low (13 birds/m²) or high (23 birds/m²) SD from week 7 until week 17 of life (rearing period). From week 18, hens were kept at an identical SD of 2.4 hens/m² until week 28 of life (laying period). At the end of each of the two periods, behavioral and immunological welfare parameters were studied. Blood and lymphoid tissue were collected and various leukocyte and lymphocyte subsets were analyzed by flow cytometry. General behavior and pecking behavior were recorded by direct observation. Statistical analysis was performed using SAS (version 9.4) and a linear mixed model.

Pullets reared at high SD showed stress-related alterations in immune cells such as reduced lymphocyte numbers which became manifest during the laying period. Of particular interest is that the number of $\gamma\delta$ T-cells was lower in the high SD group, which has not been found in any stress-related studies with chickens before. Moreover, high SD also resulted in less locomotion and comfort behavior, and in a higher incidence of feather pecking. Again, most of these effects were still evident during the laying period, although SD was identical for all hens at that time.

This highlights the significance of early-life conditions for immunocompetence and welfare throughout the whole production cycle.

Keywords: laying hen, stocking density, immunity, rearing, welfare



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P-03-05

Code 2+, a new alternative aviary system

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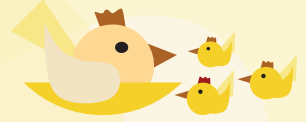
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The novel Code2+ housing system for laying hens was developed to combine the welfare benefits of aviary systems with the environmental benefits of enriched cage systems. It was established by Vencomatic Group and Boerenbond with input from Wageningen University & Research and Experimental Poultry Centre in Geel where it was evaluated.

Both intact and beak-trimmed white and brown hens (150 hens/unit) were housed in the Code2+ (12 units, 3 replicates per treatment combination). The system was compared with two aviary compartments (1920 hens capacity each), one with white and one with brown hens (intact beaks), and an enriched cage compartment (3072 hens capacity), with rows of white and brown hens (trimmed beaks), in the same hen house. Welfare, production, and emission were measured simultaneously for all three systems. The Welfare Quality® Assessment Protocol for Poultry was used to examine the hens' exterior and other welfare aspects. Additionally, extensive behavioral assessments have been carried out. Electrochemical ammonia sensors measured ammonia levels in all systems.

The exterior of the hens in the Code2+ did not differ substantially from that in the other systems ($p=0.290$). The behavior in the Code2+ resembled that of hens in the aviary system, whereas the hens' behavior in enriched cages differed from both Code2+ and aviary. Compared to the enriched cages, hens in both alternative systems showed more foraging, dustbathing, and preening ($p<0.001$). Less object pecking was observed in Code2+ compared to the aviary system ($p<0.001$). Feed conversion was better in Code2+ and mortality was lower. Ammonia emissions in Code2+ were reduced by 60% compared to the conventional aviary. In enriched cages compared to conventional aviaries, emission reductions were still higher, i.e. 75%. In conclusion, Code2+ offers the possibility to combine the benefits of an aviary system for animal welfare with the lower emissions of an enriched cage.

Keywords: Code 2+, emission, welfare, aviary, enriched cage



P-03-06

Gut microbiota activity in chickens from two genetic lines and with three ranging profiles – welfare impact

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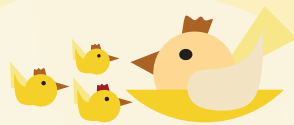
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Birds' gastrointestinal tract is inhabited by different microbes, collectively termed the microbiota. These commensal microbes are proven to constantly interact with the host.

The relationships between the housing environment and the chicken microbiome are not fully explored, especially considering the various chicken production systems and genetic strains of birds. This study investigated the differences in the composition, activity and metabolic products of the gut microbiota in 2 different lines of chicken with 3 ranging profiles (indoor, moderate and outdoor – preferring birds). Sasso and Green-legged Partridge (GLP) birds were housed separately with access to outdoor ranges. The birds' range use was video recorded to measure frequency values. With the PCR method we obtained the information about the relative abundance of *Lactobacillus* spp., *E. coli*, *Bifidobacterium* spp., and *Clostridium* spp. in the ceca. Gut microbiota activity was assessed based on the glycolytic activity of bacterial enzymes: α -glucosidase, β -glucosidase, α -galactosidase, β -galactosidase, and β -glucuronidase as well as based on the concentration of short-chain fatty acids (SCFA) in the caecal digesta. Statistical analysis was conducted by generalized linear mixed models in SAS 9.4 software. The lowest relative abundance of *Bifidobacterium* spp. was found in the cecal content of indoor-preferring Sasso birds (0.01 ± 0.001), as compared to all other birds in the experiment (ranging from 0.03 ± 0.01 to 0.11 ± 0.07 ; $P = 0.0002$). The cecal activity of α -glucosidase, β -glucuronidase and β -galactosidase was higher in GLP, as compared to Sasso ($P = 0.013$; $P = 0.008$; $P = 0.004$). Valeric acid concentrations were higher in moderate GLP than in Sasso of the same ranging profile (2.03 ± 0.16 vs. 1.5 ± 0.17 ; $P = 0.016$). The majority of the analyzed parameters confirmed that genotype and ranging profile have effect on chicken microbiota composition and properties. Additionally, in outdoor-preferring birds, the combination of outside activity had a positive effect on the birds' microbial profile and thus positive effect on gut health and birds' welfare.

Keywords: microbiota activity, free range



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P-03-07

Comparison of chickens with variable growth intensity in free range and on litter

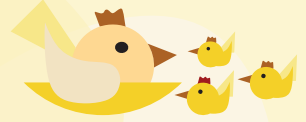
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The study was focused on comparison of performance parameters of fast growing chickens Ross 308, medium growing JA 757 and slow growing ISA Dual chickens in free range and on litter housing. All birds were kept the first three weeks on litter and then a half birds was transferred to the free range. Chickens were fattened Ross 308 35 days, JA 757 49 days and ISA Dual 70 days on litter and Ross 308 42 days, JA 757 56 days and ISA Dual 90 days in free range. Parameters of the final weight, daily weight gain, feed intake, feed conversion ratio and mortality were recorded. Statistical analyses of the results showed very high significant differences ($p < 0,001$) for type of housing in final weight, daily weight gain, feed intake, which were higher in free range. Mortality of Ross chickens was higher in free range than on litter, whereas the parameter was not affected in medium and slow growing chickens. High significant differences ($p < 0,001$) among genotypes were observed in all performance parameters. Also, significant interactions of housing system and genotype were detected in all parameters. To conclude, free range deteriorated performance in fast and slow growing chickens, medium growing chickens had similar results in both housing.

Keywords: chicken, genotype, performance, housing system



P-03-08

On-farm descriptive study on the use of winter gardens by broilers

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The implementation of physical enrichment seems beneficial for the poultry welfare but there is still not enough data on the usefulness of winter gardens (roofed area with wind net and littered floor). This study aims to provide information on the use of the winter gardens by broilers. Four flocks of broilers (JA 787; JA 987) were studied during summer (2 flocks), winter (1 flock) and spring (1 flock) in 2021 and 2022. The winter garden was enriched with a platform and straw bales. A Copeeks® video camera was installed to record the use of the winter garden (automatic counting in 3 areas: platform, straw, net, other). For 1 summer flock, resting behaviour was observed by scan sampling and other active behaviours were observed using continuous sampling of 20 birds during 5min at 26 and 40 days of age in 3 areas inside the barn and in the winter garden. Winter garden was first opened when the birds were minimum 26 to maximum 33 days, depending on the weather, for at least 13 consecutive days. On average during the whole period, we observed 12.5% of the broilers using the winter garden per day in summer, 3.3% in winter and 7.6% in spring. During the first days of winter garden access, there was no strong preference between the different zones. From day 5, the net, platform and straw areas were the most used ones (6.9, 7.2 and 8 birds/m² VS 3.6 birds/m² in the other area), except during winter where use of platforms was low. Birds tend to rest more inside than in the winter garden (70% birds resting VS 35%, p-value<0.01) and birds forage more in the winter garden than inside (16.9 occurrences VS 5.8, p-value<0.05). Our data indicate that the enrichments positively influences the animals' use of the winter garden and that the winter garden stimulates the broilers active behaviours.

Keywords: Welfare, Behaviour, Veranda



Session 4 – Bone quality

P-04-01

Keel bone damage in small-scale experimental conditions

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According to EFSA evaluation, one of the most serious welfare problems of laying hens is currently keel bone damage (EFSA AHAW, 2015). Keel bone health can be affected by fractures and deviations of various types and extent. Unlike easy to observe behavioural problems (e. g. feather pecking), birds with fractures are typically indistinguishable from healthy individuals. Causes of keel bone damage are multifactorial and high prevalence of keel bone damage can adversely affect egg quality and production.

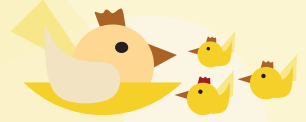
Our study aimed to explore whether, and to what extent, keel bone damage will occur even in experimental conditions with low densities and small group sizes. We compared two housing systems – enriched cages and deep litter pens, each with 4 groups of 10 White Leghorn hens. Starting age was 16 weeks, the end was 48 weeks. Within this time period, each bird was examined 6 times. Body weight and palpation of keel bone were performed. Recorded was the type of damage (fracture, deviation), affected part (cranial, middle, caudal), and the severity (scale 1–3). Palpation was done by two trained scorers.

Keel bone damage started to appear already at the age of 18 weeks in both systems and at the age of 48 weeks, 53% of hens in pens and 70% in enriched cages had either fracture or deformity. Fractures were more common in pens ($p < 0.001$) and appeared mainly in the caudal part of the bone. Deviations/deformations appeared in the middle and cranial parts and were significantly more common in enriched cages ($p < 0.01$).

Despite small groups, low density, habituation to human presence, and “stress-free” environment, the prevalence of keel bone damage was surprisingly high. This points out to the importance of finding ways to reduce the incidence of this undesirable phenomenon in terms of both productivity and welfare.

Supported by VEGA 2/0094/19.

Keywords: keel bone, fracture, palpation, welfare



P-04-02

A new dietary strategy to improve pullets' bone mineralization

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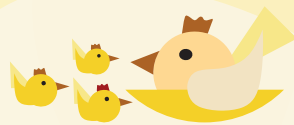
Bone mineralization in pullets is key to future laying performances and hen welfare. Recent studies have however showed that oxidative stress is detrimental to mineralization. The present study aimed at evaluating the effect of a dietary grape extract on pullet bone health.

36300 day-old pullets were randomly divided in two barns on the same farm site. Both received the same diet, with the addition of 30ppm of Nor-Grape (Nor-Feed, France) in the feed of the supplemented group (NG, n=18150) from D1 until W17. Both CTL and NG groups followed the same prophylaxis and vaccination protocol. On W17, 50 pullets were sampled from each group, euthanized and autopsied to assess keel bone deformation. A leg from each bird was also sent to an independent laboratory to measure tibia dry matter and mineral matter content, Ca and P content and bone flexibility.

Tibia dry matter and mineral matter were significantly higher in the bones from the supplemented pullets (+1.1% and +0.9% of tibia weight (TW) respectively, $p < 0.01$). This translated in higher levels of calcium (10.7% vs. 10.4% TW for NG and CTL respectively, $p < 0.10$) and of phosphorus in bones (5.0% vs. 4.8% TW for NG and CTL respectively, $p < 0.05$). Bone fracture force was also numerically increased in the NG pullets (+17%, ns.). Furthermore, the proportion of pullets presenting keel bone deviation also tended to be reduced in the NG group (18% vs. 32%, $p = 0.10$).

Overall, these results indicate a positive effect of NG supplementation on the bone mineralization of pullets during the rearing phase. This translated into a lower proportion of keel bone problems. Such improvement, before the demanding period for the skeletal system that the laying period represents, indicate a better preparation of the pullets and an improvement of their welfare linked to better bone health.

Keywords: Mineralisation, Bone, Pullet, Grape extract, Polyphenols



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P-04-03

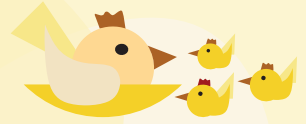
The dietary effect of dried olive pulp on the incidence of Keel Bone Damage in laying hens housed in floor system

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In modern egg industry, layers face a number of welfare problems with Keel Bone Damage (KBD) being one of the most prevalent. This study investigated the dietary impact of dried olive pulp (OP) on the incidence of KBD in laying hens. It took place in a commercial poultry farm. 180 Isa Brown layers, 20 weeks of age, were randomly accommodated in 15 fully equipped (plastic feeder, nipple drinkers and perches) floor pens (12hens/pen). After 3 weeks of adaptation, birds were randomly and equally divided in 3 dietary groups: CON, OP4, and OP6, according to the inclusion rate of OP in diet (0%, 4% and 6% respectively). All rations were isonitrogenous and isocaloric. At the age of 39 and 59 wk, KBD was assessed by palpation technique. A general score regarding the presence of KBD (fracture, deformation, or both score 1) or not (completely straight and flat keel bone, score 0) was determined. Moreover, the % of hens in each group presenting deformation, fractures or both was calculated. At the age of 39 wk, the incidence of KBD tended to be lower in OP4 hens compared to CON hens ($p=0.08$) even though the differences observed among groups were not significant ($p<0.05$). However, OP4 hens presented significantly lower ($p<0.05$) incidence of deviations (3.39%) compared to CON hens (16.67%). At the age of 59wk, OP6 hens presented significantly higher ($p<0.05$) percentage of KBD (59.32%) compared to CON (35.59%) and OP4 birds (25.42%). Moreover, at this time period, significantly lower ($p<0.05$) percentage of deviations was recorded in CON (33.90%) and OP4 (22.03%) groups compared to OP6 group (54.23%). Based on the obtained results, it could be supported that feeding laying hens, housed in floor system, with dried OP at the rate of 4% does not adversely affect the incidence of KBD.

Keywords: olive pulp, keel bone damage, welfare, layers



P-04-04

Total Replacement Technology™ (TRT) reduces keel bone damage, while improving eggshell strength and reducing mineral excretion

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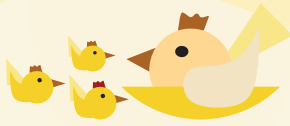
A study was performed in brown layers, over five cage-free commercial farms, to investigate the effect of organic and inorganic trace minerals (ITM) on keel and tibia traits, eggshell strength and mineral excretion. Each farm had a control and treatment group of the same breed and date of hatch. Throughout the trial, the control and treatment birds received diets of the same specifications, except for Zinc (Zn), Copper (Cu), Manganese (Mn), Iron (Fe) and Selenium (Se): control birds received a diet supplemented with conventional levels of ITM, whereas the treatment group had the ITM replaced with lower levels of organic minerals (Total Replacement Technology™, TRT) for Zn, Cu, Mn, Fe (Bioplex®) and Se (Sel-Plex®).

Eggshell strength was improved in Treatment (TRT) birds during the trial ($p < 0.05$). Zn, Mn, Cu, Ca, K and Na contents in the faeces were lower ($p < 0.05$) in TRT birds.

20 birds each from TRT and control, from one farm, were euthanized, had their keels dissected and visually scored for damage (absent/present). TRT birds had fewer damaged keels (fractures and/or deviations) than the control in the cranial, middle and caudal portions, with statistical significance in the caudal ($p = 0.004$). Keel width, Calcium ($p < 0.001$) and Phosphorus ($p = 0.005$) percentages in keel ash were greater in TRT birds, while keel height, ash percentage ($p = 0.013$) and strength ($p = 0.002$) were lower. Tibia ash was greater in the control ($p = 0.034$), but tibia strength, Ca and P were unaffected.

Despite the reduced mineral supplementation, in TRT birds there were fewer damaged keels, tibia breaking strength was unaffected and eggshell strength was improved. Keel damage was greater in the group with greater keel breaking strength, indicating that other bone structural factors, still unidentified, might help prevent keel damage. These novel findings demonstrate that TRT can simultaneously reduce keel damage, increase eggshell strength and reduce mineral excretion.

Keywords: Keel bone damage, Bone health, Laying hen welfare, Eggshell quality, Mineral excretion



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P-04-05

Occurrence of keel bone damage in German laying hens: a retrospective study

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One of the biggest welfare issues in laying hen husbandry are keel bone damages (KBD) as they affect, for instance, the productivity and behaviour of the animals and cause pain to the birds.

Data of this retrospective study were collected at 39 German herds visited several times during production, where housing system, colour of plumage, observation time and herd size were recorded. At each observation time, KBD was evaluated of 50 randomly selected animals by palpation and classified into a three-stage score (Score 0 = without deformation, Score 1 = low to moderate deformation, Score 2 = major deformation or obvious keel fracture). Statistical analyses were performed using a generalized linear mixed model (SAS 9.4).

A total of 16,868 laying hens were classified regarding the occurrence of KBD. In all herds, the percentage of affected animals from the 41 week of life was higher (Score 1 = 45.7%, Score 2 = 12.2%) than of those without KBD (Score 0 = 42.1%). At the end of the laying period, only 32.5% of the examined animals showed an intact keel bone (Score 1 = 48.9%, Score 2 = 18.6%). Model calculation showed a statistically significant influence of the type of housing system and observation time on the occurrence of KBD (both $p < 0.0001$), whereas the plumage colour and the herd size showed no effect. Overall, despite an improvement of the welfare of laying hens within the EU by a ban on battery cages and, thus, a greater freedom of movement, the present study proved KBD as a major welfare problem in egg production.

Keywords: laying hen, keel bone damage



Session 5 – Welfare challenges

P-05-01

Increased broiler growth rates are associated with reduced Footpad dermatitis development and severity close to slaughter age

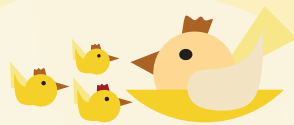
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The development of footpad dermatitis (FPD) negatively impacts broiler welfare. Its severity is related to multiple factors related to flock digestive health, dietary composition, environmental and husbandry parameters. Although increased growth rates (GR) have been associated to the development and severity of leg health related pathologies such as gait abnormalities, their influence on FPD development is uncertain. Herein, we hypothesised that increased GR *per se* does not lead to increased FPD incidence and severity as external factors related to the bird environment, are deemed more important. In the present trial, 200 broilers were allocated to 5 pens and offered the same starter, grower, and finisher experimental diets. Broiler individual BW were assessed at 9, 21 and 31 and FPD at 21 and 31 days of age. FPD was scored using a 5-point scoring system. Ascending Hierarchical Classification (AHC) was performed on BW obtained at d9, 21 and 31. Three classes were identified. C1 (51%) : medium GR (301 g, 1142 g, 2257 g), C2 (28%) : low GR (279 g; 1021 g; 194 g), and C3 (21%) : high GR, (317 g; 1275 g; 2498 g). FPD severity was compared amongst classes with the chi-squared test. At d21 FPD score distribution was similar amongst classes ($p=0.28$). On the contrary, at day 31 there was a significant effect of class on FPD development and severity ($P=0.027$). There was a lower incidence of FPD scores greater than > 3 (severe FPD development) in C3 birds, and a lower incidence of birds with a score=0 (absence of FPD) in C1 birds. In conclusion, individual bird GR is in fact associated with reduced FPD development and severity close to slaughter age. Future studies should delineate whether this phenomenon is related to altered locomotion patterns in heavier broilers.

Keywords: broiler chicken, growth rate, footpad dermatitis



P-05-02

Offspring sex ratio manipulation in Chukar partridge (*Alectoris chukar*); Effects of dexamethasone as a high-potent glucocorticoid

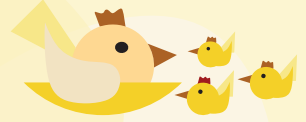
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It is well documented that birds are able to manipulate offspring sex ratio in response to different non-social and social environmental factors as well as hormones. However, the literature on the effects of environmental stress and corticosterone, (the main stress related glucocorticoid in birds) on offspring sex ratio is inconsistent. These discrepancies may occur because of differences in hormone dosage and application. For example, in contrast to oral administration, implants and injections of corticosterone may result in supra-physiological circulating concentrations followed by strong negative feedback on these concentrations. In the current study, dexamethasone, a highpotent synthetic glucocorticoid, was twice daily orally administrated for 10 days in different dosages to Chukar partridges (*Alectoris chukar*), to study its effect on sex ratio of the eggs, as well as on circulating levels of glucose, cholesterol, calcium, and aminotransferase, which have been directly or indirectly linked to sex ratio. Ninety-six female partridges were randomly assigned to four treatment groups: Untreated, Vehicle, DEX-low (1.15 µg/mL), and DEX-intermediate (20 µg/mL). DEX-low females produced more male hatchlings (72.1 %) compared to vehicle (40.5 %), and untreated (50.0 %) females ($P < 0.05$), but the percentage of sons in DEX-intermediate birds (52.9 %) was not significantly different from the other three groups ($P > 0.05$). No significant differences were found in egg production and hatchability suggesting that sex-specific mortality of embryos does not explain the effect on hatchling sex ratio. Group differences in glucose concentrations showed a similar patterns of statistical significance as that of sex ratio that in turn correlated with glucose and cholesterol over all birds. The results show that the glucocorticoid can increase the sex ratio in birds, indicate that such effects can be dose dependent, and suggest that glucose may be part of the underlying mechanism.

Keywords: sex ratio, corticosterone, dexamethasone, glucose, partridge



P-05-03

Alfalfa hay consumption in beak-trimmed and non-beak trimmed brown hens

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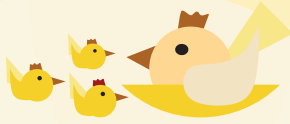
Environmental enrichment benefits the overall well-being of laying hens. Alfalfa hay bales are commonly provided, allowing hens to meet their foraging needs and to reduce pecking problems.

The current case study quantified the difference in alfalfa hay consumption behavior between beak-trimmed and non-trimmed ISA brown hens in combination with feather quality monitoring. Both intact and beak-trimmed hens (1.325 hens/group, 8 test groups, 4 replicates each) were housed in aviary systems in the same hen house. Alfalfa hay bale (Hartog Compact Luzerne, Grasdroyerij Hartog, Lambertschaag, Netherlands, 20 kg) consumption was monitored daily between the ages of 18 and 55 weeks. To gain an appraisal of the feather pecking grade, feather condition scores were measured at 9 different ages (21, 25, 30, 33, 37, 41, 45, 49, and 54 weeks) from a sample of 80 hens per experimental group using the Tauson method. Scores of individual body parts (1–4) were pooled for the whole body (up to optimal 20).

From the start of the study, alfalfa hay consumption was considerably higher in all test groups with intact beaked hens. At 55 weeks of age, intact beaks consumed on average 5,8 g/hen/day compared to 1,4 g/hen/day for trimmed beaks. Feather condition scores in hens with intact beaks were significantly lower from 25 weeks onwards compared to beak-trimmed hens ($p = 0.01$). However, hens with intact beaks still showed well-feathered hens with limited damage at 54 weeks (combined average score of 14,2 ; [95% CI: 14,1–14,3]). Beak-trimmed hens showed an average score of 14,8 ; [95% CI: 14,6–15,0]) at this age.

In summary, hens with intact beaks show apparent different behavior with up to more than 4 times higher alfalfa hay consumption than trimmed-beaked hens. Furthermore, aged intact-beaked hens still showed satisfactory feather condition scores, indicating a positive effect of alfalfa hay.

Keywords: alfalfa hay bales, enrichment, beak treatment, Isa brown, feather quality



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P-05-04

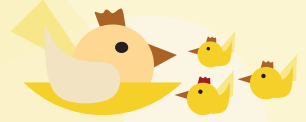
The knowns and unknowns about injurious pecking in poultry

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Poultry accounts for over 80% of the main livestock populations, making this group of species the most widely farmed land animals. Injurious pecking refers to bird-to-bird pecking that results in or has a high likelihood of causing integument injury and psychological harm to the victim. In commercial poultry flocks, injurious pecking is a major societal, economic, and animal welfare concern due to its high prevalence. There are three forms of injurious pecking: tissue pecking, aggressive pecking, and feather pecking. Additionally, there are two major views explaining the origin of feather pecking: 1) the ethological view and 2) the dysfunctional view. The ethological view emphasizes the role of the environment in creating motivational frustration leading to severe feather pecking, whereas the dysfunctional view identifies the cause of severe feather pecking as underlying neurobiological dysfunctions induced by intense or sustained stress. Finally, this presentation discusses risk factors for injurious pecking and farm management strategies that are used in an attempt to mitigate these problems.

Keywords: injurious pecking, feather pecking, cannibalism, poultry



P-05-05

Experiences with keeping turkey hens with intact beaks – a field study

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In turkey production, injurious pecking is a serious welfare problem. Therefore, beak trimming is an intervention which is intended to minimize severe pecking injuries, but which is likely to be associated with pain by the amputation.

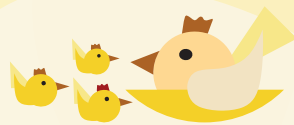
The aim of this field study was to keep beak-intact turkey hens. In order to minimise injurious pecking, housing and management were improved on six farms over three production periods. The improvement included the provision of additional enrichment materials and structural elements, a 1h-dark period mid-day, an increase of the crude fibre content and a stocking density of max. 48kg/m². Furthermore, the separation management and interventions in case of pecking events were part of the optimisation strategy.

Four farms were managed under conventional conditions. Two alternative farms with outdoor access were included with a long history of keeping beak-intact animals. Under conventional conditions, a production period with beak-trimmed hens was carried out first before introducing beak-intact flocks. Results showed that enrichments were intensively used by the turkeys, but did not prevent injurious pecking completely. In conventional beak-intact flocks, severe pecking injuries occurred more frequently, and mortality was reported twice as often due to pecking events compared to beak-trimmed flocks. The 1st week and the period of the 11th to 13th week of life as well as the finishing phase were particularly risky.

Results concerning mortality, prevalence and severity of pecking injuries in beak-intact conventionally housed turkey hens under the described optimisation programme showed the complex problems of keeping turkeys with intact beaks.

This work (Model- and Demonstration Project for animal welfare: #Pute@Praxis) is financially supported by the Federal Ministry of Food and Agriculture (BMEL) based on a decision of the Parliament of the Federal Republic of Germany, granted by the Federal Office for Agriculture and Food (BLE); grant number «FKZ 2817MDT611»).

Keywords: Poultry, turkey hen, beak trimming, beak, animal welfare



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P-05-06

Incubation conditions affect footpad dermatitis incidence and severity

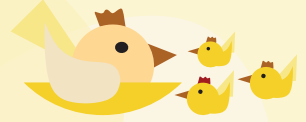
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Incubation temperature profiles (INC) affect footpad skin development in broiler and egg layer strains with long-lasting effects post-hatch. This project evaluated the effects of INC on the incidence of footpad dermatitis (FPD), a critical welfare indicator. Three factorial experiments were conducted evaluating two INC to either maintain eggshell temperature (EST) constant at $37.8 \pm 0.1^\circ\text{C}$ (Standard) with egg prewarming or have EST similar to the ones observed in multistage machines. This second profile (LowHigh), without egg prewarming, had a machine temperature constant of 37.5°C . This condition resulted in low EST ($37.4 \pm 0.2^\circ\text{C}$) for the first two days and elevated EST ($>37.8^\circ\text{C}$) after 16 days, especially during the last three days when eggs reached up to $39.2 \pm 0.2^\circ\text{C}$. In the first experiment, three Cobb genetic lines were evaluated (Cobb 500, low and high FPD incidence) in pens with fresh wood shavings. In the second experiment, Cobb 500 chicks were placed in pens with fresh wood shavings or used litter. The third experiment assessed the FPD incidence and severity in Ross x Ross, Cobb 500 x Cobb MX, Cobb 500 x Cobb Hubbard, and Athens Random Breed Cross, ARBC (Control) placed in pens with used litter. At hatch, chicks were sexed and placed in 15 replicate floor pens per treatment combination, with 14 chicks per pen. The FPD incidence and severity were evaluated in the second and fourth-week post-hatch. In all three experiments, the susceptibility to higher FPD was augmented ($P < 0.05$) more in chickens from the LowHigh INC than in those from Standard INC. The lowest FPD incidence was observed ($P < 0.05$) in chickens from Standard INC and all those placed in the new litter. The INC with elevated EST in the hatchers had higher ($P < 0.05$) litter moisture. In conclusion, INC affects broiler FPD susceptibility, and avoiding overheating in hatchers could aid in mitigating this welfare issue.

Keywords: Footpad dermatitis, incubation temperature, development, litter quality, broilers



P-05-07

Plumage cleanliness and footpad dermatitis of slow- and fast-growing broilers as affected by diets formulation

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The aim of this study was to evaluate whether dried black soldier fly (BSF) larvae combined with local ingredients affected the welfare of slow- and fast-growing broilers. Day-old chicks from a commercial broiler (FAST) and a local (SLOW) pure line (252 each) were fed 3 diets; in the corn-soybean-based diet used as Control, soybean was partly replaced with sunflower meal, brewer's dry grain, and wheat middlings (Alternative 1), and BSF (5%) was added to Alternative1 (Alternative2). Dietary soybean (as an average of starter, grower, and finisher) reduced by 6.6% in Alternative1 and 11.4% in Alternative2 compare to the Control. Birds were reared in 36-floor pens (6 pens/diet) bedded with wood shavings (14 chicks/pen, 25 kg/m²). On d 40, plumage cleanliness (PC) and footpad dermatitis (FPD) were scored on all birds. A four-scale scoring for PC (Welfare Quality©) and a three-scale for FPD were used. There were a few birds with score-2; therefore, pooled data as one-zero scoring was used for PC. Plumage cleanliness and FPD responses were analysed using Chi-square test of independence as line and diet effects were explanatory. Both line and diet effects were significant on PC. The percentage of clean birds was higher in SLOW than FAST ($P<0.0001$) whereas lower in Alternative1 ($P=0.002$). The percentage of clean birds varied with the line within Alternative1 and Control diets being higher in the SLOW ($P<0.0001$). However, in broilers fed Alternative2, PC was not different across lines suggesting an improved cleanliness in FAST. Moderate FPD was observed more often in SLOW line ($P=0.001$). Although FPD responses were independent of diets, the severity of lesions in broilers fed with Alternative2 was higher in the FAST than in the SLOW ($P=0.019$). Because intestinal health affects PC and FPD, further studies are ongoing to understand diets-microbiota relations.

Keywords: Fast-growing broiler, Slow-growing broiler, black soldier fly larvae, plumage cleanliness, footpad dermatitis



P-05-08

Relationship between feather damage measurement on-farms and at slaughterhouse in respect to feather pecking behavior in laying hens

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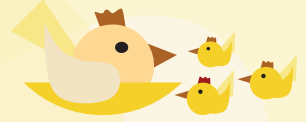
This study aimed to assess loss of feathers due to feather pecking behaviour in hens during the laying period and in the slaughterhouse. One hundred laying hens at 50, 72 and 78 weeks of age were randomly selected and subjected to the evaluation of plumage condition in six body-zones. These welfare indicators were assessed during the laying period (at 50 and 72 weeks of age) and at the slaughterhouse (at 78 weeks of age) to establish a comprehensive and systematic welfare evaluation of feathers lost by pecking behavior.

A 4-point feather score scale (from 0=no damage to 3=very damaged) was applied to six body regions: head, upper neck, back, rump, wings and tail (total feather score ranging from 0 to 18 points). At the slaughterhouse, the recordings were performed while birds were hanging on hooks for observation of feathers on the back.

During the first scoring at 50 weeks of age, the neck and tail were in worse condition than feathers in other regions. At 72 weeks, the worst feather score was observed on the tail and head; and at slaughter the wings, head and tail scored the worst. Feathers damage on the back increased with age to vary significantly ($P < 0.001$) at 50 (0.26 ± 0.56), 72 (0.68 ± 0.62) and 78 weeks (0.93 ± 0.83). Feather score on head (0.6 ± 0.69 vs. 1.26 ± 0.90 ; $P = 0.001$), rump (0.60 ± 0.73 vs. 1.10 ± 0.86 ; $P = 0.002$) and tail (0.96 ± 0.67 vs. 1.36 ± 0.66 ; $P = 0.011$) increased from 50 to 72 weeks of age, indicating their correlation with feather pecking behavior.

Feather pecking behavior remains an important welfare issue in laying hens production and can be assessed at the slaughterhouse by feather damage on the head, upper neck, back, rump, wings and tail by applying simple scoring scales such as those used in this experiment.

Keywords: laying hen, feather damage, feather pecking behavior, welfare assessment



P-05-09

Effects of a new ventilation concept on barn air quality and animal health of turkeys (*Meleagris gallopavo*)

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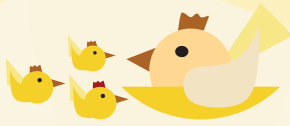
In this study, the effects of a new ventilation concept on barn air quality and health of turkeys were tested. The hypothesis was that the ventilation system leads to decreased litter humidity in winter periods and decreased air NH₃, thereby resulting in improved footpad and respiratory health.

The study was performed on three conventional turkey farms, each with two barns with 3,600 male B.U.T 6 turkeys, for six fattening periods. Two farms were equipped with Plasson bell drinkers. One farm had Plasson drinkers during the first three fattening periods and Lubing drinkers during the last three periods. In the trial barns (T), the ventilation system was modified so that fresh air could enter the barn through chimneys in the roof. By that, the air was warmed when entering the animal area. From week 5 until week 17, 50 animals per barn were examined bi-weekly for signs of pecking and footpad health via a 5-step-score. NH₃ and CO₂ were recorded continuously on one farm via a Wile 500. Use of antibiotics was documented.

Litter humidity was decreased in T with Plasson drinkers (52 vs. 56%; $p=0.04$). For Lubing drinkers there was no difference assessed (57 vs. 56%; $p>0.05$). Footpad health was increased during winter periods in barns with Plasson drinkers (0.99 vs. 1.56; $p=0.02$). Concentrations of NH₃ and CO₂ were higher in T, while still being below legal limits. No difference in signs of pecking between barns, and no difference in the occurrence of respiratory diseases and amounts of used antibiotics were found.

These results indicated that the modified ventilation system can increase footpad health of turkeys in barns with Plasson drinkers during winter. However, during this study, the trial and control barns were not changed. Therefore, above results might be related to barn effects, which could not be excluded.

Keywords: turkey, air quality, footpad health, respiratory health



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P-05-10

Are the results of different methods of beak measurement comparable?

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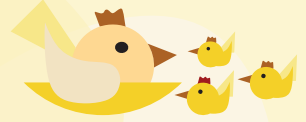
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Beak lengths measurements in poultry can be used to study the effects of beak trimming or to evaluate different methods of beak treatment. In previous research, various methods were used and different measurements on the beaks were performed. Thus, the present study compared three methods for measuring beak values using a flexible measuring tape, a digital calliper, and image analysis via ImageJ software (Version 1.51.0, National Institutes of Health, Bethesda, MD, USA). Measurements were performed on 100 severed heads of randomly selected 78-week-old slaughtered laying hens. All results were processed with Microsoft Excel (Microsoft Corporation, Redmond, WA, USA) and statistically analysed with SAS version 9.4 (SAS Institute Inc., Cary, NC, USA). Different lengths of the beaks, the height and the width were recorded and the agreement between methods was calculated using Krippendorff's alpha. For further assessment of the comparability between the measurements, a Bland-Altman plot was created and interpreted for a selection of comparable measurement values.

Comparing different methods of beak measurement of laying hens, the overall lowest difference was 0.1 mm on average, which was obtained for the length from the rostral angle of the nares to the tip of the upper beak recorded with a flexible measuring tape and image analysis. The best agreement (Krippendorff's $\alpha = 0.67$) was found between the digital calliper and image analysis, when calculating the reliability of the three different beak-measurement methods.

However, since there were rather low agreement between the three analysed methods overall, it is generally not advisable to use two different methods in one study. In addition, repetitions of the same measurement should be carried out, and recording processes should be described in detail to make the results comparable.

Keywords: beak length, beak dimensions, measurement method, measurement reliability



P-05-11

I'll beak back: mimicking and assessing pecking damage in commercial laying hens using a robotic chicken

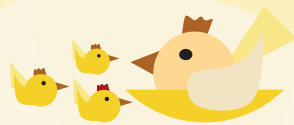
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Injurious pecking outbreaks are a welfare concern in poultry, often resulting in cannibalism and mortality. Pressure to ban beak treatment (method of reducing damage commonly performed at day-old using infra-red technology) has led to research on traits that could be selected for in breeding flocks, such as feather cover, liveability, and beak shape. It is hypothesised that blunt beaks are less successful at tearing body tissue, thereby reducing the risk of cannibalism and mortality. This suggests a relationship between beak shape and the ability to cause damage; however, the physical damage that different beak shapes cause is not fully understood. The objective of this pilot study was to quantify this damage using a robotic device. Heads from 952 laying hens were collected, and their top beak shapes were analysed using geometric morphometrics and principal components analysis. Birds with beak shapes that fell within the top (max) and bottom (min) 10 percent of the first two principal component (PC1 and PC2) distributions were selected (n=35 birds/group (PC1max, PC1min, PC2max, PC2min)). A robotic device, designed to imitate a hen's natural pecking motion, was attached to hens' heads and beaks 'pecked' into agarose gel (mimicking muscle). Three pecking forces were tested (10, 15, 20 N) with 10 pecks per force. Depth, length, width, and volume were measured from the gel indentations to quantify damage. Data were analysed as a two-way ANOVA using R. No differences in damage were found between the beak shape groups. Indentation volume at 20N was greater than at 10N or 15N. The results demonstrate that the beak shapes tested were too similar to discern differences in damage. This suggests that other beak shape characteristics beyond curvature (sharpness) contribute to tissue damage. Research examining more beak shape characteristics, such as grasping ability, and their capacity to cause damage is needed.

Keywords: injurious pecking, severe feather pecking, morphometrics, welfare



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P-05-12

Manure odour as indicator of hen health and welfare: a study on vaccination challenges in rearing hens

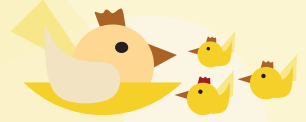
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Traditionally, laying hen farmers monitor hen health and welfare based on flock productive output, feed and water intake, climate factors, and behavioural observations. The growing number of hens per farm and the decreased availability of personnel with sufficient knowledge on poultry makes it increasingly difficult to safeguard and control bird health and welfare. This project aims to improve laying hen welfare by early stress-detection based on sensor-aided continuous assessment of predictive health and welfare indicators. During a qualitative multi-stakeholder survey, we identified that currently less than 50% of laying hen farmers, poultry veterinarians and other experts use odour in health and welfare assessment, and mostly to detect suboptimal intestinal health. To proof the relationship between hen health and manure odour, we studied the effect of a controlled stressor on manure odour in three commercial flocks of rearing hens. The stressors were salmonella vaccination during the first week of age and at the end of the flock round, and an injection vaccination at twelve weeks of age. In short, fresh manure samples were taken at eight locations on the manure belt on two sampling days before vaccination and on four sampling days after vaccination. Volatile organic compounds (VOC) were extracted from the manure samples by using nitrogen as carrier gas. VOC were captured in sorbent tubes that contained active carbon. The sorbent tubes were connected to a Thermal Desorption Gas Chromatograph Mass Spectrometer to identify and semi-quantify the VOC. We compared VOC profiles between hen manure that was produced before and after every vaccination for all three flocks. Preliminary results will be available at time of the Symposium. Ultimately, the results of this study could aid the development of odour sensors that continuously detect changes in hen manure odour as indication of suboptimal health and welfare.

Keywords: Rearing hens, Odour, Health, Welfare, Monitoring



Session 6 – Welfare of day-old chicks, including early nutrition and on-farm hatching

P-06-01

Welfare impacts of delayed nutrition at hatch: an overview

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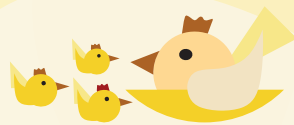
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Chicks are generally given first access to feed and water at placement in the farm. They are often between 9 to 72 hours old at this moment. At first sight, delayed feeding at hatch seems to violate some welfare criteria known in animal husbandry, such as 'Freedom of hunger and thirst'. Besides, delayed feeding early in life may permanently shape a chickens' phenotype and consequently impair welfare during later life as well. However, neonatal chicks do possess and take up residual yolk, which suggests that welfare may not be affected by delayed feeding.

A literature review was conducted and data was included from two recent PhD experiments at Wageningen University. Welfare was defined by the 5 well-known freedom criteria; 1) hunger and thirst, 2) discomfort and exposure, 3) pain, injury, and disease, 4) fear and distress, and 5) express normal behavior. For each of these criteria it was summarized which indicators were found, if long and short term effects on welfare were found, and what the knowledge gaps are.

Results provide an overview of the current knowledge of each of the 5 welfare criteria. Examples; 'freedom of pain, injury, and disease' has been studied by observing mortality and disease resilience. Delayed feeding does not seem to impair this welfare criteria in the short term, expressed by similar 1st week mortality rates, but at later ages the risk on infectious diseases increases. 'Freedom of fear and distress' may be violated in the short, expressed by alterations in corticosterone levels and behavioral, but not the long term. Findings from this literature review will be integrated and an overall conclusion on the effects of delayed feeding at hatch on poultry welfare is provided.

Keywords: Early feed, Delayed feed, Welfare, Poultry, Chick



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Session 7 – Fear and stress in poultry – identifying causes, preventive and mitigative strategies

P-07-01

Predicting stress sensitivity of laying hens by identifying genetic, incubation and rearing factors

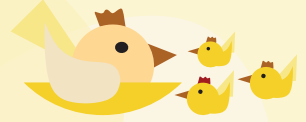
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Genetic differences exist in performance and adaptive capacity of chickens, for instance between brown and white strains. Effects of genetic, animal related and environmental factors on technical performance, health, and welfare of laying hens were determined during incubation, rearing, and laying. Hatchability was affected by strain, breeder age, egg weight uniformity, length of egg storage and season, but egg weight loss did not have a significant effect on hatchability. Predicted hatchability of brown strains was higher than that of the white strains (on average $\Delta = 2.02\%$). During the (maternal) laying phase, clutch size (CS) was included as a factor determining rearing success, while in the rearing phase of the offspring, three rearing traits (first week mortality (FWM), rearing abnormalities (RA), natural death (ND)) were included. RS was defined as the percentage of animals that survived to the laying barn relative to the number of chicks that hatched from a batch. Genetic parameters for each trait were estimated, using a Linear Mixed Model. Additionally, a Genome Wide Association Study (GWAS) was done to scan the genomes of the breeders to reveal Single Nucleotide Polymorphisms (SNPs) associated with these traits. GWAS revealed 12 different SNPs having a significant effect on RS. To further confirm the existence of these SNPs, Bayesian network analysis (BN) was used to analyze these traits and the SNPs. The results of the BN disclosed 28 SNPs associated to the traits, ten distinct SNPs each were associated with CS and RA, a single SNP with ND and seven different SNPs with FWM. It can be concluded that animal related and environmental factors are important in predicting hatchability in laying hens while CS, FWM, RA and ND are all relevant traits in investigating RS.

Keywords: chickens, hatchability, rearing success, SNPs, GWAS



P-07-02

Individual variation in movement and its association with fearfulness and exploration in laying hens

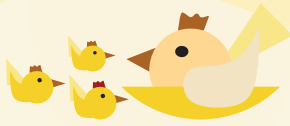
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Laying hens show high individual variation in the various areas of an aviary system including wintergarden usage. The cause of those individual differences and implications for hen welfare remain unclear. The aim of this study was to investigate the relationship between hen movement within a semi-commercial aviary and their exploration and fearfulness by means of a novel environment test. Six pens (225 hens/pen; Dekalb White) were used where 18 focal hens were selected per pen and equipped with a backpack containing a tracking device recording all inter-zone transitions for 3 weeks between 52–55 weeks of age. Based on the recorded daily duration in the wintergarden, 36 birds with a relatively high and low wintergarden use (N=72) were chosen for the novel environment test. Each focal bird was placed into the start box of the novel arena, a guillotine door was opened, and the bird's behaviour recorded by video observations for a total of 10 min. Based on a principal component analysis latency to leave the start box (PC1, loading = 0.59) and the proportion of time spent in the middle of the novel arena (PC2, loading = 0.76) were selected as proxies for fearfulness and exploration for further analysis. The latency to leave the start box was lower in birds with a high wintergarden use (median latency = 34 sec) compared to birds with a low wintergarden use (median latency = 2.6 min; Mann-Whitney-U Test, $p = 0.049$). The proportion of time in the middle did not differ significantly between high (mean = 14.95%) and low (mean = 20.33%) wintergarden users (t-test, $p = 0.067$). Those results imply that high wintergarden users may be less fearful, and the ongoing analysis will give further insight into the association of fearfulness and the use of resource-related aviary zones.

Keywords: novel environment test, wintergarden use, laying hens



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P-07-03

Spatial Mathematical Modeling of Heat Stress in Two Different Broiler Houses

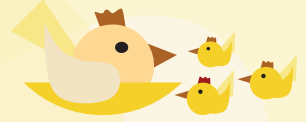
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Intense heat can have several consequences on broilers, such as discomfort, feed intake and body weight gain decrease and high mortality rate. Moreover, global warming tends to increase heat waves frequencies and intensities, which will aggravate the phenomenon. A good management of environment in broilers house is crucial to tackle this issue. An indicator combining dry bulb temperature, relative humidity and air velocity, known as Temperature Humidity Velocity Index (THVI) can be used to evaluate heat stress and the subsequent degradation of welfare over time. However, gradient of temperature, humidity and velocity can be important in broiler house and difficult to measure. Computational fluid dynamics (CFD) simulations are an efficient tool to apprehend these gradients (three dimensional distribution and magnitudes). This study proposes a demonstration of CFD modeling for assessing heat stress levels at the broiler house scale. As a first step, pre-simulations have been conducted in order to estimate the parameters to model the animal occupied zone as a porous media in function of body weight and animal density. In a second step, two representative configurations of typical French broiler houses have been designed. These configurations differ from their dimensions and ventilation modes. In each of these configurations, house scale CFD simulations have been conducted in order to estimate the THVI cartographies at animal level. Finally, from the THVI distributions, cartographies of heat stress in term of body temperature rise over time of exposure have been produced. This type of modeling can be seen as a tool able to objectify animal welfare in hot climate conditions and to investigate the animal cooling potential of additional equipments (fans, pad-cooling, ...).

Keywords: broiler house management, Mathematical modeling, Heat stress



P-07-04

Effects of early-life experiences on micro-circuitry in avian forebrain areas involved in stress regulation

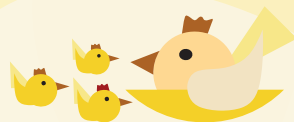
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Neuronal oscillations are correlated to a variety of cognitive functions in mammals. This neuronal activity is disrupted in neuropsychiatric conditions in which stress is a critical environmental factor. Commercial hatching of chickens takes place in a stressful environment and has a negative effect on traits important for the industry. Whilst the impact of stress on mammalian neuronal oscillations has been examined, little is known regarding the effect on avian brain neuronal dynamics. Our lab has demonstrated the ability to record cholinergic evoked gamma oscillations (20–80 Hz) in acute hippocampal brain slices *in vitro*. Using this approach, we examined the effect of early-life stress on neuronal oscillations in the chicken hippocampus. The effect of dark and green light incubation in white and brown eggs was studied as stress factors. We observed no effect of incubation conditions and genetic background on baseline gamma oscillations. Furthermore, the incubation conditions had no impact in several behavioural tests. In addition, our results showed that noradrenergic modulation has opposing effects on gamma oscillations in caudal versus rostral portions of avian hippocampus. Finally, we observed a difference in cholinergic subtype receptor sensitivity of avian hippocampal gamma oscillation compared to the mammalian equivalent.

Keywords: Neuroscience, Chicken, Stress



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P-07-05

Influence of adaptogenes on heat stress effects elimination in broiler chickens

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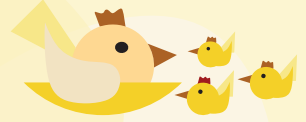
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Modern broiler genotypes are very sensitive to a variety of stressors. Globally, elimination of heat stress effects is now one of the megatrends in poultry research. This research provides the first extensive results of natural substances on mitigating the negative effects of heat stress in broiler chickens. The animals were divided into two groups, each of 171 broiler chickens ROSS 308. Both groups were fed by standard diet according to manual for ROSS 308. The feed of the experimental group was supplemented by Fortibac X Energy. The main basis of this feed additive is a combination of fatty acids esters, plant saponins and choline. Second group was the control. The experiment took place for 35 days. Animals were put under thermal stress after 14th day of the experiment (continuously 10 hours per day, 33 ± 1 °C). The experimental group showed better fattening parameters compared to the control group: weight gain (increased by 2.8 %), feed intake (increased by 0.7 %), final weight (increased by 2.3 %), and fattening efficiency index (increased by 3.1 %). The feed conversion of the experimental group decreased by 2.0 % compared to the control group. In addition, the antioxidant status of experimental group showed increased activity of glutathion peroxidase, catalase, (increased by 14.5 %, 6.8 %, respectively) compared to the control group. Levels of reduced glutathione were increased (increased by 53.3 %), and levels oxidized glutathione were decreased (decreased by 18.4 %) in the experimental group compared to the control group. From the above results, it follows that feed supplements based on phytogenic substances and esters of fatty acids can reduce the effects of heat stress from the point of view of performance and at the same time improve the antioxidant parameters of the organism.

Keywords: phytoadditives, heat stress, performance, antioxidant indicators



P-07-06

Fasting, but not early life stress, increases hypothalamic neuropeptide Y mRNA expression in Japanese quail (*Coturnix japonica*) chicks

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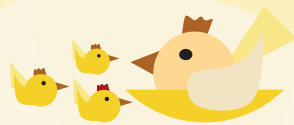
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The prenatal period can shape adult phenotype. Appetite is governed by the melanocortin system and regulated by neuroendocrine mechanisms that are vulnerable to stress. By investigating the neurobiological mechanisms of early life stress programming on hypothalamic feeding circuits we aimed to determine appetite development in birds and the impact of pre-natal stress. We investigated how changes in nutritional status, food availability, and early life stress programming effect the hypothalamic appetite pathways in Japanese quail (*Coturnix japonica*). Nine-day old female and male chicks (19L:5D), were randomly assigned to FED (control, ad libitum feeding) or FASTED (food removed at lights off and 4h fast from lights on) and FED CORT (ad libitum feeding, in ovo corticosterone injection at incubation day 5) groups. FASTED chicks had lower body and gut weights and lower blood glucose compared to FED control or FED CORT; no sex differences were observed. Behavioural analysis of video recordings showed that FED, FED CORT and FASTED chicks rested and rarely fed or drank during darkness. Following lights on, all chicks exhibited food-seeking behaviour (increased visits to feeding station during the first 2h of lights on), with more food seeking behaviour in FASTED chicks compared to FED and FED CORT chicks. RT-qPCR showed increased hypothalamic neuropeptide-Y (NPY) mRNA expression in FASTED compared to FED and FED CORT chicks. There were no differences in agouti-related peptide (AgRP), or proopiomelanocortin (POMC) and no differences in NPY, AgRP and POMC mRNA between FED control and FED CORT groups. These findings suggest that there are some functional differences in the neuropeptide mechanisms regulating food intake in young birds compared to adults and that early life stress does not alter feeding related neuropeptides in young quail.

All work was performed under UK Home Office licence, ARRIVE guidelines and ethical review. Research supported by BBSRC (BB/5015760/1).

Keywords: Feeding, Stress, Appetite, Hunger, Hypothalamus



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P-07-07

Development of corticotropin-releasing factor brain systems and relationship to variations in stress response with age in chicken

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Understanding the neural mechanisms that regulate the stress response is critical to know how animals adapt to a changing world and is one of the key factors to be considered for improving animal welfare. Corticotropin releasing factor (CRF) is crucial in regulating physiological and endocrine responses, triggering the activation of the sympathetic nervous system and the hypothalamo – pituitary – adrenal axis (HPA) during stress. In mammals, telencephalic areas, such as the amygdala and the hippocampus, regulate the autonomic system and the HPA. These centers include subpopulations of CRF containing neurons that, by way of CRF receptors, play modulatory roles in the emotional and cognitive aspects of stress. CRF binding protein also plays a role, buffering extracellular CRF and regulating its availability. CRF role in activation of the HPA is evolutionary conserved in vertebrates, highlighting the relevance of this system to help animals cope with adversity. However, knowledge on CRF systems in the avian telencephalon is very limited, and no information exists on expression of CRF receptors and binding protein. Knowing that the stress response changes with age, with important variations during the first week posthatching, the aim of this study was to analyze mRNA expression of CRF, CRF receptors 1 and 2, and CRF binding protein in chicken telencephalon throughout embryonic and early posthatching development, using in situ hybridization. Our results demonstrate an early development of CRF and its receptors in pallial areas regulating sensory processing, sensorimotor integration and cognition, and a late development in subpallial areas regulating the stress response. However, CRF buffering system develops earlier in the subpallium than in the pallium. These results help to understand the mechanisms underlying the negative effects of noise and visual stress during prehatching stages in chicken, and suggest that stress regulation becomes more sophisticated with age.

Supported by H2020-MSCA-ITN-2018-812777



P-07-08

Heavy broilers' surface temperature and behavioral responses to air velocity under heat stress

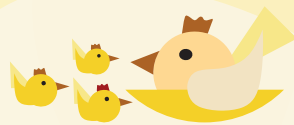
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Heavy broilers exposed to hot summer conditions or heat waves experience fluctuations in surface temperature due to heat stress, which leads to decreased performance and welfare. In addition, broiler chickens exposed to heat stress conditions adapt to their environment by various behavioral changes attempting to regulate body temperature, which is one key welfare parameter. This study aimed to evaluate the effect of two air velocity (AV) treatments on heavy broilers' surface temperature dynamics and behavioral patterns from 42 to 61 days of age. Five experiments were conducted in environmental chambers during the summers of 2017, 2018, and 2019 as a part of a comprehensive study on the effectiveness of wind chill applications in mitigating heat stress on heavy broilers. Two dynamic AV treatments (high and low) were applied in three chambers per treatment and 44 broilers per chamber for a final density of < 40 kg/m². The surface temperature of birds was recorded periodically from 35 to 61 days of age with infrared thermography in the morning, noon, evening, and night. Video cameras inside each chamber recorded different bird activities like feeding, drinking, and resting. The number of chickens posing for each activity was counted. The correlation between birds' surface temperature and air temperature was investigated. Results indicated that the overall mean surface temperature was $35.89 \pm 2.37^{\circ}\text{C}$ and the birds exposed to high AV had lower surface temperatures ($P < 0.05$) than those under low AV. A regression model ($R^2 = 0.51$) was built to predict heavy broiler surface temperatures from the bird's age, temperature-humidity index, and AV. Besides decreasing the broiler surface temperatures, the high AV increased ($P < 0.01$) the number of birds eating, standing, and walking compared to those under low AV. It was concluded that high AV helped heavy broilers release body metabolic heat by adjusting behavior under heat stress.

Keywords: Heat stress, heavy broilers, air velocity, surface temperature, behavior



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P-07-09

Thermal acclimation and lighting during embryogenesis: Behavioral responses of broilers to post hatch heat stress

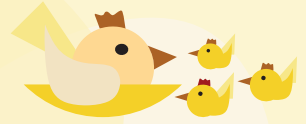
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Thermal acclimation and light exposure during embryogenesis may have significant impact on behavioral responses of broilers to heat challenge. Two consecutive studies were conducted to investigate if thermal acclimation (TA) (38.8°C for 6 h/d between embryonic days 11–16) in darkness or combined with 16L:8D photo-schedule (TA+Light) affected behavioral responses of broilers to heat stress (HS). In the first trial, broilers from TA or control (37.8°C) incubation were used. In the second, broilers were from either lighted (L) or TA+Light incubation. There were 128 broilers (16 pens of 8 birds) in each trial. The half of the broilers were exposed to HS from 32 to 36 d ($30 \pm 1^\circ\text{C}$ during 11:00–15:00 h) while remaining were kept at 22 °C. Panting and wing elevation were observed at pen levels by scans of 10 minutes interval on the first and the last (4th) days of the experiments. Threshold temperature for panting were recorded. In both trials, HS significantly increased panting and wing elevation irrespective of the incubation treatments ($P \leq 0.05$). TA reduced percentage of broilers expressed panting at the 1st but not at the 4th d suggesting an adaptive change in thermoregulatory behavioral responses to chronic HS. Wing elevation did not differ with incubation treatment at 1st d while TA broilers had lower percentages at 4th d. In the 2nd trial, TA+Light significantly reduced panting compared with L at 1st d of the heat challenge ($P \leq 0.05$). However, the effect disappeared at 4th d. The wing elevation behavior was not different between the incubation groups at both ages. Either TA in darkness ($30.38 \pm 0.15^\circ\text{C}$) or TA+Light ($29.94 \pm 0.19^\circ\text{C}$) increased the threshold for first panting as compared to control in darkness ($29.80 \pm 0.15^\circ\text{C}$) and L incubation ($29.36 \pm 0.19^\circ\text{C}$), respectively ($P \leq 0.05$). Our preliminary results suggest that both TA and photoperiod (16L:8D) during embryogenesis may alleviate thermoregulatory behavioral responses of broilers to HS.

Keywords: broiler, heat stress, thermoregulatory behavior, lighted incubation, thermal acclimation



P-07-10

Hippocampal immediate–early gene induction and HPA-axis activation in response to acute stress in chickens

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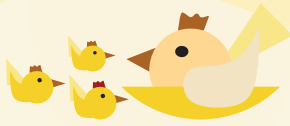
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Changes in hippocampal anatomy (e.g. the number of doublecortin-containing neurons) have recently been used as markers of chronic stress in poultry. However, to know what the functional consequences are of these changes for further stress responses, we have to better understand the role of the hippocampus in the regulation of the stress response.

This study aims to extend the understanding of the avian Hypothalamo-Pituitary-Adrenal (HPA) axis functioning and to shed light on the role of the hippocampus in the regulation of stress in birds. Using in-vitro tract-tracing techniques, we therefore evaluated which part of the hippocampus is connected directly or indirectly to the HPA axis. In addition, we exposed 10-day-old broiler chicks to social isolation for 20 minutes. The detected increases in plasma corticosterone concentration validated our treatment as an acute stressor. We then used immediate early gene expression profiles to determine which subdivision of the hippocampus is activated during and after the acute stress induction. Our study provides a better understanding of the neuroendocrine stress response in birds and aims to provide valuable information about the subregional organization and functional specialization of the avian Hippocampal Formation.

This project receives funding from the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 812777

Keywords: hippocampus, stress, chicken, HPA, anatomy



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P-07-11

Research gaps in poultry welfare research

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Despite substantial progress over the last decades in the understanding of poultry welfare, critical research gaps still abound. In this study we review key areas where evidence on the welfare of broiler chickens and egg-laying hens is missing. Research gaps fall into different categories. First are those related to time. Although affective experiences unfold over time, little attention has been paid to understanding the time course of important challenges affecting poultry (e.g., gait-impairing conditions, cardiorespiratory abnormalities) or the extent to which their onset is delayed with improved genetics or management. This knowledge is critical to understand how long animals spend in negative affective states of various natures. A second class of gaps relates to evidence on the degree of aversiveness associated with different harms, as perceived by animals. Studies involving ‘demand elasticity’, ‘willingness to pay’ and other preference tests are needed in multiple areas. Third, major data gaps are present on the prevalence and frequency of welfare challenges in commercial conditions. Where commercial data is available, it is overwhelmingly from countries where welfare legislation is stricter and/or from farms agreeing to take part in research studies, suggesting an important bias (‘healthy farm’ effect) towards better welfare in published research. Likewise, the generalizability of conditions in experimental research (e.g., better stockmanship, management) to representative commercial scenarios is often not clear, suggesting a similar bias. Finally, research is needed to determine the extent to which the salience of pain can be modulated by factors such as fear (fear-induced analgesia is known in many species) or by engagement in highly motivated behaviors (endogenous analgesia may be also promoted in such cases). Evidence in this direction would have important implications for understanding the impacts of behavioral deprivation on welfare and the design of effective interventions in different areas of poultry welfare.

Keywords: research gaps, poultry welfare, endogenous analgesia, broilers, egg-laying hens



P-07-12

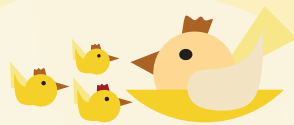
Evaluating thermal comfort of broilers fed dried olive paste

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Heat stress is a major welfare problem of poultry affecting birds' performance and health. This study investigated the dietary impact of dried olive pulp (OP) on broilers' thermal comfort. It was carried out in a commercial poultry farm during summer months, using 108 Ross-Cobb male broilers, 19 day-old. Chicks were randomly assigned into 3 groups CON, OP3 and OP6, according to the inclusion rate of OP in the ration (0%, 3%, and 6% respectively). Several indicators were measured to assess birds' thermal comfort. Broilers' body weight (BW) and body temperature (BT) was determined at 19, 26, 33 and 40 day of age. Body weight gain (BWG), feed consumption (FC) and feed conversion ratio (FCR) per bird, was calculated on weekly intervals and during the whole experiment (13–41 day of age). BT was evaluated with a digital rectal thermometer. Quality behavior characteristics (panting, lifting up wings, moving and sitting) were estimated at 26, 33 and 40 day of age. Temperature (T) and humidity (H) in the poultry house were recorded daily. From the values obtained TH Index (THI) was calculated at weekly intervals classified as follows: <27.8 = absence of heat stress, 27.8–28.9 = moderate heat stress, 29.0–30.0 = severe heat stress, > 30.0 = very severe heat stress. THI values were: 32.67 the 1st week, 28.43 the 2nd week and 26.39 at the 3^d week of the trial. At 26 d OP6 broilers had significantly ($p<0.05$) lower BT ($40.55\pm0.06^{\circ}\text{C}$) compared to CON ($40.78\pm0.09^{\circ}\text{C}$). At 40d a significant ($p<0.05$) lower % of birds presenting panting behavior was recorded in OP6 (61.11%) group compared to CON (86.11%). Growth performance of chicks did not differ significantly ($p>0.05$) among groups. OP feeding at the rate of 6% seems to be beneficial for broilers thermal comfort without compromising their growth performance.

Keywords: olive pulp, thermal comfort, welfare, broilers



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P-07-13

Functional and informative interaction between Avian Influenza factors discovered by the applications of Bayesian networks

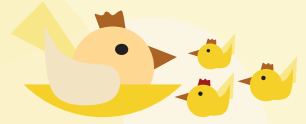
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Avian influenza (AI) is a zoonotic disease that represents a threat to the health of poultry species and humans. An alternative to traditional epidemiological methods is the application of Bayesian networks (BN), a useful graphical model that displays statistical relationships between a given set of variables. The aim of this study was to explore variables related to AI and get further insights into it by using BNs. Two publicly available datasets on AI were collected from zenodo.org (DOI: 10.5281/zenodo.6400451 and 10.5281/zenodo.6780350) and combined into a larger dataset considering those variables shared by the two datasets. Variables and their corresponding levels were selected based on their frequency to create a complete case dataset (no missing values). H5N1 was selected as the AI strain in addition to Type (Primary/Secondary), Country (e.g., Germany), Sampling program (e.g., Surveillance Active), Holding production category (e.g., Commercial), Source (e.g., Indirect wild bird), Species, Production category (e.g. Breeding), Increased mortality (No/Yes), Presence of clinical signs (No/Yes), Season (e.g., Autumn), and number of susceptible animals, coded as a binary variable. The structures of BNs were learnt using the open-source R package “bnlearn”, and after visualizing the searches, the top highest scoring network was selected as the consensus BN. The network connected all variables via 17 arcs. Some arcs confirm expected relationships, such as between clinical signs and mortality, while others such as those involving Type—whether the outbreak was due to close contacts with other birds or due to between farm contacts—might provide guidance for preventing future outbreaks from happening/spreading, e.g, its relationship with country and with presence of clinical signs. As a first approximation to AI and BNs, the findings are promising when it comes to understanding key variables related to the zoonotic disease. However, better ways of collecting and reporting data are needed.

Keywords: Avian influenza, poultry, Bayesian networks, epidemiology



Session 8 – Open session

P-08-01

A qualitative survey among the Flemish poultry sector on how birds fit for transport to the slaughterhouse are selected, caught, and loaded

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Broiler chickens and spent laying hens deemed fit-for-transport are caught, loaded, and transported to the slaughterhouse at the end of their productive period. Although this pre-slaughter stage is a high-risk phase for animal welfare issues and production losses, detailed up-to-date information about commercial practices is scant. Therefore, representatives of the Flemish poultry industry involved in this stage were surveyed to obtain an in-depth understanding of the current selection, catching, and loading practices, of the actions taken to prevent animal welfare issues or production losses, and their opinion about these practices.

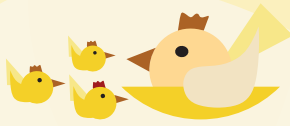
Flemish poultry farmers (broilers, n=5; laying hens, n=5), catchers (broilers, n=5; laying hens, n=5), transporters (n=5) and slaughterhouse representatives (n=5) were questioned during a face-to-face interview. The number surveyed was matched to saturation.

The most reported advantages of selecting poultry unfit-for-transport during the production cycle were less feed waste (5%), chicken uniformity (10%), feather pecking avoidance (10%), restraint of prolonged suffering and spread of disease by moribund animals (15%). The main disadvantages concerned more labor (extra costs) (15%) and inadequate knowledge (which animals) (10%).

For catching and loading, 100% of all the participants reported 'in the dark' as the best moment to catch and load in order to minimize stressing the birds. The majority (57%) were opposed to catching during the daytime, whereas 13% were in favor and 30% expressed no preference. The motivation for catching at night was the belief that the birds are calmer and less at risk of being exposed to heat stress during summer.

In conclusion, this survey provided in-depth knowledge of how broilers and spent hens fit-for-transport are selected, caught, loaded, and transported, what the main welfare risks are, and which actions are and could perhaps be taken by the actors to reduce these risks.

Keywords: unfit-for-transport, catching, loading, poultry, welfare



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P-08-02

Meat quality parameters for the assessment of animal welfare in the electrical water bath stunning of broilers

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As part of the CasStunn project (Implementation of a controlled atmosphere stunning as an alternative to an electrical bath stun for broilers), an investigation of broiler carcasses was conducted in a slaughterhouse. Meat quality parameters were collected from 16 flocks of each light fattening, heavy fattening and organically reared animals. 30 broilers of each flock (10 for organic broilers) were comparatively examined. The parameters of hemorrhages and petechiae were examined purely by visual inspection, and any hemorrhage ≥ 0.5 cm was considered positive (present). Fractures were examined both by visual inspection and palpation. Bones that showed a separation between both ends and/or crepitation were considered fractured (positive). For all parameters there was only the expression "present" or "absent", and additionally "left" or "right" for fractures.

In general, petechiae were most common on the muscle and fat base of the leg, and hemorrhages were most common on the wing, wing tips, fillets and tenderloins. Fractures were most commonly found on the bones of the anterior wing tip and the furcula, whereby fractures in the organically reared animal were overall less often found than in the conventional ones.

Hemorrhages and fractures may occur as a result of stunning, but are not necessarily due to it. In our project, however, it was not possible to examine the animals before stunning, therefore the results show a status quo survey of the entire production process. The problem of (stunning-related) carcass damage is present in all fattening methods we investigated. Since these differ in their frequencies, further investigations are necessary to determine the influence of body weight, fattening method and technical settings at the electrical water bath.

Keywords: Electrical Water Bath Stunning, Broiler Chickens, Animal Welfare, Meat Quality, Fractures and Hemorrhages



P-08-03

Assessing the impact of head removal following cervical dislocation on latency to insensibility and death

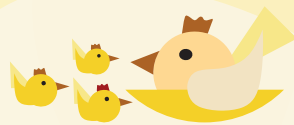
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The welfare impact of accidental head-removal following euthanasia by cervical dislocation is currently unknown. To investigate this, latency to insensibility and death was evaluated following head-removal during cervical dislocation. Broilers ($n=180$; 66 male and 114 female) were assigned either cervical dislocation with head-removal (HR) or a control treatment of only cervical dislocation (CD) over three trials. For the HR treatment, birds were decapitated within 10s post-CD to simulate head-removal. Broilers at 35, 44, or 50 days of age, with an average weight of 2.2, 2.8, and 3.7 kg, respectively were used. Responses recorded were latency to loss of nictitating membrane reflex, indicating latency to insensibility, and latency to total cessation of movement indicating latency to death. Data were analyzed via two-way ANOVA for main effects of treatment and sex, with age as block effect (RCBD, PROC Mixed SAS 9.4). Data were (Log+1) transformed for normality. Differences were considered significant at $P \leq 0.05$. No effect of treatment, sex, or interaction was found for either the nictitating membrane reflex ($P = 0.91, 0.21$, and 0.86 , respectively) or cessation of movement ($P = 0.65, 0.07$, and 0.10 respectively). Latency to loss of nictitating membrane reflex was 7 s regardless of the method used. Cessation of movement averaged at 150 s when HR was performed and 159 s for CD. Over three trials, latency to loss of nictitating membrane reflex and cessation of movement ranged from 0 s to 60 s and 81 s to 258 s in HR then 0 s to 75 s and 93 s to 600 s in CD, respectively. To conclude, head removal during cervical dislocation did not affect the latency to insensibility and death, implying that accidental head-removal following broiler euthanasia via cervical dislocation does not impact bird welfare.

Keywords: nictitating membrane reflex, cessation of movement, decapitation, welfare, euthanasia



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P-08-04

Optimise stunning condition for Taiwan native chicken

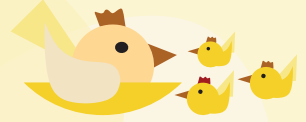
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Red-feather native chicken is one of the slow-growing chickens in Taiwan. It is deeply loved by the Taiwanese because of its meat quality and its variety in local cuisine. The consumption is second only to white broilers. The main and only humane poultry slaughter method is through water-bath stunning in Taiwan. Most research evaluated the humane electrical stunning conditions based on white broilers, scarce research uses slow-growing broilers. The body size, body composition and age of red-feather native chickens are different from white broilers. The stunning conditions which suitable for white broilers might not be applicable to red-feather native chickens to achieve good stunning efficiency. Therefore, the purpose of this study is to evaluate the stunning conditions for red-feather native chickens to achieve good welfare in slaughter. One hundred and twenty female red-feather native chickens are divided into three treatments with low, medium and high constant-voltage in direct current and stunned for 7 seconds in the water bath. Electroencephalography (EEG) and physical reflexes, such as corneal reflex, spontaneous eye blinking, wing flapping, epileptic activity, rhythmic breathing, and pain stimulus, were collected. The results showed that the EEG power was correlated with corneal reflex and spontaneous eye blinking. The number of chickens with inhibited total brain power was more in the high voltage group than the others. The recovery time is longer in the high voltage group. The lower the voltage, the less effective stunning achieved. In conclusion, corneal reflex and spontaneous eye blinking might be good indicators for effective stun onsite. Due to the difference between Taiwan native chickens and white broilers, the different electrical conditions should be applied to achieve higher welfare in slaughter. Further study is required to optimise electrical water-bath stunning conditions to achieve good stunning efficacy.

Keywords: water bath stunning, native chicken, humane slaughter, physical reflex, electroencephalogram



P-08-05

Which methods of loading broilers are used in Germany – and why?

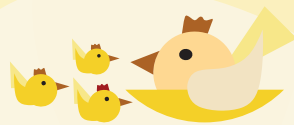
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German broiler farmers were asked in an online survey to name the used loading methods and their advantages and disadvantages. 10 farmers completed the questionnaire. Of the ten, two used a catching machine and eight used manual methods to load their broilers. Reasons named for using catching machines when loading broilers were, occupational health and safety reasons (100%), animal welfare reasons (100%), cost reasons (50%), time reasons (50%), and/or personal reasons (50%). Marketing reasons (e.g., less condemnation at the slaughterhouse) were not mentioned. It is apparent that above all hygiene and biosecurity of the farm were important points for their decision. Of the two farmers who decided in favor of the machine, they did so for reasons of hygiene and better independence from catching crews, or because they were dissatisfied with the hygiene standards of manual catchers. Farmers who performed manual loading mentioned different reasons for not using a loading machine. In particular, lack of contractors (62.5%), structural conditions (37.5%), financial profitability (25%), better animal control in manual catching (25%), lack of information (25%), and/or no interest in mechanical catching (12.5%) played a role. Farmers who have not used a machine so far would choose to do so if there were no more colleagues to assist with catching, if empirical values could be gathered that the use of a catching machine would guarantee animal welfare-friendly loading, if there was safety in functionality and if the financial outlay was worthwhile. Also, catching machines are necessary, which can be used e.g. in smaller or double-deck barns. In order to enable the use of catching machines and to give people the necessary safety, further research and the transfer of information, as well as international experience, are very important.

Keywords: loading, broilers, transport, methods



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P-08-06

Pre-slaughter handling related injuries in broilers

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This study aimed to assess the welfare of commercially reared broilers in slaughterhouses based on the presence of bruises in 5 body zones (left wing, right wing, left leg, right leg and breast), extensive injuries and fractures that occurred during the pre-slaughter period. The welfare measures were collected in 3 different slaughterhouses (A, B and C) in 70 batches of transport. Records were made regarding the presence of recent bruises/trauma/hematomas (wings, legs and breast) and fractures in samples of 100 broilers per batch and the corresponding percentage for the flock was calculated.

In general, bruises were observed on wings (1.918%), followed by legs (1.344%) and breasts (0.439%). Fractures were observed in an average of 0.098% of birds, at the wings and legs level and extensive injuries in 0.11% of slaughtered animals. The colour of the bruises was mostly red-purple, suggesting that this type of injury occurred around 12h before slaughter, and could be associated with inadequate catching, placing of many birds simultaneously in cages and rough handling of cages. Furthermore, the time elapsed between capture and slaughter was on average $11.5h \pm 3.2h$, according to the results presented.

Although bruises are an indicator used in the implementation of the Broiler Directive, in this study it was found that these types of lesions were mostly related to catching, crating and transport and do not provide information about on-farm welfare. Thus, it is crucial to analyse the colour of traumatic lesions to check when they occurred and to apply action plans and training to catchers and drivers' teams when the percentage obtained for these lesions was high.

Keywords: broiler, bruises, fractures, extensive traumatisms, handling



P-08-07

The role of the body composition of Red-feathered native chickens on electrical stunning efficiency and carcass quality

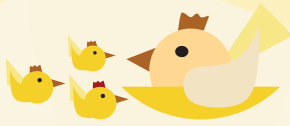
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Red-feathered native chicken is one of the slow-growing broilers in Taiwan and its consumption is second only to commercial broilers. The rearing period is approximately 12 to 13 weeks with a market weight of 2.3–3.0 kilograms. Compared to commercial broiler lines, it is heavier and with different body composition. Many studies have focused on the efficiency of electrical bath-stunning in commercial broiler lines to achieve good welfare, yet scarce research on the stunning efficiency in slow-growing chickens nor the effect of body composition in stunning efficiency. To improve the effectiveness of the stunning protocol for Taiwanese native chickens, this study aimed to investigate the physiological factors, such as weight and body fat content, influencing the efficiency of electrical stunning and the quality of Red-feathered native chicken carcasses. A total of 120 female Red-feathered native chickens were divided into three different voltage treatments with the same intensity and frequency. After stunning, the state of unconsciousness and carcass defects were evaluated. The weight of eviscerated carcass and the fat removed from the proventriculus surrounding the gizzard down to the cloaca were collected. The results showed that as the stunning electrical voltage increased, the carcass defects also increased. Under the same voltage condition, the chicken with higher fat content showed less recovery time after proper stunning. In conclusion, body composition, especially fat content, plays a role in affecting stunning efficiency. Further study is required to achieve high stunning efficiency and good welfare in slow-growing native chickens.

Keywords: Humane Slaughter, Native chicken, Fat content, Body weight, Carcass defects



P-08-08

Stunning effectiveness for the evaluation of animal welfare in electrical water bath stunning for broilers

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According to the Council Regulation (EC) No 1099/2009, animals must be treated at the slaughterhouse during care, stunning and slaughter in such a way that no more than unavoidable excitement, pain, suffering, or damage is caused to them.

The first objective of the “CasStunn” project was to verify the stunning effectiveness of the electrical water bath stunning of broilers prior to the implementation of a CO₂ stunning system, in order to assess the current state of animal welfare at the slaughterhouse.

For data collection, the stunning effectiveness of broilers from 15 flocks of conventional light fattening, 16 flocks of conventional heavy fattening, and 15 flocks of organically reared broilers was examined.

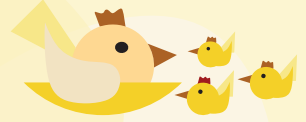
The study was conducted at a slaughterhouse in Germany where 4,800 broilers are slaughtered per hour. For each flock, a minimum of three rounds of assessment was performed.

Behavioral parameters such as wing-flapping, movements, and respiration were recorded after electrical stunning as “indications of miss-stunning”. A total of 15 broilers at a minimum per flock were examined for reflexes (pupillary and corneal) after debleeding. When animals were not immersed in the water bath or showed clear signs of consciousness, such as raising the head, these were scored as “miss-stunning”.

The organically reared broilers showed significantly more wing-flapping (0.43%) and respiration (2.26%) after bleeding than the heavy (0.11% and 1.80%) and light (0.12% and 1.04%) fattened ones. In addition, light-fattened broilers showed the least (1.7% for corneal reflex and 1.91% for pupillary reflex), whereas heavy-fattened (3.11% and 4.27%) and organically reared broilers (4.94% and 4.00%) showed higher values.

Overall, the results indicate that there may be an influence of the fattening method on the stunning effectiveness. Light-fattening broilers showed a significantly lower percentage of positive animals for both indications of miss-stunning and in the case of miss-stunning.

Keywords: Stunning effectiveness, Electrical water bath stunning, Broilers, Slaughter, Miss-stunning



Session 9 – Poultry behaviour and environmental enrichment

P-09-01

The impact of wing use and disuse on the musculoskeletal system and behaviour in laying hens

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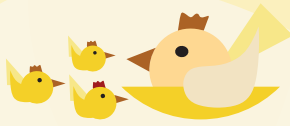
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Laying hens may live in high stocking densities, where there may be a lack of space to flap or stretch both wings at once, restricting muscle usage. Notably, reduced wing activity and accompanying muscle weakness are hypothesized risk factors for keel bone fractures and deviations.

To understand the impact of wing use and disuse on laying hens' ability to locomote and the musculoskeletal system, we used radio-frequency identification (RFID) to measure duration spent at elevated resources (feeders, nest-boxes), ultrasonography to measure muscle thickness (breast and lower leg) changes, radiography and palpation to determine fractures and deviations, respectively, following no, partial (one-sided wing sling) and full (cage) immobilization in white- and brown-feathered birds (n=60).

Following five weeks of partial immobilization, brown-feathered hens saw a 42% reduction in time spent in the elevated nest boxes (week 5 versus week 0; $p=0.035$). No change in feeder/nest box use was detected in white-feathered hens. Additionally, white-feathered hens showed a 17% reduction in pectoralis thickness (week 5 versus week 0; $p=0.0002$). Altogether, white and brown-feathered birds differed in their behavioural and physical adaptations to wing immobilization. Keel bone fractures and deviations were not affected by the wing immobilization treatments.

Keywords: flapping flight, keel bone, resource use, wing use, laying hens



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P-09-02

Investigation into the use of cognitive challenges in the chicken's environment

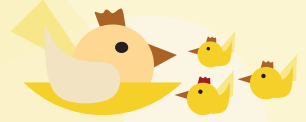
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With the increasing demand on adapting modern livestock farming to higher animal welfare standards, a comprehensive understanding of a species' cognitive abilities is essential in designing adequate housing systems and identifying the animals' adaptive capabilities. With the chicken being the world's most numerous bird in livestock farming, it is mandatory to identify possibilities and limitations of its (cognitive) abilities in order to meet its needs and create a high welfare environment. In this study, we investigated if chickens are able to use a cognitive enrichment tool by using the reflective properties of a mirror to find a hidden food reward. In total, 18 female chickens of two different breeds (7 dual-purpose hybrid Lohmann Dual, 11 purebred Japanese Bantam) were tested in a mirror-mediated spatial location task. Five out of seven Lohmann Dual hens and six out of eleven Japanese Bantam hens solved the task successfully and thus, probably exploited the correlation between the reflection in the mirror and the real food reward. We found no differences in performances on a breed level, but on the individual level, with the animals needing different amounts of time until they understood the representation of the rewards in the mirror image ($p < 0.05$). The results imply sophisticated cognitive abilities in chickens, assuming they can understand how mirror images represent objects in the real environment, and to make use of it during foraging. Tasks like this that challenge the abilities of these animals and give them control over their environment should be implemented in the design of appropriate housing systems to meet not only the physical but also the mental needs of the chicken.

Keywords: chicken cognition, mirror-use, welfare



P-09-03

Innovative evaluation method of behavioural reactivity for ducks reared for the production of 'foie gras' and its association to working conditions for farmers

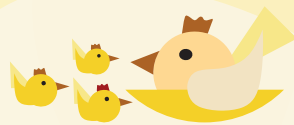
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The substantive improvement of the welfare of farm animals must necessarily be based on scientifically validated assessments. To date, the scientific literature still lacks a behavioural assessment methodology covering all animals at all rearing stages, especially to monitor critical handling phases, such as the overfeeding stage in 'foie gras' farms. The purpose of our study is to implement an objective measurement of behavioural responses in ducks during overfeeding. In 2021, trials were conducted in an experimental station at two separate sites and repeated four times. Behavioural and health data were recorded for 307 male mule ducks three times throughout the duration of overfeeding. Both behavioural and health data were collected 2h before overfeeding and during overfeeding for each day of observation. Ducks housed in collective cages were more excited and struggled more than ducks housed in pens altogether interpreted as a higher reaction to a stressful situation than ducks housed in pens ($p < 0.05$). We also found that ducks housed in collective cages showed more discomfort behaviours while ducks housed in pens expressed more natural behaviours ($p < 0.05$). The results obtained before and during overfeeding are complementary and consistent. In addition, the before overfeeding results reinforce even more those obtained with the creation of the new behavioural metric. Therefore, this innovative evaluation method conducted during overfeeding allows us to discriminate animal reactivity. Moreover, as ducks express these behaviours while being handled, it is relevant to also take them into account when considering the day-to-day work conditions of farmers. Our preliminary data are encouraging in terms of reproducibility and repeatability of measurements, and we have implemented them in current trials to evaluate the individual behaviour and rearing conditions of ducks throughout overfeeding. The measured reactivity tackles the welfare of both the animals and farmers, and is thus consistent with the 'one welfare' approach.

Keywords: Behaviour, Duck, Overfeeding, Welfare



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P-09-04

Comb size, complexity, and laterality of laying hens reared in environments varying in resource choice

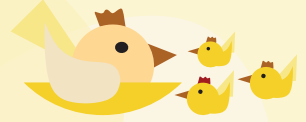
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The comb is an ornament involved in signalling condition and social status in domestic fowl. We hypothesised that comb size, complexity, and laterality are influenced by allostatic load, especially during the pre-pubertal stage when comb growth surges. In a 2 x 2 factorial crossover experiment investigating the role of resource choice, layer pullets were exposed to multiple (M) vs single (S) litter and perch types during Weeks 1–4 (Period 1) and/or Weeks 5–15 (Period 2) of rearing prior to transfer to standard laying pens and commencement of photostimulation (n=4 pens/treatment combination: SS, SM, MS, MM). In Weeks 16 and 27, feather scores (related to feather pecking) and body weights were registered as indicators of allostatic load. Combs were also photographed in Week 27, and comb laterality (hanging on left or right side) was noted. We used a bespoke image analysis programme to capture comb area and complexity (perimeter length/area) from photographs of 6 randomly selected hens/pen. Feather condition in Weeks 16 and 27, and growth from Week 16–27, were enhanced by M in Period 2 ($P < 0.05$). Therefore, we predicted that hens kept in the M (vs S) environment during Period 2 would have larger, more complex, and left-side-biased combs reflecting reduced allostatic load. The predicted comb side bias was speculated to result from head posture/movements associated with increased right eye/ear use and left-brain hemispheric dominance. We found no associations between treatment in Period 1 or 2 and comb area, complexity, or laterality of the adult hens ($P > 0.2$). Furthermore, the comb measures were not correlated with the hens' feather scores or growth rate. Thus, although the M treatment in Period 2 promoted adaptability to the laying environment as indicated by improved plumage condition and growth, the variation in pre-pubertal allostatic load was insufficient to modify the trajectory of comb morphological development.

Keywords: laying hen, pullet rearing, environmental choice, animal welfare, comb development



P-09-05

Maternal care and damaging behaviour of the Japanese quail

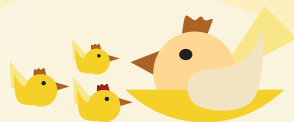
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Impaired maternal care has been shown to lead to cognitive and behavioural deficits in offspring in many species, including birds. The aim of this study was to examine the effects of maternal care on damaging behaviour in Japanese quail. New-born quail chicks were reared with adoptive mother hens ($n = 50$) or under the ceramic brooder lamps ($n = 50$) during the first 3 weeks of age. After that time chicks were moved to deep litter pens and the occurrence of damaging behaviour was assessed from video recordings at 5, 8 and 12 weeks of age. The fear level of young chicks was also tested using the emergence test and the novel object test during rearing and again later in adulthood. Rearing with the adoptive mother significantly reduced the incidence of damaging behaviour, more specifically given ($F_{1,238} = 5.61$; $P < 0.05$) and received toe pecking ($F_{1,238} = 12.02$; $P < 0.001$), received severe feather pecking ($F_{1,238} = 4.96$; $P < 0.05$), received aggressive pecking, and given gentle feather pecking ($F_{1,238} = 14.08$; $P < 0.001$). On the other hand, the effect of maternal care on fear level of chicks was not confirmed (emergence test $F_{1,37} = 0.40$; $P = 0.530$; novel object test $F_{1,37} = 0.42$; $P = 0.5212$). Our results confirm the important role of the mother in the early life of young quails. Her presence positively influenced the behaviour of the chicks in terms of reducing the occurrence of damaging behaviour and thus helped to improve their welfare.

Keywords: maternal care, Japanese quail, damaging behaviour, fearfulness



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P-09-06

Chicken amygdala: from neural mechanisms regulating emotions to welfare

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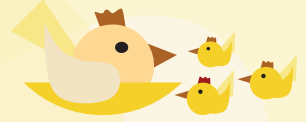
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To improve animal welfare it is crucial to know how animals perceive and respond to different stimuli, and how they regulate emotions. In mammals, the amygdala is one of the major actors regulating the endocrine, autonomic and behavioral responses upon exposure to a stressing stimulus. The amygdala is an extremely complex and heterogeneous structure, with multiple subdivisions, cell subtypes and intricate connectivity patterns. Two major neural populations of the central extended amygdala (EAc), containing the neuropeptides somatostatin or enkephalin, play opposite roles, as they become active (on) or inactive (off) during stress; thus, they represent a double switch for the regulation of the stress responses.

One major question in the study of the neural bases of stress in poultry is whether the chicken amygdala is similar to the mammalian one from structural and functional points of view. However, identification of the chicken amygdala has been extremely challenging due to divergent forebrain evolution in vertebrates. This problem has started to be overcome by using an evolutionary developmental biology approach, analysing combinatorial gene expression patterns during development to identify homologous brain divisions and cell populations across species. Using this approach, we identified the chicken EAc and used this information to further investigate its neuronal subpopulations and connections. We found neurons resembling the mammalian on and off cells, as well as local connections and outputs to the hypothalamus compatible with a complex direct and indirect regulation of the endocrine response. Moreover, we found activation of two subdivisions of the chicken EAc during social isolation acute stress. Our results suggest the existence of similar neural mechanisms for stress regulation in chicken and mammals, providing new information that could be used to improve poultry welfare.

Supported by H2020-MSCA-ITN-2018-812777

Keywords: Chicken brain, Stress regulation, Amygdala



P-09-07

Better with charcoal? Preferences of organic laying hens for pecking blocks with and without charcoal particles

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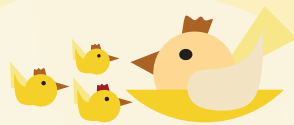
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One of the most commonly used enrichment materials in German laying hen husbandry is the pecking block. Different additional ingredients can be mixed in these, usually calcium carbonate based blocks to rise attractiveness or promote health. In this on-farm study, pecking blocks with charcoal-addition (CB) as well as usual calcium-carbonate-only (UB) blocks were offered to two groups of laying hens to analyse potential preferences for one material. The two types of pecking blocks were presented simultaneously, in addition to normally offered enrichment, in the winter gardens of an organic farm to two groups of about 2,400 laying hens (Lohmann Brown Lite) each. From the age of 40–73 weeks, counts of hens occupied with each pecking block were assessed on photographs of the winter gardens. In general, the hens preferred the UB with a mean of 2.49 (SD: 2.39) hens per block and 1.5 (SD: 1.59) hens at the CB ($p < 0.005$) over the whole observational period. The interest in both block-types was highest in the beginning and decreased steadily until the end of observations. While the UB started with a mean of 4.73 (SD: 2.92) hens and ended with 0.69 (SD: 0.9) hens per block ($p < 0.005$), the interest in the CB was lower in the beginning with a mean of 1.94 (SD: 1.9) hens and decreased significantly until the end with 0.56 (SD: 0.83) hens per block ($p < 0.005$), too. Daytime also significantly influenced the mean number of hens around the blocks. At both blocks, most hens were counted in the evening hours, followed by afternoon hours and least hens in the time until 2 p.m. (all $p < 0.005$).

This work was financially supported by the Federal Ministry of Food and Agriculture based on a decision of the Parliament of the FRG, granted by the BLE.

Keywords: laying hens, enrichment, behaviour



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P-09-08

Early provision of increased environmental choice influences the gut microbiota and physiology of layer pullets

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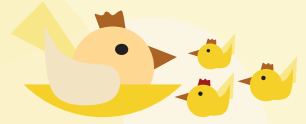
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This study explored the role of the gut-brain-axis and other physiological axes on the effects of variation in resource choice during rearing in layer pullets. Pullets (n=364, 16 pens) were reared with one (“Single-choice”) or four (“Multi-choice”) litter and perch types during early (day 1-week 4) and late rearing (week 5–15). In week 5, the environment in half the pens was switched, resulting in four treatments. Treatment effects on faecal microbiota (genus), immunological variables (lysis and agglutination against sheep red blood cells (SRBC)), heterophil/lymphocyte (H/L)-ratio and cognitive abilities (working memory in a hole-board test) were evaluated around week 15. The early rearing environment influenced the pullets’ microbiota composition (ANOSIM, $P=0.007$). Pullets that switched from Multi-choice to Single-choice had higher H/L-ratios than pullets reared continuously with Multi-choice ($P=0.02$) or Single-choice ($P=0.05$), implying an underlying chronic stress response presumably arising from the reduction in environmental choice. Furthermore, these pullets also tended to have higher concentrations of natural antibodies against SRBC than individuals from the other treatments ($P<0.1$). The early exposure to several types of litter and perches, potentially mediated by the gut microbiota, may have facilitated functional activation of the antibody response when the birds were challenged by the switch to a less favourable environment. Pullets with Multi-choice during late rearing showed a tendency for higher lytic activity than those with Single-choice ($P=0.08$), suggesting increased activity of this first barrier against pathogens. No treatment effect was found on working memory, but pullets reared continuously with Multi-choice were more successful locating baits in the hole-board test than pullets with Single-choice during late rearing ($P<0.05$). The results provide some support for the interpretation that a variation in litter and perch types during rearing stimulates the gut-brain-axis, which interacts with other physiological pathways such as the HPA-axis and can thereby influence pullet behaviour and immunocompetence.

Keywords: Development, Rearing, Microbiota, Immune response, Hole-board test



P-09-09

Do commercial turkeys dust bathe? An insight into the underlying behavioural pattern

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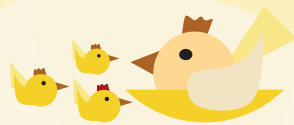
Once provided with dry and clean litter, turkeys often use the opportunity to dust bathe. While extended knowledge about this behaviour in other birds exists, commercial turkeys have yet remained relatively unexplored. The aim of this study was to analyse the process of dust bathing in commercial turkey hens shortly after refreshing the substrate.

The trial was conducted during one production cycle in two barns. For this study, twelve groups of B.U.T. 6 turkey hens (Aviagen Ltd., Tattenhall, UK), were observed using video cameras. Each group of 71 hens shared a 17.5 m² compartment, which included one dust bath (1 m²) available at all times during the fattening phase. The dust baths were refreshed once a week using rock flour, sawdust and sand in four groups each. The analysis was carried out by focal sampling (software BORIS v. 7.13.6, University of Torino, Italy) of four dust bathing birds per substrate, which entered the dust bath between the 3rd and 30th minute after refreshing, in the 6th and 10th week of life.

The average time spent in the baths was 391.8 sec (± 336.0 sec). Of that time, 32.4% (127.0 sec (± 165.8 sec)) were filled with the dust bathing process, which included perching down, scratching the ground and performing vertical wing shakes until standing up again. Overall, the birds shook up the substrates with their wings 4.1 (± 3.6) times on average. The remaining 67.6% of the time in the dust bath was spent with exploratory and resting behaviours such as foraging and sitting.

This work (Model- and Demonstration Project for animal welfare #Pute@Praxis) is financially supported by the Federal Ministry of Food and Agriculture (BMEL) based on a decision of the Parliament of the Federal Republic of Germany, granted by the Federal Office for Agriculture and Food (BLE); grant number «FKZ 2817MDT611»).

Keywords: turkey farming, dust bathing, farm animal behaviour, animal welfare



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P-09-10

Provision of enrichments to meet the behavioural needs of broilers

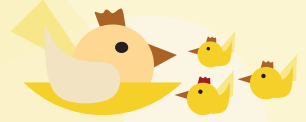
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To meet the behavioural needs of broilers is fundamental to increase their welfare. The provision of physical enrichment in the barn is a way to meet these needs. The aim of the study was to assess the relevance of enrichments from the animal point of view. First, a working group described 11 behavioural needs based on the knowledge of *Gallus g. domesticus* and red jungle fowl : forage, perch, move, rest, preen, dustbath, sunbath, stretch, interact, play, use of cognitive ability. Then, 5 broiler farms were monitored in Western France (Ross308 or JA987; 1 week before slaughter, 28–38 days; 19–22 broilers/m²) in Spring 2021. The enrichments were designed to meet the following behavioural needs: foraging (pecking blocks, strings, chains, straw bales, balloons, bags of pellets), perching/resting (platform perches, linear and straw bales), dustbathing (tray with sand), use of the cognitive abilities (more complex environment with vertical panels). Resting and foraging behaviours were collected through 5 scans (at 1min intervals), while other active behaviours were collected through 5min continuous sampling, twice a day, in an enriched and a non-enriched areas. Data were analysed using non parametric tests. More broilers were foraging with a pecking enrichment than in the empty area (10% of animals/scan VS 2%, $p < 0.05$), and seemed to prefer the pecking blocks, strings and straw bales compared to the other items. Platforms and straw bales were preferred by broilers to perch on compared to linear perches ($\approx 60\%$ of animals/scan VS 10%, $p < 0.001$), even if no difference was observed on the percentage of resting birds. Vertical panels were also used by birds to rest along. No difference was observed on the dustbath behaviour with or without the tray. The addition of suitable enrichments improves the welfare of broilers and it is important to provide different enrichments so that the broilers can express their full behavioural repertoire.

Keywords: Behaviours, Welfare



P-09-11

Effect of environmental enrichments on the proximity of broiler chickens to a human and novel object

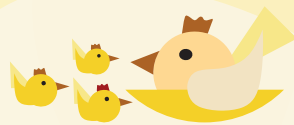
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Providing broilers with environmental enrichments can improve their welfare, and evaluation is important to ensure welfare is positively affected. The objective of this study was to evaluate the effect of supplemental lighting and platform enrichments on the fearfulness of broiler chickens. The study was a 2x2 factorial block design with 16 pens (N=25 birds/pen) within 2 rooms randomly assigned to one of four treatments (N=4 pens/treatment): no enrichments (C), platform (P), spotlight (S), and a combination of spotlight and platform (SP). Enrichments were positioned in the center of each pen. In the SP pens, P was positioned directly beneath S, so that S illuminated the greatest surface area of P. The Avoidance Distance (AD) Test and Novel Object (NO) Test were performed when the birds were 3 and 5 weeks old. Distance (cm) from the object and human during NO and AD Tests were partitioned into zones. Birds in each zone were counted and proportions of birds within each zone were calculated from the total birds in each pen. Data were analyzed with ANOVA, with the fixed effects of treatments and their interaction and random effects of time and pen nested within room. Video was recorded during the tests. There were few enrichment effects in the data collected. There was a greater proportion of birds near the human during the AD Test in S compared with P and SP pens at week 3 ($P=0.02$). Bird proximity to the NO and human decreased from week 3 to week 5 ($P\leq 0.05$). The results indicate that the environmental enrichments had minimal impact on fearfulness of broilers in this study.

Keywords: broiler, environmental enrichment, novel object, avoidance distance, fear



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Free Papers

P-FP-01

Natural essential oils work as the residue-free repellent in poultry red mite management

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Poultry red mite (PRM) is the most prevalently hematophagous ectoparasite of laying hens. Blood sucking by PRM usually causes irritation, restlessness resulting in feather pecking and cannibalism. Heavy infection level can lead to anaemia or even mortality. That are hence, posing a serious welfare issue. Chemicals used for PRM control have turned on public health alert due to the risk of residue and resistance. Therefore, natural solutions are on demand. The essential oils (STEOs) from *Cymbopogon nardus* and *Eugenia caryophyllus* was documented for their repellent capacity through modulating hens' body odor, consequently making them less attractive to PRM. This study aims to demonstrate the interest of using theses STEOs as residue-free repellent via feed in managing PRM.

First, the repellent effect was assessed. 2 groups of layers were designed: STEOs group (n=4) fed standard feed supplemented STEOs at 5000ppm; CT group (n=5) received the same feed without supplementation. The repellent effect of hens' body odor was determined by letting 50 starving female PRMs freely choose hens for blood through an olfactometer.

Second, the residue-free in eggs was studied by an adapted GC-EI-MS method. 10 egg samples were collected from hens supplemented STEOs for 10 months (n=5) with and without supplementation (n=5). 2 major volatile compounds of STEOs, geraniol and eugenol, were targeted.

The binomial test revealed that up to 78% of tested PRM was recorded feeding on CT hens while only 14% of PRM chose STEOs hens. That showed significant repellent against PRM of STEOs hens compared to CT hens ($P < 0.001$). In which, there was 8% of mites stayed starving without moving on hens for blood. Besides, no aromatic compound was detected in all eggs from both tested groups. Indicating that the likelihood for EOs residues accumulation in eggs laid by hens regularly supplemented in feed with EOs for 10 months duration was absent.

These results showed the massive interest using STEOs in managing PRM in layers via feed without the risk of residues in eggs promoting *Dermanyssus gallinae* hens' welfare without compromising egg's quality.

Keywords: Poultry red mite, Natural repellent, Essential oils, animal welfare



P-FP-02

Effect of pasture intake on meat quality of fast-, medium- and slow-growing chickens

Darina Chodová¹, Eva Tůmová¹, Jan Tyl², Mohamed Ketta¹

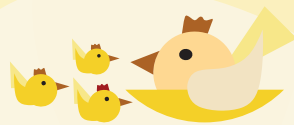
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Recently, the consumer interest in food safety and animal welfare has been growing. In the case of chicken meat production, grazing is one of the possible enriching elements improving animal welfare, which can also affect chicken meat quality. The aim of this study was to evaluate the effect of pasture intake on carcass value and physical meat characteristics in fast- (Ross 308), medium- (JA757) and slow-growing (ISA Dual) chickens. In the experiment, total of 210 chickens in conventional system and 300 chickens in free range system from each genotype were used. On 22 day, 300 chickens from each group were assigned to free range system with a free access of birds to grassy paddock. At live weight of 2 kg, 20 chickens per each group (male to female ratio 1:1) were slaughtered for carcass value and breast meat quality. There was no interaction of production system and genotype for the dressing out percentage or breast percentage. Fast- and medium-growing chickens had lower pH value on pasture ($P < 0.001$), while slow growing chickens had higher pH when birds had outdoor access. There was detected an interaction of production system and genotype for the meat colour intensity (a^* , b^* ; $P < 0.001$ for both). The meat of the slow birds became redder and less yellow when the birds had outdoor access compared to conventional system, however, this was not detected in the fast- or medium growing chickens. Interaction of production system and genotype was also in water holding capacity ($P < 0.001$). The medium- and slow-growing chickens with pasture intake had higher ($P = 0.001$) drip loss and lower ($P = 0.002$) cooking loss than those fattened in conventional system. In conclusion, pasture availability improved meat colour intensity and had positive effect on heat-treated meat parameters.

The study was supported by the Project NAAR No. QK1910387.

Keywords: pasture, genotype, carcass composition, meat quality



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P-FP-03

Bird reactivity and semen production in the Pepoi and Robusta Maculata Italian chicken (*Gallus gallus domesticus*) breeds

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The presented research was aimed to investigate birds' individual reactivity and quantitative semen production in Pepoi (PEP) and Robusta Maculata (RMA) roosters. Tonic Immobility (TI) and Emergence (ET) behavioural tests (fear response) were used. Twenty-seven roosters, 15 PEP and 12 RMA, were housed in single cages in controlled environment. Semen collection was performed twice weekly at 20–24 months of age. Tested parameters: body weight (Kg; LW), semen volume (ml; VOL), semen concentration (n sperm/mL semen volume; CON); total sperm output (n sperm.; VOL*CON; TSO), ratio semen volume/live weight (mL/kg; VOL/LW; VWR). In TI: number of inductions (N, max 3; TINI), TI duration (s, max 180 s, TIDU), number of vocalization (n, TIVO). In ET (max latency time 180s): head emergence out of the box latency (s, ETHE), first step out of the box latency (s, ETFS), bird's complete emergence out of the box latency (s, ETCE), number of vocalization (n, ETVO), defecation (n, ETDE). GLM and Bivariate Correlate procedures of SPSS® were applied; breed was the source of variation in GLM (Bonferroni test; $P \leq 0.05$). PCA was performed using Past® 4.05 software. Significant differences were found in LW, ETVO and semen quantity parameters. Lower LW was recorded in PEP birds (1.957 kg vs 4.303 Kg). Higher ETVO frequency was recorded in RMA birds (17.25 vs 2.0). Significant Inverse correlations between LW and VOL, CONC, TSO and VWR were found. PC1 and PC2 described the 60.27% and 24.5% of the variance respectively, and they were both influenced by TIDU and ETHE. PC3 described the 14.16% of variance based on ETEM and ETFS. PCA reveals the effects of birds' reactivity on roosters' distribution. Results underline the relevance of breed based behavioural characterization in order to optimize semen collection procedures for productive and conservative purposes and to improve birds' welfare.

Keywords: Biodiversity, Rooster welfare, Rooster behaviour, Artificial insemination, Heritage breeds



P-FP-04

Effects of dietary energy levels on intestinal morphology, corticosterone in blood, meat quality, and indication of welfare effects of broiler

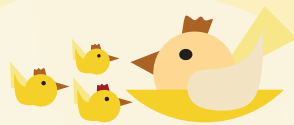
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This study was determined the effects of dietary energy levels on intestinal morphology, corticosterone in blood, and meat quality of broiler in animal welfare rearing environment. A total of 775 one-day-old Ross308 male chicks were randomly allotted to five treatments with five replicates per treatment and 31 birds per replicate. The 5 feeding programs (starter, grower, and finisher phases) were: CON (3025, 3100, 3150 kcal/kg), L200 (2825, 2900, 2950 kcal/kg), L100 (2925, 300, 3050 kcal/kg), H100 (3125, 3200, 3250 kcal/kg), H200 (3225, 3300, 3250 kcal/kg). The control diet was complied with the nutritional requirement of Korean Feeding Standard for poultry (2017). Feeding with H200 increased ($P<0.05$) jejunal villus height 9.9% compared to CON group, while crypt depth and villus height/crypt depth ratio were presented similar by the diet energy level. Additionally, the diet energy level did not affect the corticosterone level in blood of broilers. The pH of meat was lower ($P<0.05$) in L200 than the others, and the cooking loss was highest ($P<0.05$) in L200, which leads to negatively affect meat quality. The L^* of breast meat colour was increased ($P<0.05$) and a^* of meat was decreased ($P<0.05$) with increasing dietary energy. While b^* of breast meat was not affected by diet energy levels. The share force of meat was linearly increased ($P<0.05$) according to diet energy levels, the L200 and L100 groups were higher ($P<0.05$) compared to the others. In conclusion, intestinal morphology and breast meat quality except for corticosterone might be adjusted to dietary energy level, then dietary energy may be strategized to improve value to breast meat and animal welfare rearing environment.

Keywords: dietary energy, animal welfare, broiler, intestinal morphology, meat quality



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P-FP-05

The relationship of housing system for laying hens and egg quality and egg safety under commercial conditions

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The aim of the study was to compare enriched cages, aviaries and organic production in egg quality, eggshell contamination, faecal microbiota, air contamination in laying hens kept on commercial farms. All selected parameters were evaluated over the whole laying cycle of Lohmann Brown layers kept under controlled conditions. The significantly highest egg weight was detected in aviaries (63.1 g) and the lowest in organic housing (56.7 g). The same trend was in the Haugh Units score. The albumen and shell percentages were the highest ($P \leq 0.001$) in organic production, yolk percentage in enriched cages ($P \leq 0.001$) and eggshell strength in aviaries ($P \leq 0.001$). Housing system does not affect cuticle deposition and faecal microbiota composition. Eggs and air from organic production showed the significantly highest shell contamination by total number of microorganisms. In the organic production the significantly highest correlations among eggshell contamination and microbiota and air were observed (0.67–0.89). The results of the study showed an important effect of housing system on internal and external egg quality parameters, on eggshell and air contamination and also on correlations among eggshell contamination and air composition, faecal microbiota and eggshell contamination correlations between microbiota composition and air contamination.

Keywords: laying hens, housing, egg contamination



P-FP-06

Is there a need to maintain the production of day-old male chicks from laying hens as a source of feed for endangered species?

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On 19 July 2021, France and Germany presented a proposal for an EU-wide ban on the systematic culling of male day-old chicks. To date, only Germany has approved this ban with force from 1 January 2022, which has led to a reduction in the number of laying hens in German farms and the cessation of hatching of chicks in several smaller hatcheries in Germany. However, France, in its regulation for the introduction of this restriction from 2023, signed by the Minister of Agriculture mentions exceptions, one of which is the use of culled chickens as feed. They are an irreplaceable and natural source of nutrition, especially for birds of prey. If these animals are fed on other biologically based food (e.g., rodents), they will be treated in conflict with the legislation and may therefore be subject to cruelty.

Alternative methods of sexing embryos and culling them will only increase the number of animals killed in general. At the same time, it will be a significantly more costly and less environmentally friendly way of obtaining feed for captive animals. The culling of chickens by the approved CO₂ euthanasia method is seen as controversial in some European countries in relation to ethics and animal welfare. Day-old male layer poultry are neither waste nor a secondary product of hatching in the sense of the above arguments.

When drafting legislation to prohibit the culling of day-old chicks and ducks, it is essential that this exception be stated as it is in the French decree. The ban should not apply to hatcheries that are able to supply chicks from their own production, which are not used for further breeding, for the purpose of feeding the animals needed. This is the production with the lowest carbon footprint and the lowest demands on agricultural land use.

Keywords: killing of day-old males, irreplaceable feed, endangered species, carbon footprint



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