



Prof. Dietmar Flock,  
Editor

## Editorial

This month, the World's largest book fair, the Frankfurter Buchmesse, brought together more than 250,000 people – publishers, authors, teachers and interested readers – to update information on recent publications and monitor trends in the global business of information and entertainment. While the number of publications continues to grow, the competition is getting tougher. The growing share of E-books offers new opportunities for buyers and sellers.

Eight years ago, when we started to publish Lohmann Information in digital format, we were curious how many readers would go along. It turned out that we lost only a handful of readers who would still prefer a printed edition. And despite the change from German to English language the number of readers continues to grow, even in the German speaking countries. Our address list for direct mailing currently has 2,772 readers in 132 countries.

The global business of multiplying printed material has come a long way since Johannes Gutenberg developed the first known printing machine in 1448, and you may or may not give Bill Gates or Steve Jobs credit for their contributions to a “better” world. A fact is that more people can read and write with the help of modern digital techniques, with positive and negative consequences: you can benefit from available information anywhere at any time, but you are also in danger of being flooded with unwanted e-mails, advertising and biased publications.

Back in 1966, when I was offered a position as geneticist in the poultry industry, my major professor, Jay Lush, offered two encouraging arguments: (1) the company was focused on a scientific approach to breeding and offered more potential for continued learning in modern poultry breeding than any university and (2) the company would also encourage publications in scientific journals. As editor of this journal, I try to contribute to the continuing flow of useful information. In case you don't find at least one article of interest in this issue, I suggest to visit the “archive” for previous papers or to drop me a note with suggestions which topic you would like to see covered in a future issue

This issue of Lohmann Information starts with two papers on poultry health, followed by two papers on poultry nutrition and three papers of special interest in connection with poultry welfare, environmental sustainability and hatching egg treatment to optimize hatchability. Common to all 7 contributions is the basic question: how can current knowledge be applied to achieve the best possible combination of bird welfare, protection of the environment and sustainable economics?

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**LOHMANN**  
TIERZUCHT

1. **Prof. Dr. med.vet. Dr. h.c. Erhard F. Kaleta** worked all his life with poultry and just published a book in two volumes, describing the history of Poultry Medicine at the University of Gießen (ISBN: 978-3-8359-5994-1) and Research on Poultry Diseases (ISBN: 978-3-8359-5995-8), which are recommended for German speaking readers. The paper **DISINFECTION IN POULTRY MEDICINE – AIMS AND MEANS** should be of general interest for people working with poultry.
2. **Prof. Hebert Trenchi**, University of Montevideo, Uruguay, explains principles of **IMMUNOLOGY AND DISEASE PREVENTION IN POULTRY** with focus on applicability under field conditions in different parts of the world. Keeping birds alive and healthy is obviously of common interest from the viewpoint of bird welfare, efficient use of resources and economics.
3. **Dr. Murdo MacLeod**, University of Edinburgh, Scotland, well-known as nutritionist and past editor of British Poultry Science, takes a critical look at **NUTRITION-RELATED OPPORTUNITIES AND CHALLENGES OF ALTERNATIVE POULTRY PRODUCTION SYSTEMS**, reviewing advantages and disadvantages of alternative systems and suggesting answers to current conflicts between limitations for organic production and sustainable use of resources.
4. **Prof. Dr. Dr. h.c. Heinz Jeroch** and **Dr. habil. K. Kozlowski**, University of Olsztyn, Poland, document in their article **IMPROVING THE NUTRITIVE VALUE OF POULTRY FEEDSTUFFS: THE RAPESEED PRODUCTS EXAMPLE** how the competitiveness of rapeseed as a component of poultry feed has been improved and suggest that further improvements can be expected from joint efforts of plant and poultry breeding as well as technical treatments of feed components.
5. **Dr. Klaus Damme** and **Stefanie Urselmans**, Kitzingen, Germany, address a „hot topic“ in European poultry welfare, based on recent experimental results, in their article **INFRARED BEAK TREATMENT – A TEMPORARY SOLUTION?** Although the message seems clear, the question remains whether public opinion and political decisions in Germany will be impressed by these research results.
6. **Dr. Ilkka Leinonen** and **Ilias Kyriazakis**, Newcastle University, UK, report on the results of their analysis **QUANTIFYING THE ENVIRONMENTAL IMPACTS OF UK BROILER AND EGG PRODUCTION SYSTEMS**. Large differences were found in many categories of environmental impacts, reflecting mainly differences in feed efficiency.
7. **Dr. Dinah Nicholson *et al.***, Aviagen Ltd., summarize the results of a series of designed experiments to improve hatchability after prolonged storage by application of **SHORT PERIODS OF INCUBATION DURING EGG STORAGE – SPIDES**. The technique essentially simulates what a broody hen does while adding an egg to her clutch every day.

With kind regards,



Prof. Dietmar Flock,  
Editor

## Disinfection in poultry medicine – aims and means<sup>1</sup>

E. F. Kaleta, Giessen

### Introduction

Veterinary activity is focused simultaneously on the animal itself and the suitable environment. Animal care includes detailed diagnostics, prophylactic immunization and, in some cases at least, effective treatment. In the environment, practically all plants, animals and humans are colonized by bacteria, fungi, viruses, prions but also by single cell coccidia and multicellular parasites. As a logical consequence, a permanent fight exists to control such agents and their debilitating effects on animal health, welfare and productivity. Effective measures are essential to combat these pathogenic microorganisms with the target to reduce or even to eliminate their deleterious effects. Such measures are applied on non-living objects and generally summarized under the term “disinfection”.

Highly desired effects of disinfection are (i) all pathogenic microorganisms, all oocysts of coccidia, all eggs of internal and external parasites are totally destroyed in all areas, (ii) re-introduction of pathogens is permanently prevented, (iii) losses due to transmissible diseases are minimized, (iv) profitability of animal farming is enhanced and (v) a positive cost-benefit balance is assured.

Proper disinfection is usually associated with undesired effects. These effects may be (i) irritation of skin and eyes of workers, (ii) slippery surfaces may cause accidents, (iii) persistence of disinfectants in rooms and on surfaces, (iv) some disinfectants discolour painted walls, (v) spill of disinfectant into the environment, open waters, creeks etc., (vi) toxicity for fish, crustaceans, arthropods, plants etc., (vii) damage to electrical wires, to engines, equipment and (viii) corrosion on metal surfaces.

Various definitions have been given to characterize “disinfection” and to discriminate it from related or similar measures. The following delineates some selected definitions.

Disinfection is a

- method applied to prevent transmission of disease causing agents (Schließer, 1981)
- procedure used for inactivation of certain microorganisms (Böhm & Straub, 2002)
- abolition (abrogation) of disease causing agents (Duden, 2006)
- method that destroys infection-producing agents (Blood *et al.*, 2007)

It is clear from these four selected definitions that the prevailing target is the elimination or at least reduction of such agents that (may) cause disease. It is never attempted to obtain an environment that is devoid of virtually all microorganisms. Also, the aim is prevention of transmission and infection by destroying the vital properties of pathogenic organisms.

The term “disinfection” requires demarcation from other more or less related technical means and procedures.

Some examples are:

- cleansing** means mechanical removal of dirt, manure, dust, vermin etc.
- sterilization** is the application of dry heat for several hours on solid materials
- filtration** means separation of organic matter in a liquid phase by membrane or asbestos filters of variable pore size
- pasteurization** includes repeated cycles of heating and cooling of liquid matter for variable times and temperatures
- antiseptics** is the application of germicidal substances on and in animals

<sup>1</sup> Based on a presentation at the annual meeting of the Poultry Veterinary Study Group in Vilnius, Lithuania, on May 27, 2013

## On the history of disinfection

Some historical remarks should be added at this point on means to correct environmental pollution of air and surfaces and on preservation of human food. Elevated but appropriate room temperature for hatching chicks was achieved in old Egypt in so-called Pharaoh hatcheries by burning of plant material. The developing smoke contains phenolic compounds that condense at surfaces in the hatchery compartments and exert their disinfecting effects. Another example: The common plant thyme (*Thymus vulgaris*) is frequently used in herbal medicine. It contains high concentrations of thymol which is chemically o-isopropyl-m-cresol, a substance with a high disinfecting property. Thirdly, even in contemporary times smoke and smell are generated by smouldering of the resin derived from the tree *Boswellia serrata* which contains (among many others) a high concentration of phenolic substances and terebinthene which have disinfecting properties and also emit a pleasant smell. Frankincense is commonly used in religious ceremonies e.g. in catholic churches - without realizing the real disinfecting effects of this practice.

I mention these practices to illustrate different germ reduction strategies which have been used for centuries without realizing the basic modes of chemical reaction and without realizing and using the contemporary term “disinfection” which means the opposite of “infection”.

In the past and in some areas even today, various means are practised to maintain and to preserve edible food for human consumption. Such measures include reduction of the water content of food by drying and salting or by adding sugar or keeping food on ice.

Another example of traditional “disinfection” is the rather common use of sunshine to dry and bleach cloths etc. on green meadows. Housewives interpret this as an effective measure to turn the greyish appearance of cotton to splendid white and to obtain dry and nice smelling cloths. Actually, the most important effect of “drying” in sunshine is directly associated to inactivation of the infectivity of microorganisms. It is well known that UV-light destroys the genome of pathogenic microorganisms within a short time, and drying and bleaching are just welcome additional side effects.

Historically, the most important prerequisite of disinfection was the development of techniques for isolation and identification of microorganisms, especially bacteria and viruses. Pioneers in this field were Louis Pasteur (1822-1895) in France (*Pasteurella* spp.), Robert Koch (1843-1910) in Germany (*Mycobacterium tuberculosis*), Theodor Escherich (1857-1911) in Austria (*Escherichia* spp.), Georg Theodor August Gaffky (1850-1918) in Germany (*Salmonella* spp.) and Friedrich Loeffler (1852-1915) in Germany (*Foot and mouth disease virus*). These and many other outstanding scientists paved the way to microbiology and fundamentals of the theory of infectious diseases.

Equally important for the promotion of microbiology and disinfection were chemists who isolated and identified chemicals and developed techniques for their synthesis which might be useful as disinfectants. Outstanding in this respect is August Wilhelm Hofmann (1818-1892) who discovered in 1867 (among many other inventions) the structure of formaldehyde and developed the synthesis of this chemical.

Initial studies on inactivation of microorganisms by available chemicals were undertaken some hundred years ago. Robert Koch was the first to test phenol for the inactivation of spores of *Bacillus anthracis* and published his results in 1884. He contaminated threads of silk by sporulated bacteria that cause anthrax, dipped these threads into a phenol solution and tried to re-isolate these bacteria after several time intervals. More recently, the German Society for Hygiene and Microbiology produced the first guideline in 1958 which contains already suspension and carrier tests and selected bacteria and fungi as test organisms.

Currently, the fourth edition of the guidelines provided by the German Veterinary Society (DVG) forms the basis for any testing of disinfectants in Germany. Besides suspension and carrier tests, a reference disinfectant and a reference test virus are included. All disinfectants must be examined by at least two independent persons who were approved by the board of the DVG. On an international level, various organisations are involved in the development of testing procedures for the evaluation of disinfectants.

In the following, past and current procedures for testing and evaluation of chemicals for disinfection in the veterinary field are reviewed. Various governmental and non-governmental organisations

developed guidelines for this purpose. Internationally operating organisations include OECD, FAO, EFSA, FDA, national organisations include the British Standards Institute (BSI); in Germany the Robert-Koch-Institute (RKI), the Veterinary Society (DVG), the Society for Applied Hygiene (VAH) and the German Agricultural Society (DLG). National organisations for testing and evaluation of chemical disinfectants exist also in The Netherlands, France and Italy, to name only a few countries. All these organisations developed procedures that are similar in testing principles, but the details of methodology, the agents used and the evaluation and subsequent recommendations differ quite markedly. Since 1989 the European Committee for Standardization (CEN) is working on harmonization of test procedures and their efficient evaluation of results.

The results obtained on the basis of a firmly established testing protocol are of paramount importance for the producer, for the testing establishment and finally also for the user of a disinfectant. Producers wish to obtain (i) reliable and reproducible test results for efficacy and safety reasons, (ii) a low working concentration of a disinfectant because low working concentrations have a strong bearing on the market price and (iii) results that are acceptable in almost all countries in order to expand their international market without re-testing of their products in various countries. The users prefer a broad spectrum of efficacy at low cost. The testing laboratories need a testing procedure which (i) yields results that must be reproduced in their own and in other laboratories, (ii) is efficient and not too elaborative and (iii) can be obtained within acceptable times.

### **The sub-committee for disinfection of the German Veterinary Society (DVG)**

Around 1970 Theodor Schließer in Giessen promoted the development of guidelines for testing of chemical compounds as disinfectants for virucidal, bactericidal, levoricidal and antiparasitic efficacy. The initial testing protocols were further refined during the following decades. Currently, the fourth edition (2004) of the guidelines for testing and evaluation of chemical compounds for application in the fields of veterinary medicine and food production are in use. Both guidelines require suspension and carrier tests and contain lists of reference bacteria, viruses, fungi and parasites. The results of testing that were generated by two laboratories are evaluated by the sub-committee and subsequently published at regular intervals in the *Deutsches Tierärzteblatt* and are accessible on the internet. Two separate lists are currently produced. One list contains data on disinfectants intended for use in the field of animal production; the second list aims at use in food hygiene. Both lists contain all essential background information (concentration, time, temperature) and the proven efficacy against viruses, bacteria, fungi and parasites.

### **The European Committee for Standardization (CEN)**

This Committee started its work in 1989 during the first meeting in the building of the British Standards Institution (BSI) in London, UK. The committee is composed of a maximum of three delegates per European country. In Germany, the delegates are selected and approved by the German Institute for Standardization (DIN) in Berlin.

The CEN has the following structure:

Technical Committee TC 216 – evaluates content and form of all drafts for standards and makes final decisions. The TC 216 inaugurates and installs three working groups:

Working group 1 is responsible for drafts on standardization in human medicine

Working group 2 is responsible for drafts on standardization in veterinary medicine

Working group 3 is responsible for drafts on norms in areas of food, drinks, cosmetics, hospitals, kitchens etc.

The Technical Committee 216 may create Special Task Groups for defined topics and workloads such as “surface test task group”, “sporocidal test task group” or “virucidal task group”. Such groups have to perform specified experiments and to report on the results to the Technical Committee 216 within a fixed deadline.

General duties of the CEN are to further improve guidelines that contain detailed prescriptions for test organisms (including proven purity, identity and infectivity titres), necessary diluents for test

organisms, materials, size and surface structure of germ carriers (e.g. poplar or linden wood, steel, glass, plastics etc.).

Even now a large number of questions on the testing procedure are still open to debate. In order to answer at least some of the very pressing questions, members of the Committee are asked to perform specified experiments “at home” and report the obtained results to all members of the Committee prior to each session, to comment on the results in writing and answer questions during the sessions. It appears that all members consider this procedure as essential to obtain further progress.

The content and structure of the testing protocol is also important. Required are data on the method used, the microorganisms, the organism and disinfectant used as references.

So far, the CEN produced a large number of preliminary reports. The circulated drafts are termed “preliminary European Norm, preEN” and an approved final text represents a European Norm (EN). Quite a number of such ENs exist already and are in full use in Europe. Comprehensive texts of all approved EN are available on the internet.

At any time in the future the work on standardization of testing of disinfectants and evaluation of procedures for disinfection will be completed and all results are then published. At this future stage further work is planned for harmonization of aims and means in cooperation with non-European countries (e.g. USA, Japan, India) and internationally operating organisations such as OECD, FAO, WHO.

## Areas that may need disinfection

Practical experience and solid scientific evidence prove the existence of highly different microbial populations at different locations. In the veterinary field, such locations require different disinfectants and carefully selected modes of application.

Such locations may be:

- Stables, including roads and gateways
- Liquid and solid manure
- Hatching eggs, hatcheries, chick trucks
- Slaughterhouse, transport vehicles
- Milk production: teats, tubes, vessels
- Breweries: in process applications
- Hands, towels, offices, door locks, ...
- Oil production and conservation
- Machineries lubricating oils, cooling devices, ...

These and most likely other “locations” require correct disinfection in specified situations. The selection of the most appropriate chemical disinfectant for any contaminated location leads to the question of prevalence of specific pathogenic microorganisms and available chemical compounds.

## Microorganisms as test models

Two conditions must be considered for the use of a microorganism for testing purposes. These are the multiplication of an organism to high concentrations (titres) under laboratory conditions and the relative resistance to chemical inactivation which is known from the results of previous experiments. After long debates during committee meetings of the DVG, DIN and CEN the following mandatory test organisms were approved and published in several EN for quantitative suspension and carrier tests:

Bacteria: *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Enterococcus hirae*, *Staphylococcus aureus*

Mycobacteria: *Mycobacterium avium*

Levuricidy: *Candida albicans*, spores of *Aspergillus niger*

Viruses: Newcastle disease virus, vaccinia virus, reovirus, bovine enterovirus type 1, others if desired

Parasites: *Ascaris suum* eggs, *Eimeria tenella* oocysts

In addition to these approved test organisms additional agents of interest may be tested on an optional basis. More recently, these are avian influenza A viruses (subtypes H5N1, H1N1, H6Nx H9Nx) West Nile virus, calicivirus, strain Norwalk. For these and any other optional viruses the CEN-approved testing procedure must be followed. The general pathway consists of the following phases and steps:

- Phase 1: laboratory testing of chemicals for antimicrobial efficacy
- Phase 2, step 1: quantitative suspension test
- Phase 2, step 2: quantitative carrier test
- Phase 2, step 3: quantitative surface test under field conditions

### Basic tests on chemical disinfectants

All active substances intended for use in disinfectants must be listed in Annex I or IA to Directive 98/8/EC. The commercial product is classified as product type 3 (Veterinary hygiene biocidal products).

For conformity, the following information and material is required:

- Chemical composition of the disinfectant, identity and quantity of the active substance, minimum purity of the active substance, absence of impurities.
- Physical and chemical properties (e.g. pH at various concentrations in water of standardized hardness; solubility in water of standardized hardness)
- Freedom of contamination by infectious agents and other cell type of primary or permanent cell cultures. Permanent cell lines can be obtained from the American Cell Culture Collection, Manassas, Virginia, USA, the Cell Culture Bank, FLI, Insel Riems or from well-known virological laboratories. Primary cell cultures are produced from SPF chicken eggs, VALO, Cuxhaven.
- Pure and well characterized microorganisms (viruses, fungi and bacteria) are maintained and distributed by National Reference Laboratories, the American Type Culture Collection, and in Germany by the Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig. Additional sources are published by the Deutsche Veterinärmedizinische Gesellschaft e. V., Giessen in the 4<sup>th</sup> edition of the "Richtlinien für die Prüfung chemischer Desinfektionsmittel".
- There is no formal source for ascaride eggs. These eggs are collected from the intestine of pigs at slaughter. Oocysts of *Eimeria tenella*, strain Houghton, are maintained and distributed on formal request by the Institute for Animal Health, Compton Laboratories, UK.
- Toxicological profile for man and animals including metabolism (e.g. determined cytotoxicity in approved cell cultures)
- Effectiveness against target organisms (e.g. minimal inhibitory concentration of test microorganisms)
- Ecotoxicological profile including environmental fate and behaviour
- Measures necessary to protect man, animals and the environment

If these conditions are fulfilled, a more specific question arises which refers to the intended uses of disinfection. Under field conditions, the following groups of pathogens are of major interest:

- Bacteria including *Chlamydia* spp. and *Mycoplasma* spp.
- Viruses (and possibly prions)
- Protozoa: coccidial oocysts, flagellates (e.g. *Histomonas* spp., *Trichomonas* spp.)
- Eggs of round and tape worms

The inherent biological properties of these organisms vary extremely from highly sensitive to highly resistant. Therefore, the disinfectant must be tailored according to the predominant and mostly prevalent target microorganisms. In other words, a meaningful selection of an available disinfectant is necessary.

### Chemicals useful as disinfectants

Surprisingly, all currently used chemical disinfectants are "old" compounds and their chemical actions are known for many decades. These chemicals include:

- Aldehydes – formaldehyde, glutaraldehyde, glyoxal
- Aliphatic alcohols – ethanol, propanol, isopropanol

- Aromatic alcohols – phenols including alkyl-, aryl- and halogen derivatives
- Organic acids – formic, acetic, propionic, citric acid
- Hydrogenperoxide – H<sub>2</sub>O<sub>2</sub>
- Guanide – biguanide
- Iodophors – alcoholic solution of potassium iodide
- polyvinylpyrrolidone solutions of iodine
- Chlor – organic and inorganic compounds
- Alkalines – NaOH, CaO, Ca(OH)<sub>2</sub>, bleach
- Peroxyacetic acid (plus alcapur)
- Quaternary ammonium compounds

Some of these chemicals are now considered as obsolete; some others are applied under limited conditions. Beside a reliable mode of fast action, presently more impetus is given to the fate of a disinfectant after its application. Desirable are (i) rapid degradation without residues in the area of application, (ii) low level of toxicity to farmed animals and men, (iii) easy to handle during application, (iv) effective across a wide range of low and high environmental temperatures and (v) low price.

In recent years organic acids and especially peroxyacetic acid (plus alcapur) apparently gained more interest and widespread use under farm conditions. Various phenol derivatives maintain a strong position as antiparasites. Almost all commercially available products contain not only an active ingredient, but also additives to enhance dispersion on surfaces (surface-active compounds).

### **Formalin as a traditional disinfectant**

Formaldehyde is the gaseous form and its solution in water is named formalin. Formalin is on the market either at a concentration of 35 - 37% or as crude formalin at various concentrations. Since the discovery of formaldehyde by A. W. Hofmann in 1867, this compound was recognized as a disinfectant of superior value. The aqueous solution of formaldehyde was initially used to prevent fouling of animal skins prior to tanning. It was soon realized that repeated formalin exposure of the skin of tanners resulted in hypersensitivity, inflammation and pruritus. Since these early observations, formalin is considered as a potent allergenic compound. It does cause epithelial hypertrophy in the respiratory tract of rats that were experimentally exposed to high concentrations for prolonged times. More recently rumours spread that formalin may cause also cancer in experimental animals. Definite proof for this assumption is still lacking. However, the Institute for Risk Assessment in Berlin argues that a “certain risk for cancer development cannot be excluded.”

Despite this debate with pros and cons, formalin experienced a world-wide use as topical disinfectant especially for the disinfection of shells of hatching eggs. Rather recently, formalin was replaced by other compounds in hatcheries for safety reasons. It seems to be appropriate to list some of the major facts on formalin:

- 1867: first synthesis by August Wilhelm Hofmann in Giessen, Germany
- Technical synthesis today in Germany: approx. 500,000 metric tons per year by catalysis: CO<sub>2</sub> + H<sub>2</sub>O = HCHO
- Automobiles in Germany emit ca. 35,000 tons CO<sub>2</sub> per year (2004 statistics)
- Naturally present in plants, cigarette smoke and exhaust of gasoline and diesel engines
- Overwhelming amount is used for the production of plastics, waxes, glues, insulation foams for buildings, panel wood etc.
- Small fraction of the total production is used in medicine, anatomy, histology, pathology, production of inactivated vaccines, cosmetics, deodorants, creams ...
- Polymerisation of formaldehyde to paraformaldehyde, so-called „dry alcohol“ is used by campers
- Maximal concentration at working places is fixed by the German Institute for Risk Assessment, Berlin, at 0.6 mg/m<sup>3</sup>, equal to 1 ml/m<sup>3</sup>, equal to 0.5 ppm. Experienced people cannot smell this level

To complete the list of known facts on advantages and disadvantages of formalin, the following should be kept in mind:

**Advantages** of using formalin as a disinfectant are the following facts:

- Effective at rather broad range of temperatures
- Effective against bacteria, fungi, yeasts, some viruses
- Effective in liquid and gaseous forms
- Effective at pH 4.0 to 9.0
- Penetrates porous surfaces, e.g. egg shells
- Oxydation to formic acid:  $2 \text{HCHO} + \text{O}_2 = 2 \text{HCOOH}$
- Production is cheap, favourable cost-effect ratio

Major **disadvantages** of formalin are:

- Not listed in Annex I and IA of the Biocide Directive 98/8/EC. Legal use in the fields of medicine and animal production is not permitted anymore or requires special permit
- Causes irritation of conjunctiva and respiratory tract of humans and animals
- Causes allergic contact dermatitis after prolonged exposure
- Evaporates for long times from treated wood, panel wood, furniture etc.
- Hardens plastics – electrical cables etc.
- Polymerisation is prevented by methanol which is highly toxic
- Is inactivated by proteins
- Assumed to cause nasopharynx carcinoma after long-time exposure of rats

These and possibly more advantages and drawbacks may exist in various fields of technical and biological applications. In any case, it is worthwhile to look for promising alternatives to formalin as chemical disinfectants.

### Detection of formaldehyde by its smell

Some people maintain that they are able to recognize the rather characteristic smell (odour) of formaldehyde even at extremely low levels, e.g. evaporating from newly acquired furniture made of wood panel. These people consider formaldehyde as a dangerous product to their health and wellbeing. In an attempt to confirm the ability to detect this gas, I asked my veterinary students to determine the lowest concentration which they are able to detect by smelling briefly opened petri dishes. For this purpose dilutions of formalin were produced in distilled water in the range of  $10^{-2}$  to  $10^{-5}$  and two millilitres of each of the dilutions were placed on filter paper in closed petri dishes. A separate petri dish was offered to the students that contained two millilitres of distilled water. Beginning with the highest dilution of formalin, the students were asked to write a protocol on their ability to detect the smell of formaldehyde in each of the petri dishes. This experiment – performed on a voluntary basis – was done with veterinary students of the fifth and seventh semester. The results are presented in the following Tables 1 and 2.

**Table 1: Recognition of formaldehyde (FA) by 44 veterinary students, 5<sup>th</sup> semester. MAC  $0.6\text{mg}/\text{m}^3$  ( =  $1 \text{ ml}/\text{m}^3$  =  $0.5 \text{ ppm}$ ). N. t. = not tested**

Dilution in log10	FA concentration		FA recognition	
	%	ppm	Yes	No
0	37.0	370 000	N. t.	N. t.
1	3.7	37 000	44	0
2	0.37	3 700	44	0
3	0.037	370	27	17
4	0.0037	37	29	14
5	0.00037	3.7	23	21
Control <sup>1</sup>	0.0	0.0	27	17

<sup>1</sup> – distilled water

In medicine, anatomy, histology and pathology, phosphate buffered formalin is generally used in concentration between 9% and 10%. All students detected in correct manner formaldehyde at concentrations of 3.7 and 0.37%. However, lower concentrations were not recognized by all students. The maximum working concentration (MAC) of formaldehyde is in Germany fixed at 0.5 ppm. Obviously, approximately half of the students detected this low level by smelling. Some of them believed that they smelled the odour of formaldehyde even in distilled water. Thus, the errors of correct detection increased at low levels.

The same arrangement of the test was repeated with students of the seventh semester. The results are quite similar to the first test. Again, high concentrations were recognized in a correct manner. At low concentrations the number of students increased that came up with obviously wrong results. A few students (3 of 27) recognized formaldehyde at a concentration of 3.7 ppm. In contrast to the first test, all students interpreted the control sample that consisted of distilled water in a correct manner. In conclusion, formaldehyde at levels well above to the MAC value can be detected only by a few students but the rates of failure are high and therefore unreliable.

**Table 2: Recognition of formaldehyde (FA) by 27 veterinary students, 7<sup>th</sup> semester. MAC = 0.6mg/m<sup>3</sup> (= 1 ml/m<sup>3</sup> = 0.5 ppm). N. t. = not tested**

Dilution in log10	FA concentration		FA recognition	
	%	ppm	Yes	No
0	37.0	370 000	N. t.	N. t.
1	3.7	37 000	27	0
2	0.37	3 700	26	1
3	0.037	370	20	7
4	0.0037	37	8	19
5	0.00037	3.7	3	24
Control <sup>1</sup>	0.0	0.0	0	27

<sup>1</sup> – distilled water

### Alternative approaches for the inactivation or elimination of pathogens

Ironically, nothing – including pathogens – live for ever. This common saying applies also for pathogens. An inactive approach to get rid of pathogens according to the advice “just wait, time helps” may be effective under certain circumstances. However, under modern life style conditions active actions are generally preferred. Almost no commercial, but mainly hobby-type activities were and are practised in some selected alternative areas for the inactivation or elimination of pathogens. These may include the following:

#### Biologically:

- composting of carcasses
- production of biogas
- addition of harmless bacteria to decaying plants and carcasses
- addition of cultivated earth worms (*Eisenia foetida* or *Lumbricus terrestris*) or fungi

#### Physically:

- enhanced heat generation during composting
- Heat plus elevated pressure (autoclave)
- Sunshine or UV light
- Gamma radiation

#### Chemically:

- Oxidation by exposure to open air
- Denaturation by addition of lime stone or calcium hydroxide

These measures have their own merits and under certain circumstances and meaningful use in both, under extensive poultry production in developed and in developing countries with small scale productivity. At least some of these germ reduction strategies will still be applied in the future. Major applications of disinfection are established following approved testing of efficacy and likely side-effects. Such testing is performed in suspension and germ carrier tests. This is demonstrated by the methodology provided by the German Veterinary Society.

### **Quantitative virucidal suspension test**

The quantitative virucidal suspension test includes

- Four test viruses: cytopathogenic Newcastle disease virus (NDV), vaccinia virus, strain Elstrey, human reovirus type 1, enteric cytopathogenic bovine orphan virus (ECBOV)
- Several temperatures, at least recommended 4 and 10°C
- With protein (40% foetal calve serum, FCS) and without protein load
- Several concentrations of disinfectant under test
- Quantitative determination of residual virus by titration
- Minimum requirement to pass: at least 4 log<sub>10</sub> reduction as compared to control

Each assay has to be performed in duplicates and the final report must contain details of all methods and a recommendation for practical use. The quantitative virucidal suspension test is considered as an initial test to provide orientation of the efficacy of the tested disinfectant. A quantitative carrier test must follow.

### **Quantitative virucidal carrier test**

The major aim of the quantitative virucidal carrier test is to confirm or to disprove the results of the suspension test. It consists of the following components:

- Four test viruses: cytopathogenic Newcastle disease virus (NDV), Vaccinia virus, strain Elstrey, human reovirus type 1, enteric cytopathogenic bovine orphan virus (ECBOV)
- Carrier: mandatory is poplar wood, optional are linden wood, steel, concrete, others
- Mandatory are temperatures of 4 and 10°C, optional are several other and room temperatures
- Required is a protein load of 40% fetal calve serum (FCS)
- Several concentrations of disinfectant under test
- Quantitative determination of residual virus by titration
- Minimum requirement to pass: at least 4 log<sub>10</sub> reduction as compared to control

### **Number of listed disinfectants per indication in 13<sup>th</sup> list of DVG, 2011**

The sub-committee of the DVG on disinfection in the veterinary field, division animal farming, approved in 2011 a total of 103 commercial products (Table 3). According to the intension of the producers, these disinfectants were not examined for efficacy against all test organisms. Most of the commercial disinfectants (84 products, 81.6%) were examined for virucidal efficacy. Second and third rank bactericidal and fungicidal efficacy. Rather few products were examined for their tuberculocidal and antiparasitic efficacy.

The active compounds of disinfectants intended for use in the veterinary field (animal production) are contained in Table 4. The peroxyacetic acid and combinations of this acid with other organic acids is the most frequently listed compound for the field of animal farming. Second rank aldehydes (various combinations that were not specified in the 13<sup>th</sup> list) either as mono preparation or in combination with quaternary ammonium compounds (QUACs) and alcohols. Various aldehydes – not specified in the 13<sup>th</sup> DVG list – represent also a major group of disinfecting compounds (Table 4). Aromatic circular hydrocarbons such as derivates of phenol and cresol find their prevailing applications for disinfection of parasites (eggs of round- and tapeworms and oocysts of Eimeridae). The organic acids, formic and acetic acid in particular, are now more frequently listed as compared to previous lists. Disinfectants

**Table 3: Number of commercially available chemical disinfectants for use in the veterinary field according to targets of their intended use (13<sup>th</sup> DVG-list, published in 2011)**

Targets for efficacy testing	No. of commercial disinfectants	% of total 103
Virus	84	81.6
Bacterium	79	76.7
Fungus	78	75.7
Tubercle	15	14.6
Parasite	13	12.6

**Table 4: Active compounds in commercial disinfectants for use in the field of animal production according to the 13<sup>th</sup> DVG-list, published in 2011**

Active compounds in commercial disinfectants	Total number disinfectants in the 13 <sup>th</sup> list (2011)	% of total 103 commercial disinfectants
Peroxy compounds	7	6.8
Peroxyacetic acid	16	15.5
Peroxyacetic acid + organic acids	7	6.8
Peroxyacetic acid + quaternary ammonium compounds	4	3.9
Peracetic acid + H <sub>2</sub> O <sub>2</sub>	3	2.9
Aldehydes	17	16.6
Aldehydes + quatern. comp.*	19	18.4
Aldehydes + alcohol	1	1.0
Glutaraldehyde + quatern. amm. comp.	5	4.9
Glutaraldehyde + formalin	1	1.0
Organic acids	4	3.9
Chloramin T	2	1.9
Phenols and cresol derivates	15	14.6
Others	2	1.9

\* - quaternary ammonium compounds

that contain glutaraldehyde or chloramine T are currently listed at rather low rates. Phenols and cresols are of major relevance for their antiparasitic effects and only a few producers of these compounds are listed.

Peroxyacetic acid (PAA) in higher concentrations is explosive, inflammable and corrosive on surfaces of metal. The explosiveness and corrosiveness is now prevented by alkalisation with alkali phosphates and subsequent shift of the pH to  $\geq 8$ . PAA exerts a broad spectrum of efficacy against bacteria, fungi and viruses. Its action is not inhibited by low temperatures and the presence of proteins (with the exception of blood). Other peroxide substances such as performic, perpropionic and perphthalic acids found so far no applications in the veterinary field. PAA alone or in combination with either hydrogen peroxide or organic acids (mainly formic and acetic acid) have a strong oxidizing power. It seems that PAA and combinations with other active compounds gained market shares compared to former years.

Both compounds are also highly germicidal against almost all pathogenic agents.

Obviously, the listed active disinfecting compounds differ markedly according to their intended fields of application in the veterinary fields as compared to food hygiene. The chemical compounds used and listed for use in the fields of food hygiene, production and processing of food are shown in Table 5. Almost half of the listed disinfectants contain quaternary ammonium compounds (QUACs) as active ingredients. This group of chemicals are soluble in water and contain positively-charged hydrophilic radicals. Distinct antimicrobial activity of QUACs is evident even at low concentrations. Most of the gram-positive bacteria are inactivated at concentrations of 50 - 100 mg per millilitre whereas gram-negative bacteria need more than 200 mg/ml. QUACs are not effective against *Mycobacteria* spp. and spores of bacteria. Their action is partially inhibited by the presence of proteins and iron. Hard water reduces the efficacy. QUACs are frequently used in food hygiene due to its easy modes of application and absence of negative effects on surfaces.

Sodium hypochloride and various aliphatic alcohols are popular for application on plane surfaces, tubes and appliances. Their effectiveness is rather broad but alcohols tend to evaporate at room and higher temperatures.

**Table 5: Proportions of DVG-listed chemical disinfectants for use in the fields of food hygiene, production and processing of food**

Active compound in commercial disinfectants	Total number of disinfectants in the 7 <sup>th</sup> list (2011)	% of total 220 commercial disinfectants
Quaternary ammonium compounds	108	49.1
Sodium hypochloride	30	13.6
Alcohols including 1- and 2-propanol	24	10.9
Products containing peracetic acid including and H <sub>2</sub> O <sub>2</sub> or organic acids	22	10.0
Alkylamines	22	10.0
Other compounds	14	6.4

### **Recommended disinfectants for specific indications (13<sup>th</sup> list DVG, 2011)**

Most of the chemicals in disinfectants are known since many decades or even centuries. Nowadays a large number of commercially available products are present on the market. The large number of products for use in hygiene and food processing (total 220 products) and for use in the veterinary field (103 products) reflects the economic efforts of various companies, but not necessarily their creativity. Admittedly, many companies try hard to improve secondary properties of their products such as better adherence to vertical surfaces, spreading on uneven areas, and reduction of surface tension.

In contrast to the large number of available products, the search, invention and synthesis of new chemicals with germicidal properties is lacking. In addition, the effective concentrations and necessary times appear to be rather similar.

Table 6 summarizes recommended concentrations and times to achieve bactericidal, tuberculocidal, fungicidal, virucidal and antiparasitic effects for a selected number of compounds. Not mentioned in Table 6 are additions to the final product that enhance penetration, spreading on surfaces by tensides and related chemicals. Also omitted are properties which affect handling, smell, corrosiveness and other characteristics.

**Table 6: Grouping of selected chemical compounds in relation to their activity against infectious agents**

Active Compound	Target									
	Bacterium		Tubercle		Fungus		Virus		Parasite	
	%	h	%	h	%	h	%	h	%	h
Peroxyacetic acid	1	1	2	2	1	1	1	1	3	1
Organic acids	1	1	4	4	1	2	1	1		
Aldehydes	1	2			1	2	1	2		
Choramine T	3	2			3	2	3	2		
Quaternary ammonium compounds	3	2			6	2	2	2		
Cresols	2	2	4	3					4	2

% - required concentration of disinfectant during application  
h - minimum time of effective exposure of disinfectant

### Antiparasitic disinfectants

Among the targets for disinfection are also the developmental stages of round- and tapeworms and oocysts of coccidia, mainly of the family Eimeridae. All these forms have long survival times in the environment. Consolidated scientific data and practical experience have proven that only few chemicals destroy the viability of these parasitic forms. Most common and listed in the 13<sup>th</sup> list are three specified chemicals. These are p-chlor-meta-cresol, chlor-methyl-phenol and o-hydroxydiphenyl fatty acid eutectic peracetic acid (Table 7). The 13<sup>th</sup> list of disinfectants for use in the veterinary field contains in addition to these four compounds seven disinfectants without disclosure of their chemical names.

From the data in Table 7 it is obvious that rather high concentrations and at least two hours of exposure are needed to inactivate worm eggs and oocysts. It is important to note that two hours is the maximum time for listing of a product. Also, only a small number of producers were listed (see Table 5).

**Table 7: Listed compounds for inactivation of eggs of flat- and round worms and coccidial oocysts**

Active compound in disinfectant	Embryonated eggs of <i>Ascaris suum</i>		Embryonated oocysts of <i>Eimeria tenella</i>	
	%	h	%	h
o-hydroxydiphenyl fatty acid -eutectic peracetic acid (listed twice)	2 A + 1.5 B	2	3 A + 1.5 B	2
p-chlor-m-cresol (listed 4 times)	2 - 3	2	3 - 4	2
Chlor-methyl-phenol (listed twice)	3	2	3	2
Cresols - chemically not specified) (listed 7 times)	2	2	4	2

% - required concentration of disinfectant during application  
h - minimum time of effective exposure of disinfectant

## Criteria for selection of a disinfectant

Advantages and limitations of a given disinfectant should be known by all users. In addition, it is a special duty to investigate and evaluate the areas and structures of a farm prior to application of a disinfectant. Here, local specifications of buildings and their structural components (wood, aluminium, steel, plastics) and likely undesired side effects (corrosion, vapour), the presence of animals and/or humans must be considered.

Beside the structure of a farm, the specific cause of contamination is essential because bacteria, fungi, viruses or parasites demand products of different modes of action, qualities and applications. In case of the confirmed or putative presence of epidemic, notifiable diseases, legal recommendations / prescriptions must be followed.

## Who should disinfect?

The selection from a large number of available products of an appropriate disinfectant and the timely and correct application is a major issue for use in practice. A consulted veterinarian may provide professional advice in this respect. Further candidates for this selection may be the farmers themselves or their employees. None of these persons will guarantee the effectiveness of the performed disinfection. More likely, they will deny any legal responsibility. To prevent any queries, it seems to be wise, to delegate the disinfection to a professional company. Such company will perform disinfection in a professional way, will guarantee the proper performance and will compensate for any possible failure. In Germany, quite recently the company *Gesellschaft für Seuchenvorsorge mbH*, Cloppenburg, Germany, has been founded which will provide professional advice and logistic support in all issues related to disinfection and monitoring of the effect.

In view of any likely failure, it is recommended to monitor the effects of disinfection right after its completion. However, this is rarely done in practice. If it is done, cotton swabs from various representative surfaces should be collected and assayed for residual infectious agents.

## Common errors of disinfection

Reliable statistics on types and frequencies of errors during disinfection are not published and remain unknown to outsiders. Some more general points will draw the attention to possible failures. Among these are (i) a wrong product was used for the intended purpose, (ii) the dilution of the concentrate of a disinfectant was false, e.g. too low or too high, (iii) the exposure time was too short, (iv) applied disinfectant rinsing off or dripping off from vertical walls, (v) not all contaminated areas were reached, (vi) the environmental temperature was too low, (vii) re-contamination occurred at or soon after disinfection.

## Conclusions

Disinfection is a valuable tool in disease control and should be an integral part in prevention and elimination of transmissible diseases. Any disinfection must be done professionally, preferably by experienced companies. Selection of an appropriate disinfectant must be tailored according to a recognized, farm-specific problem. Inherent properties of disinfectants must be known for the selection of the best possible product. For safety and efficacy reasons, instructions of the producer should be followed. Generate a detailed work-plan and time schedule prior to any activity. Duration, time, temperature, concentration of any process of disinfection must be realized to guarantee success. Keep records on all steps of disinfection.

## Summary

Disinfection of the surroundings where farm animals and poultry are kept is an important part of maintaining and/or restoring health and production. The selection of suitable disinfectants from a large number of basic substances available and commercial products with proven benefits and their correct application in specific situations requires extensive knowledge and experience. Technical possibilities and goals for disinfection in different areas of poultry farming are described. Health risks from using disinfectants for animals, man and the environment are also addressed.

## Zusammenfassung

### **Desinfektion als Voraussetzung für erfolgreiche Geflügelhaltung: Ziele und Methoden für den Fachtierarzt**

Die Desinfektion des Lebensraums landwirtschaftlicher Nutztiere ist ein bedeutsamer Teil zur Erhaltung bzw. zur Wiederherstellung der Gesundheit und Leistung. Die Auswahl geeigneter Desinfektionsmittel aus einer großen Zahl verfügbarer Grundstoffe und als wirksam befundener Handelspräparate sowie die fallbezogene korrekte Anwendung erfordern große Sachkenntnis und Erfahrung. Beschrieben werden die technischen und räumlichen Möglichkeiten und erreichbaren Ziele von Desinfektionsmaßnahmen im Rahmen der Nutztierhaltung. Auf gesundheitliche Risiken für Tier, Mensch und Umwelt beim Umgang mit Desinfektionsmitteln wird hingewiesen.

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## Immunology and Disease Prevention in Poultry

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### Introduction

One of the golden rules in the poultry industry is: “only with healthy birds it is possible to get high productive efficiency and with it, economic profitability”.

The first step to prevent sanitary risks is to design a correct plan for biosecurity. In addition, a correct immunization schedule has to be designed and followed for each flock, considering the health challenges expected in the region and the production conditions on a particular farm.

Birds, like all type of animals, have **non-specific** ways to protect themselves in order to maintain good health conditions. Intact skin and mucosa are important factors. The scan action of cilia in the respiratory tract and the low pH in the digestive tract are additional barriers to possible challenges.

In addition to these mechanisms, the normal intestinal flora acts as a kind of barrier for other potentially pathogenic bacteria competing for receptors in the gut wall and the nutritive elements they depend on. Another important point to consider is that the mucosa in the digestive and respiratory tracts as well as in the skin, secrete substances as lysozymes with an important bactericide action. All these mechanisms are part of the non-specific defense system as they act in a similar way against any challenge coming from a pathogen whatever it could be: viral, bacterial or parasite.

In a wide range of different tissues there is a type of cell called macrophage, which does not belong to the immune system but works in association with it. Macrophages have the special capacity to engulf and transform or destroy what they recognize as a foreign agent which may enter the internal media. The result of their action is subsequently introduced to immune cells.

To develop **specific protection** in birds against potential aggressors (bacterial, viral or parasitic), we apply different types of vaccines. To reach a good level of specific antibodies, we need to consider two different factors:

- 1) peculiarities of the avian immune system
- 2) the antigen (vaccine) we are using

The following questions must be answered to optimize the vaccination program:

- which elements of the immune system are involved in the defense against an aggression?
- how do they act?
- what kind of results do we expect from the type of vaccine applied?
- what is the ultimate target for using a particular vaccination?

### The Avian Immune System

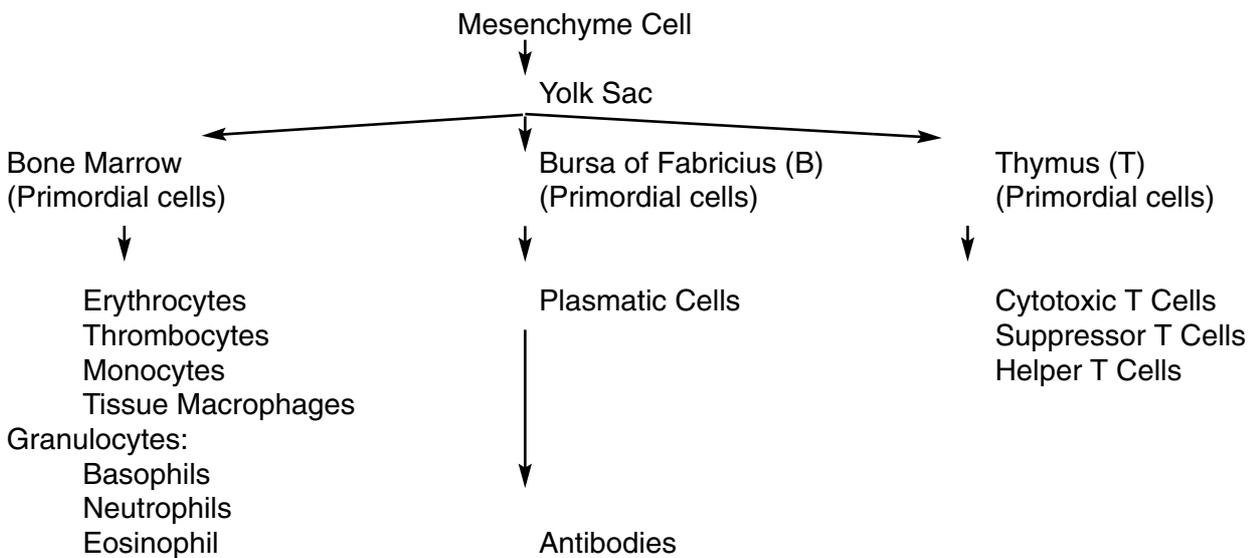
Veterinarians familiar with other animal species need to understand that the avian immune system has peculiar characteristics that make it quite different from mammals.

All immunity cells arise from undifferentiated mesenchyme elements from the yolk sac during the embryonic period. They migrate prior to hatch and during the first 3 days of life to other locations in the future bird as origin of different cellular strains.

After the chick has hatched, the lymphoid organs are classified as primary and secondary. In the first group we find:

- 1) **B**ursa of Fabricius, a close sac-like extension located above the cloaca. It is organized in follicles which are filled with **B** lymphocytes (modulated in the Bursa) where plasmatic cells have their origin. They produce the specific antibodies, which are circulating in the blood stream.
- 2) **T**hymus, a paired organ placed in the neck at both sides of the trachea formed by each five lobules. **T** cells multiply there and are responsible for the cellular immunity.

**Table 1: Migration from the yolk sac and differentiation of cells**



The avian immune system differs from that of mammals as they do not have lymphoid nodules. That is why at a regional level the so called secondary lymphoid tissues are organized as lymphoid cell accumulations. The cecal tonsils, placed in the origin of both ceca are an example of it. In other cases they are in definite structures like spleen.

Finally there are lymphoid tissue accumulations spread in the mucosa. They have a common origin as they have been colonized during the embryonic development by both type of cells, **B** and **T**.

As shown in Table 2, their position is important when trying to reach the best way to get an immune response from live vaccines.

**Table 2: Position of lymphoid tissues associated with mucosa and spread in different organs**

Mucosa Associated Lymphoid Tissues	
Name	Position
<b>Galt</b> (Gut Associated Lymphoid Tissue)	Meckel’s diverticulum Peyer’s Patches (intestinal wall) Esophagus - proventriculus joint Cecal tonsils (gut – ceca joint)
<b>Halt</b> (Head Associated Lymphoid Tissue)	Harderian gland (behind the eyes) Paranasal and lachrymal tissue Conjunctiva Nasal cavity
<b>Balt</b> (Bronchial Associated Lymphoid Tissue)	Primary and Secondary Bronchia Lamina Propria
<b>Scattered</b>	Gall bladder Liver Pancreas Kidney Oviduct

## Immunity and Protection in Poultry

When baby chicks hatch, they have specific immunity against different pathogenic agents called **passive immunity**. These antibodies came from their mothers through the yolk sac. The protection given by these antibodies is short as they disappear from the blood stream within about 10-15 days. Thereafter, each bird has to produce its own specific antibodies actively against different challenges from their environment.

**Active immunity** is a specific reaction to an aggression from any kind of antigen. This is the goal of vaccination, i.e. to challenge the immune system with specific antigens under controlled conditions (e.g. Newcastle disease virus) to get the highest possible level of immune response. Vaccines can be prepared with **live agents** (modified or not) which have the capacity of multiplying in the organism or with **inactivated agents** (killed by physical or chemical procedures) which obviously are not able to multiply in the avian organism.

## Development of the Immune Response

The immune response has different stages. It begins when the antigen has overcome non-specific natural barriers as we have mentioned previously. Then:

- 1) the antigen is recognized as “foreign” to the organism
- 2) a proliferation of different cells from the immune system starts (humoral and cell-mediated)
- 3) the antigen is eventually eliminated
- 4) from that time the birds have “memory cells” which allow a quicker response to that particular antigen in case of another exposure to it

In the first stage macrophages and heterophils engulf the antigen and attract T lymphocytes and inflammatory cells to the place. Antigen is processed and presented to immune cells. Cytokines are chemical messengers secreted by **T** helper and other cells to attract **B** lymphocytes to the site. Then **B** cells proliferate and produce specific antibodies.

After that, memory cells lead to a quicker and higher antibody production if a new aggression from the same antigen occurs.

This complex mechanism starts each time we revaccinate.

As shown in Table 3, different kinds of immunoglobulins with diverse functions are produced after contact with the antigen.

**Table 3: Avian immunoglobulin characteristics**

Immunoglobulins in Poultry			
	Ig A	Ig G (Y)	Ig M
<b>Function</b>	long lasting circulating	early protection	mucosal immunity
<b>Time to appear after contact with antigen</b>	3 – 7 days	2 – 5 days	3 – 7 days
<b>Time to get maximum level</b>	18 – 23 days	5 days	5 – 7 days

## Objectives of Immunization Plans

After understanding how the avian immunity system works, it is time to focus on our objectives when organizing a vaccination schedule for a particular flock. First of all, it must be kept in mind that we are working with large populations and not with single birds; therefore our main interest is to achieve good immunity. In other words, we are not trying to get strong immunity for each individual bird, but a large number of well immunized birds in the population.

Our defined targets may be different, for example:

**Target 1:** Prevention of economic losses associated with clinical diseases.

When we are talking about vaccination benefits, our first thought is usually **to keep birds alive and to prevent a drop in production**. Everybody understands the necessity to have good levels of protection against Newcastle Disease or Infectious Bronchitis, because their direct effects are easy to see and calculate.

**Target 2:** Prevent subclinical diseases.

Financial losses can happen in an indirect way, e.g. due to Infectious Bursal Disease (Gumboro) with immunosuppression. We observe an impaired development of the flock, with reduced weight gain and increased mortality, caused by opportunistic agents. We can also see a very poor response to the vaccination against other pathogens, evaluated by routine serologic tests using ELISA. Although the vaccines are correctly applied, it is not possible to get satisfactory antibody titers.

### A special case: breeder flocks seroconverting

In this case the target is to avoid vertical transmission of pathogens through the hatching egg to the offspring. Breeders may be infected during their production period with or without any clinical signs. If this happens, they will transmit the agent through fertile eggs. When the baby chicks hatch they will show clinical signs of the disease and high rates of mortality during the first and second week. Therefore, vaccination of parent stock against Chicken Anemia and Avian Encephalomyelitis is strongly recommended.

In this case our goal is **not only** to transmit maternal antibodies to the offspring, but to expose all birds in the breeder flock to those viruses at a time when they do not show clinical symptoms and develop active immunity against them prior to onset of lay. Therefore, as they are seropositive (immune to the virus) they are not going to shed it through the hatching eggs to the day-old chicks.

**Target 3:** Displacing very aggressive field strains

Some pathogens are very difficult to control once they get into “problem farms”. This is typically the case in areas with high poultry concentration and/or poor biosecurity conditions. Examples for this could be *Mycoplasma gallisepticum* or Infectious Bursal Disease.

Multi-age layer farms are common in many countries, sometimes combined with the practice of molting. In addition, genetic improvement of the persistency of rate of lay and shell quality has encouraged egg producers to keep their flocks longer. Under those conditions Mycoplasmosis is a real problem. Vaccination with the mild F strain over a sufficient number of years could be a strategy to displace and eventually eliminate a virulent Mg field strain.

Another example are the so called “hot houses” with Gumboro (IBD) where an option is to use a more pathogenic strain for vaccination, accepting the risk of damaging the bursa. If this helps to displace the field strain, it is possible to go back to milder vaccines.

**Target 4:** Transfer of maternal antibodies to offspring.

In some cases transferring high levels of antibodies via the yolk sac from breeders is an option to protect baby chicks better during their first days of life.

It is necessary to reach really high levels of antibodies in breeders because only a part of them go through the yolk sac to baby chicks.

Sufficiently high levels could be reached using inactivated vaccines prior to the onset of lay. It is common to use one or two live vaccines during the breeder’s rearing period and then a booster vaccination a few weeks before laying starts.

Another booster with an inactivated vaccine is common in breeder flocks at about 45 weeks of age to be sure there is no decline in the antibody level. This has been a common practice in controlling Gumboro Disease, but makes it difficult to determine the best time for vaccinating the chicks for the first time. If vaccination is too early, maternal antibodies will interfere, if vaccination is too late, the field strain will have the possibility to damage the Bursa.

New technology can help to solve this problem. It is no longer necessary to know the antibody level, because the interference between vaccine and maternal antibodies has been eliminated. Using a different virus as vector (usually HVT of Marek's disease), only a part of the genetic information of Gumboro virus is expressed.

The immune system recognizes and produces antibodies only against the segments included in the vector without interfering with maternal antibodies.

Later the technicians working in the field will decide whether it is necessary to use a live vaccine to booster the immunity. This discussion is still going on.

Against most common poultry diseases, maternal antibodies cannot protect young chicks against field virus challenge.

### Different types of immunity to pathogenic agents

Finally, we must consider the different types of immunity generated by diverse pathogenic agents. Before deciding which vaccine to use and how to vaccinate, it is necessary to understand what type of immunity will be induced.

**Table 4: Poultry pathogens and predominant type of immunity generated**

Agent	Systemic	Local	Cellular
Fowl Pox			+
Infectious Bronchitis	+/-	+	+
Gumboro (IBD)	+		+/-
Laringotracheitis		+/-	+
Lymphoid Leukosis			+
Marek Disease	+		+
Mycoplasmas		+	+
Newcastle	+	+	+
Fowl Cholera			+
Reovirus	+/-		
AE	+/-		+
Fungal Diseases			+
Parasitic Diseases			+

The summary in Table 4 shows, for example, that high protection against Infectious Bronchitis in layers is achieved with strong local immunity using a live vaccine via eye drop as a primer. In that way the immune tissues located in the head of the birds will easily be reached. An alternative could be spray vaccination to reach the immune tissues in the respiratory tract with the droplets.

Consequently it is possible to get a good immunity, both local and cellular, especially if we repeat the vaccination at two or three different times. Layers must have a high level of circulating antibodies to secure a long lasting protection. It is necessary to booster the effect with inactivated vaccine, applied subcutaneous or intramuscular. In that way enough Ig G (Y) will be present in the blood stream.

Obviously, to get the best possible protection for our birds, a combination of cellular, local and systemic immunity is needed according to the different pathogens. However, the choice of possible alternatives may be limited by the high cost of hand labor or availability.

Individual applications, like eye drop with live vaccines for respiratory diseases, have excellent results but hand labor is extremely expensive in some countries. To find the necessary balance between efficiency and cost, drinking water or spray application could be preferable. These collective ways of vaccination result in lower antibody titers, but require minimal labor cost compared to the individual options.

In other words: the type of vaccine available and route of application will be limiting the result obtained. Against Avian Influenza, only inactivated vaccines are available. That is the reason for using sentinel birds or DIVA strategy (differentiate infected from vaccinated animals) to find out what is really happening in the flock, if there is still viral activity without clinical evidence.

The avian immunity system is quite complex, so prevention plans must be designed according to the objectives we are aiming for. They must be adapted to local conditions.

The knowledge of a specialized technician in poultry diseases is always required and particular conditions in the field must be evaluated if we want the best results in each case.

## Summary

The avian immune system has peculiar characteristics that make it quite different from mammals. After the chick has hatched, the lymphoid organs are classified as primary and secondary. The first group includes the Bursa of Fabricius (containing B lymphocytes producing antibodies) and the Thymus (containing T cells responsible for the cellular immunity). The secondary lymphoid tissues are organized as lymphoid cell accumulations in various locations of the chicken body (e.g. caecal tonsils, mucosa associated lymphoid tissues). Understanding the functions of the immune system is essential when deciding about the antigen presentation of a vaccine or the route of application.

## Das Immunsystem und Krankheitsprophylaxe beim Huhn

Das aviäre Immunsystem hat besondere Eigenschaften, die es deutlich von dem der Säugetiere unterscheiden. Nachdem ein Küken geschlüpft ist, werden die lymphatischen Organe als primäre und sekundäre eingestuft. Zur ersten Gruppe zählt die Bursa of Fabricius (mit Antikörper produzierenden B-Lymphozyten) und der Thymus (mit T-Zellen verantwortlich für die zelluläre Immunität). Die sekundären lymphatischen Gewebe sind in lymphoiden Zellhaufen in verschiedenen Orten des Körpers (z.B. Blinddarm Tonsillen und Schleimhaut assoziierten lymphatischem Gewebe) organisiert. Das Verständnis der Funktionen des Immunsystems ist wichtig bei der Entscheidung über die Antigenpräsentation eines Impfstoffs oder der Applikationsart.

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## **Nutrition-related opportunities and challenges of alternative poultry production systems**

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### **Introduction**

A number of alternative systems for poultry have been developed or revived in recent years, largely in response to demand for improved poultry welfare (Fröhlich et al., 2012). All of these systems have nutritional implications, especially if they are combined with a demand for “organic” production. For those who are less familiar with the term, “organic” production involves requirements such as: low stocking density; access to “outdoors”; late slaughter (lower growth rate); feedstuffs preferably of local origin and grown with strictly limited use of chemicals; no use of synthetic amino acids; no use of genetically modified organisms; restricted use of pharmaceutical products. Organic feed is governed by EU directives such as EC889/2008, which can be accessed on-line at EU or national government sources. A summary of rules regarding organic feed is given in MacLeod and Bentley (2012).

We must be alert to the possibility that some alternative systems may not be in the best interests of sustainability. However, at a time of concerns about climate change, the environment and human population growth, we should be optimistic about what poultry have to offer. Publicising some of the positive features of poultry might help to counteract some of the negatives that have been allowed, almost by default, to become accepted opinion. Objective scientific analysis (Life Cycle Assessment) of the carbon, nitrogen and energy impacts of poultry meat and egg production shows that these are among the most sustainable forms of animal agriculture. It is an inherent biological advantage of poultry that the overheads of reproductive, rearing and maintenance costs are relatively low because of rapid sexual maturation, numerous offspring per parent and short time to slaughter. These features result in low biological, environmental and economic overheads compared with other agricultural species. The changes in genetics, nutrition and husbandry which poultry science and industry have produced over the years have enhanced these inherent advantages.

A dilemma for proponents of alternative systems is that breeding or feeding for lower growth rates or altering environmental conditions or behavioural opportunities may act against these benefits. Most challenges and opportunities related to alternative systems are likely to involve the application of existing knowledge rather than the development of novel nutritional principles. Many of the nutritional challenges arise because feed for some alternative systems may be governed by rules which are not evidence-based. Organic schemes are the most demanding nutritionally but there is a range of others, including certification schemes run by some large retailers. For instance, it is possible to formulate “organic” diets without animal protein, genetically modified organisms and synthetic amino acids but it is difficult to attain nutritional optima. This may lead to sub-standard performance and may even compromise health and welfare (Hadorn *et al.*, 2000). Furthermore, nitrogen excretion and the associated environmental impact will be greater if an imbalanced protein mixture has to be used to attain nutritional requirements.

### **Nutritional costs of alternative systems**

Much of the dietary energy consumed by poultry is used for “maintenance”, i.e. to sustain the processes which keep the bird in a steady state. Any alternative system is likely to affect the bird’s maintenance requirement, particularly if locomotor activity or the thermal environment are altered by the system of housing, husbandry or nutrition. Even in the confined conditions of chamber calorimeters, about 12% of the energy expenditure of a light layer strain was attributable to locomotor activity, compared with about 5% for broilers (MacLeod *et al.*, 1982). Activity can also be predicted to produce differences in energy requirements and food intakes between different housing systems. Pre-oviposition behaviour increases heat production by about 60% over the resting value (MacLeod and Jewitt, 1985), similar to treadmill measurements of the cost of walking. Feeding, drinking and preening activity have each been shown to increase heat production by about 25% (MacLeod and Jewitt, 1985). Environmental temperature and plumage condition must also be considered in alternative systems.

A 1°C reduction in temperature will raise the energy requirement by approximately 20 kJ/d, equivalent to 1-1.5% more feed in well feathered hens in temperate climates. This effect may be twice as great if feather condition is poor (Tullett *et al.*, 1980). With increased activity and thermoregulatory costs, feed intake could, therefore, be 10-20% higher in outdoor systems. Since poultry are not always the most active of birds, differences among indoor systems may not be so great. A recent life cycle assessment of conventional and less intensive indoor systems of broiler and egg production, based on feed intake and fuel use, indicated that there was little difference in environmental impact, especially when heat exchanger ventilation was used (Leinonen *et al.*, 2013). This was a rather limited study, even in the authors' opinion, and further assessment is needed.

### **Qualitative control of feed and nutrient intake**

Selecting among food sources so as to obtain the appropriate mixture of nutrients is essential for birds living under natural conditions. This ability is of such fundamental evolutionary advantage that it seems unlikely to have been eliminated from domestic poultry by generations of breeding on compound diets. The persistence of this ability has been tested many times in poultry, with variable results (Rose and Kyriazakis, 1991; Henuk and Dingle, 2002), although choice feeding was common practice before requirements had been sufficiently well defined to allow the formulation of nutritionally complete diets. However, the re-ascendancy of free-range poultry husbandry raises the possibility of birds obtaining a supplementary source of feed items from the range or pasture.

#### **Supplementary range feeding**

A much-desired advantage of access to outdoor areas is the availability of supplementary feed, whether animal, vegetable or mineral. However, this advantage can be difficult to quantify since it depends on ecological factors, such as the quality and biodiversity of the "range" area, stocking density and also on behavioural factors such as the readiness and ability of the birds to move over the area and select from its resources. Knowing the intake and composition of forage has the potential to allow fine-tuning of the main (farmer-provided) diet, although there is so much scope for variation between and within farms that it may not always be economically justifiable to do so.

Assessing the contribution of foraging to nutrient intake may have to rely on methods such as sampling of crop contents (Antell and Ciszuk, 2006). Horsted *et al.* (2007) used this technique to assess the intake of different forages when hens were given either a typical organic layer concentrate (184 g crude protein /kg dry matter) or a nutrient-restricted diet consisting of whole wheat (120 g CP/kg DM) and oyster-shell grit. The latter diet was intended to encourage foraging and did indeed produce significant effects, being associated with greater crop contents of plant materials, oyster shell, insoluble grit and soil. There was no significant difference in intakes of animal matter, such as earthworms and larvae, which might have been expected if the birds were "adjusting" their nutrient intake. However, the authors suggested that the range area had already been depleted of such items before the measurements started, illustrating a source of variation which can potentially be controlled if sufficient land is available.

Results from an invertebrate-rich pasture are described by Sun *et al.* (2013). A suitably managed pasture can be seen as a source of materials other than the obvious macro-nutrients. Ponte *et al.* (2008) studied some of the effects of a legume-rich pasture (clover, etc.) on broiler performance and meat quality and found generally positive effects. Positive effects of forage plants on egg quality have also been described (Hammershøj and Steinfeldt, 2012). However, poorly designed or poorly managed systems can lead to overloading of the pasture with nitrogen and phosphorus and increase losses of nitrogen and phosphorus to the environment (Dekker *et al.*, 2012).

#### **Yolk colour**

Yolk colour is an aspect of product quality that can be expected to improve with access to suitable pasture. Especially when diets are based on wheat or barley, synthetic or concentrated xanthophyll supplements may be added to the feed in conventional systems, to give the preferred intensity of yolk colour (Nys, 2000). The plant pigments are natural derivatives of  $\beta$ -carotene. They are present at high concentrations in plant materials such as marigold meal and some species of algae but are also present at practically useful concentrations in many potential forage plants.

**Whole grain feeding**

Feeding whole grain may occur as part of the nutritional strategy in alternative systems. This has several potential advantages: it provides a form of environmental enrichment for the bird (Picard *et al.*, 2002), it encourages muscular development of the gizzard and it reduces feed processing costs. Grain (e.g. wheat, barley, oats) can be provided separately in a choice feeding system, mixed with mash or fed at alternating times to a compound diet (sequential feeding; Rose *et al.*, 1995). Starch digestibility is improved by the addition of whole wheat (Hetland *et al.*, 2002). The gizzard has a well-developed ability to grind down larger particles such as whole grains and increased gizzard size and activity may increase the opportunity for enzymatic digestion. However, not all whole grain systems have given positive results (Bennett and Classen, 2003). It should be noted that simply adding whole cereal grains to an existing compound diet will dilute many nutrients. This may be advantageous if maintenance energy requirements have increased (e.g. under more extensive systems), since energy intake will be allowed to increase without excessive additional intake of the more expensive components of the diet. Umar Faruk *et al.* (2010) noted that loose-mix feeding of grain with a compound “balancer” diet had no effect on ME intake. However the loose-mix treatment reduced feed and protein intake due to lower intake of the balancer diet, resulting in lower egg production and lower egg and body weights than sequential feeding. Sequential feeding of whole grain and a concentrate resulted in similar egg-laying performance to conventional feeding and thus could be used to advantage in situations where it is applicable.

**Specific nutrient appetites**

It may be possible to cater for specific nutrient appetites in some alternative systems. A calcium appetite is particularly clear in the laying hen (Mongin and Sauveur, 1979) and separate feeding of a calcium source is one form of free choice feeding that is reliably successful. It has the advantage over feeding calcium only as part of a complete compound diet that the intake of calcium is dissociated from energy and protein intake and can occur at the time of maximum physiological demand (e.g. for egg shell deposition).

**Nutrient effects on behaviour**

It has sometimes been asserted that a lack of animal protein in the diet makes pecking damage more likely; this has not been supported by controlled experiment (McKeegan *et al.*, 2001). However, an imbalanced diet (independently of whether animal protein is included) may induce such behavioural effects (Elwinger *et al.*, 2008).

**Feeding programmes in alternative systems**

Diets and feeding programmes are usually devised from tables of recommended nutrient concentrations. Such tables have wide applicability for conventional systems, because these use standardised environments, common genotypes, consistent feed presentation and well defined ingredients, none of which are always guaranteed in alternative systems. This severely reduces the direct application of general nutritional tables to alternative systems, which require a more flexible, iterative, approach, involving reliable feedback about performance from the producer to the nutritionist (MacLeod and Bentley, 2012). This may allow the formulation of diets specifically for an individual flock but this will depend on the scale of the production and feed mill operations. Tailoring the diet for a specific flock may often entail adapting the use of a standard commercial feed.

**Environmental impacts of nutrition in alternative systems**

This subject is discussed in greater detail in MacLeod and Bentley (2012). Organic poultry meat and egg production increase “fuel” energy use by about 30% and 15%, respectively, compared with conventional systems (Williams *et al.*, 2006; Bokkers and de Boer, 2009; Leinonen *et al.*, 2012). This is because the lower energy cost of producing organic feed is counteracted by lower bird conversion efficiency, which results in higher feed intake. Providing optimally balanced protein is usually practicable only with supplemental amino acids (currently not permitted in organic diets) and is well known to reduce nitrogen (N) losses. (e.g. Kim and MacLeod 2001; Table 1):

**Table 1: Nitrogen retention and loss by broiler chickens on diets with the same lysine concentration but a wide range of crude protein content**

Diet	1	2	3	4	5
Metabolisable energy MJ/kg	13.4	13.4	13.4	13.4	13.4
Crude protein (CP) g/kg	180	210	240	270	300
Lysine concentration g/kg	11	11	11	11	11
Lysine : CP ratio	0.061	0.052	0.046	0.041	0.037
N intake (g/bird.d)	4.10	4.18	5.29	5.90	6.18
N retention (g/bird.d)	2.68	2.43	2.60	2.61	2.60
N loss (g/bird.d)	1.41	1.75	2.68	3.29	3.59
Efficiency of N retention	0.66	0.58	0.49	0.44	0.42

This experiment showed N retention efficiency (N retained in body/N intake) falling from 0.66 on a near-ideal protein to 0.42 on an imbalanced diet. N retention was held constant, because of a constant and limiting dietary lysine concentration, but there was a 2.5-fold increase in N excretion.

There are further possible environmental consequences of restrictions on the use of “non-organic” raw materials. IFEU (2002), for example, showed that 1 kg synthetic DL-methionine requires only 16% of the energy needed to provide the same amount of methionine from soybean or rapeseed meal. The degree to which a perfectly balanced (ideal) protein is used is an economic or legislative matter, because the relevant science is clear. As well as reducing nitrogen losses to the wider environment, it may improve bird welfare by improving floor and litter conditions and may also reduce ammonia concentration in the house environment.

## Conclusions

All the classical rules of nutrition apply to alternative systems, but there are differences in the way they have to be applied. There is certainly a need for good channels of communication between the producer and the nutritionist. Because of the variables affecting alternative systems, such as climatic environment and locomotor activity, the optimal application of nutritional principles requires observation and recording of flock performance against defined targets, with iterative adjustment of nutrition as required. There are nutritional advantages or opportunities to be tested in alternative systems. These include such things as: supplemental feeding on plants and invertebrates by free range poultry; effects of supplemental feedstuffs on product quality; choice or sequential feeding to meet the birds’ varying requirements; whole grain feeding. Organic nutrition has the undoubted advantage of avoiding the release of pesticides and herbicides into the environment but also has environmental costs; there is increased environmental impact because of reduced feed conversion efficiency related to deliberately reduced rates of production; current regulations prevent the use of supplemental amino acids, usually resulting in increased nitrogen losses and pollution. The strategy which should benefit all husbandry systems is to continue breeding for efficient utilisation of nutrients. Genetic selection may be less immediate than nutritional methods but it has the advantages of “permanency” and, potentially, a degree of independence from diet composition. The latter may be particularly valuable when there are impediments to formulating a balanced amino acid composition, such as might occur with organic diets or if there is an increasing tendency to use imbalanced protein co-products from biofuel production.

## Zusammenfassung

### Bedarfsgerechte Ernährung von Geflügel in alternativen Haltungssystemen - eine Herausforderung für Forschung und Praxis

In den letzten Jahren wurde die Haltung von Legehennen in vielen Betrieben von Käfighaltung auf Bodenhaltungssysteme umgestellt, vor allem um Forderungen nach mehr Tierwohl zu erfüllen. Das erfordert Anpassungen in der Fütterung, die vor allem bei der Produktion von Bioeiern nicht einfach zu erfüllen sind. Angesichts steigender Weltbevölkerung und drohender Erderwärmung scheint die Forderung nach optimaler Nutzung begrenzter Ressourcen schwer mit der Bioproduktion vereinbar, aber die Produktion von Geflügelfleisch und Eiern wird ihren Wettbewerbsvorteil gegenüber anderen Formen der tierischen Veredlung hinsichtlich Nachhaltigkeit behalten. Dank der hohen Reproduktionsrate und des schnellen Wachstums sind der Erhaltungsbedarf und damit die Umweltbelastung relativ gering. Die biologischen Vorteile konnten durch Selektion und verbesserte Ernährung und Haltungstechnik weiter ausgebaut werden.

Ein Dilemma für die Befürworter alternativer Haltungsverfahren ist, dass mit der Forderung nach langsamerer Gewichtszunahme, Verzicht auf leistungssteigernde Futterkomponenten und mehr Bewegung die Erfolge von Züchtung und Ernährung teilweise aufgehoben werden. Die meisten Fütterungsempfehlungen für alternative Haltung beruhen auf der Anwendung bekannten Wissens und sind nicht ganz neu. Viele Probleme ergeben sich aus gesetzlichen Vorgaben und Spezialanforderungen großer Handelsketten, die keine wissenschaftliche Basis haben. Biofutter muss sich an die EU Richtlinie EC889/2008 halten; eine Zusammenfassung der Regeln wurde kürzlich von MacLeod und Bentley (2012) veröffentlicht. Z.B. kann man "Biofutter" ohne tierisches Eiweiß, GM Getreide und synthetische Aminosäuren formulieren, aber die Optimierung bleibt schwierig. Das kann dann zu Minderleistung und sogar Beeinträchtigung der Gesundheit und des Tierwohls führen (Hadorn u.a., 2000). Außerdem führt die Einhaltung der Bio-Richtlinien zu einer höheren Umweltbelastung infolge höherer N-Ausscheidung, weil zur Bedarfsdeckung mehr Protein verfüttert wird als bei ausgewogenem Aminosäureprofil erforderlich und bei Intensivhaltung üblich ist.

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## Improving the Nutritive Value of Poultry Feedstuffs: the Rapeseed Products Example

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### Introduction

Commercial brown-egg layers have the genetic potential to produce 26.5 kg egg mass per hen housed (405 eggs with 65.5 g average weight) in a prolonged laying period of 16 months, with a feed conversion ratio of 2.1 kg feed per kg egg mass (Lohmann Tierzucht, 2011). Rapid growing broilers reach market weight of 2.1 kg live weight in 5 weeks, with a feed conversion of 1.6 kg feed per kg live weight gain (ROSS, 2012). The genetic potential to convert feed nutrients efficiently into eggs and poultry meat (broiler, turkey, duck) for human consumption can only be fully utilized if the birds are well managed, remain healthy and receive highly digestible, concentrated and well balanced feed rations (Jeroch *et al.*, 2013). The challenge for commercial feed formulation is to optimize feed composition from a reduced choice of raw materials, limited inclusion rates and special attention to anti-nutritive substances.

Table 1 shows the annual use of components currently used in poultry rations in Germany. The dominating raw materials are cereals (mainly wheat and corn) and soybean meal, which are also used for human consumption and the production of bioethanol (cereals).

**Table 1: Annual demand for raw materials of poultry rations in Germany (DVT, 2013)**

Raw materials	Layer rations		Fattening rations		Source
	Mio t	%	Mio t	%	
Cereals (Legumes)	1.4	61	1.6	62	Germany, EU,
Soybean meal	0.3	13	0.5	19	South America, USA, (EU)
Other by-products of oil manufacture (Rapeseed meal, sunflower meal)	0.15	6.5	0.1	4	Germany, EU
By-products of the food industry	0.15	6.5	0.15	5.5	Germany, EU
Oils and fats	0.1	4.3	0.15	5.5	Germany, EU, other
Minerals (phosphate)	0.2	8.7	0.1	4	Germany, EU, other
Total	2.3	100	2.6	100	

DVT= Deutscher Verband Tiernahrung

The available resources are limited and have to be utilized efficiently in animal feed. Poultry nutrition is focused on converting feed protein to egg and poultry meat protein. According to own calculations, feed protein is converted to edible protein at ratios of 33% in eggs, 26% in broiler meat, 24% in turkey meat and 20% in duck meat (Jeroch *et al.*, 2013). In the following outline, we will examine possibilities to improve the conversion ratio further and to include other components.

## Goals and methods to improve feed value

The main **goals** are:

- Species-appropriate feed, higher feed intake, lower mortality
- Higher content of valuable and lower content of indigestible components
- Reduction of antinutritive and undesirable components
- To optimize digestion and intestinal health
- Improved resorption of nutrients and enhanced nutritional value of feed and
- Improved feed hygiene in source components and finished feed

Classical methods have been improved over the years and continue to be used, sometimes with “new” refinements. Of special importance are:

- plant breeding
- biological treatments (e.g., fermentation)
- use of feed additives (e.g., feed enzymes, probiotics, organic acids, phytobiotics)
- chemical treatments (e.g., decontamination)
- technical treatments (e.g., cleaning, milling, shelling, pelleting, toasting, decontamination, extruding, expanding).

## Rapeseed as an example for systematically improved the nutritive value

### **Results of successful genetic improvement of rapeseed**

During recent decades, plant breeders in Europe and Canada improved the quality of rapeseed for human and animal nutrition significantly, as shown in Table 2. Starting from technical oil with about 50% of the problematic erucic acid (C 22:1, which is deposited in body fat and may cause heart problems), a valuable product for human consumption and component of animal feed has been developed. The by-products of oil extraction have become useful for monogastric animals after significant reduction of glucosinolate (GLS). The conventional rapeseed varieties had negative effects on thyroid function - glucosinolates inhibit iodine uptake by the thyroid - and performance in poultry if included at low levels of 2-3% rapeseed meal in poultry rations. These by-products could only be used in ruminant rations.

**Table 2: Results of genetic improvement of rapeseed<sup>1</sup>**

Variety, breeding goal	Quality assessment
Traditional varieties	High content of erucic acid in oil, high concentration of antinutritive substances in fat free dry matter, especially glucosinolate, sinapin and others
0-Varieties (improved single quality): 1 <sup>st</sup> step in quality breeding: 0-Winter variety of rape seed in Germany since 1973	Varieties of winter rape seed in Europe, summer rape seed (Canola) in Canada and Northern Europe with minimal content of erucic acid (from 50 % down to <1 % of total fatty acids)
00-Varieties (improved double quality): 2 <sup>nd</sup> step in quality breeding: 00-Winter variety in Germany since 1981; 00-Summer variety of rape seed already earlier (Canada and Northern Europe)	Varieties with < 1% erucic acid and reduction of glucosinolate content (from about 100 µmol to <18 mol/g seed)
00-New 00-Varieties for winter and summer rape seed	< 10 µmol glucosinolate/g seed
000-Varieties (triple quality, yellow), available for summer rape seed (Canola)	20-30% less crude fiber in grain due to reduced shell content, 50% less lignin

<sup>1</sup> BECKER *et al.*, 1999; RÖBBELEN, 1997 and 2001, Lie *et al.*, 2007; Bartkowiak-Broda *et al.*, 2011

As shown in Table 2, the glucosinolate content has been further reduced in the new double quality varieties. This achievement of plant breeding, combined with the toasting process in modern oil mills, has reduced the content of glucosinolate in the rapeseed meals (RSM) to a relatively low level. A 4-year study (2005-2008) of rapeseed meal quality in Germany showed glucosinolate levels between 6.8 and 9.3  $\mu\text{mol/g}$  RSM (WEBER, 2009). Plant breeders in Poland and Canada are aiming for further reduction of glucosinolate levels in rapeseed and corresponding rapeseed meal. RSM with lower GLS content can be included in poultry rations without any negative effects, as shown by the results in Table 3.

**Table 3: Results with rapeseed meals (RSM) from low glucosinolate (GLS) rapeseed in rations of growing turkeys and laying hens**

Literature	Birds	RSM-content %	GSL-content $\mu\text{mol/g}$ RSM (88 % DM)	Comments
Mikulski <i>et al.</i> (2012)	Male turkeys	0/6/12/18	4.4	Little effect on growth and carcass value; no increase in foot pad lesions
Campbell <i>et al.</i> (1999)	White-egg Layers	0/10/20	1.8	Same performance in all groups, no effect on functioning of organs
Rodehutscord <i>et al.</i> (2012)	Brown-egg Layers <sup>1</sup>	0/5/10/15	6.0	No negative effects on performance and egg quality

<sup>1</sup> without tainter gene

A new goal for plant breeders are varieties of rapeseed with reduced total fiber and lignin content (so-called triple quality, shown in Table 2). With this, the digestibility of crude protein and amino acids should be improved and the energetic value of feed increased. In Canada experimental yellow varieties of rapeseed with reduced shell content are currently being tested. They contain less fiber and oligosaccharides per kg dry matter (DM) compared to the conventional black varieties and more crude protein and amino acids. Preliminary results suggest also an improved digestibility of amino acids and a higher content of energy (Table 4).

**Table 4: Feed quality parameters of rapeseed meal from black and yellow rapeseed varieties<sup>1</sup>**

Parameter	Birds	Rapeseed meal from	
		Black variety	Yellow variety
Fiber-/lignin content (g/kg DM)		301/71	241/37
Oligosaccharide content (g/kg DM)		36	21
Crude protein (g/kg DM)		438	498
Average pc AA-digestibility (%)	broilers	83	89
Metabolizable energy (MJ AME/kg DM)	broilers	7.98 (100)	9.18 (115)
	turkeys	8.40 (100)	9.08 (108)

<sup>1</sup> Slominski *et al.* (2007, 2011), Jia *et al.* (2013)

Additional goals for rapeseed breeders are the reduction of other antinutrients, e.g., phenolic substances (sinapin), tannins and phytin acid, while further reduction of glucosinolate continues.

**Positive effects of technical treatments**

Several technical solutions have also been developed to reduce the fiber content of rapeseed cake and rapeseed meal. **Peeling the seeds** before processing (Kracht *et al.*, 1998) improves the nutrient composition (Table 5). The fiber content decreases, especially lignin is reduced (by 50%), while the content of crude protein, amino acids and other nutrients is enhanced. The AME<sub>N</sub> content depends mainly on crude fiber reduction by peeling and varies with the age of the birds (Table 6).

**Table 5: Contents (g/kg DM) of rapeseed meal from not dehulled (nd) and dehulled (d) seeds of identical rape varieties**

Authors		Crude ash	Crude protein	Crude fat	Crude fiber	NDF <sup>1</sup>	Lignin	Lysine
Kracht <i>et al.</i> (2004)	nd	77	396	21	117	268	88	19
	d	82	424	21	72	193	44	22
Huang <i>et al.</i> (2007)	nd	79	386	14	118	n.a.	n.a.	13
	d	78	468	9.5	56	n.a.	n.a.	21

<sup>1</sup> neutral detergent fiber

**Table 6: Effect of dehulling rape seed on energetic feed value of rapeseed meal and rapeseed cake (Jeroch *et al.*, 2001)**

Feed source	Birds	Not dehulled seed		Dehulled seed		AME <sub>N</sub> -increase %
		Crude fiber g/kg DM	AME <sub>N</sub> MJ/kg DM	Crude fiber g/kg DM	AME <sub>N</sub> MJ/kg DM	
Rapeseed meal	Broilers	117	6.94	72	8.27	19
	Layers	117	8.08	72	9.91	23
Rapeseed cake	Broilers	102	11.42	61	13.05	14

Dehulling before processing also improves the amino acid (AA) digestibility in by-products of rape seed oil production included in rations for fattening pigs and broilers (Kracht *et al.*, 2004; Zuprizal *et al.*, 1992). The results with broilers are shown in Table 7. The digestibility of the meal from peeled rape seed is close to that of soybean meal.

**Tabelle 7: Effect of dehulling rapeseed on digestibility of amino acids of rapeseed meal in broilers (Zuprizal *et al.*, 1992)**

Amino acid	Rapeseed meal		Soybean meal
	Not dehulled	Dehulled	
Lysine	76	82	84
Methionine	84	90	89
Cystine	76	80	80
Threonine	78	82	84

Other technical treatments of rapeseed (hydrothermic conditioning, micronising, infrared treatment) and of rapeseed meal (expansion, extrusion) showed no improvement of energetic feed value for broilers (rapeseed, rapeseed meal) and laying hens (rapeseed), whereas treatment of rapeseed with **hot air** (Jet sploding) at 125 °C was successful (Dänicke *et al.*, 1998).

**Chemical-hydrothermal treatment** of rapeseed (Brettschneider, 2006) reduced the glucosinolate content more effectively than toasting of RSM in modern oil mills. This treatment reduced the glucosinolate content from 13.8 µmol/g (91 % DM) to 1.5 µmol/g. It should be pointed out, however, that a very high inclusion rates of expanded rapeseed in layer rations can have negative effects on thyroid function (Jeroch *et al.*, 2008), probably caused by degraded glucosinolates.

Sinapin (in layer feed responsible for the „fishy“ taint of eggs from brown-egg layers with insufficient activity of trimethylamine oxidase) has been treated successfully with a combination of **chemical** (10% sodium bicarbonate solution) and **hydrothermal treatment** (expander) to minimize the negative effects (Jeroch *et al.*, 1999). Rapeseed products have been included up to 7.5 % in rations for brown-egg layers with tainters (Dänicke *et al.*, 2006). Since then, primary breeders have eliminated the tainting gene, which makes the feed treatment unnecessary.

### The potential of added feed enzymes

Rapeseed has essentially no phytase activity in the seeds and therefore low utilization of total phosphorus (with its high content of phytin) in the by-products of oil extraction. Oloffs *et al.* (2000) estimated 28 % phosphorus (P) utilization in laying hens for meal and 22% for cake, compared to 46-49 % utilization for phytase-rich wheat. **Phytase supplementation** improved the P utilization in broilers und laying hens significantly, as shown in Table 8 (Dänicke *et al.*, 1998). Therefore rations with rapeseed by-products should be supplemented with a microbial phytase preparation.

**Table 8: Effect of phytase supplementation with rapeseed by-products on P-utilization in 43 meat type and egg type chickens (Dänicke *et al.*, 1998)**

Birds	Rapeseed by-products	Phytase	P utilization (%)
Broilers	Meal	without	
		with	62
	Cake	without	35
		with	52
Layers	Meal	without	6
		with	32
	Cake	without	22
		with	40

The high content of cell wall carbohydrates (non-starch polysaccharides, NSP) and indigestible oligosaccharides suggested testing of products with **NSP degrading enzymes**. The results of different authors (Kocher *et al.*, 2000, 2001; Slominski *et al.*, 2003, 2007; Fang *et al.*, 2007, Zdunczyk *et al.*, 2011) vary and cannot be summarized as a recommendation to add enzymes to feed with rapeseed products. The lack of enzyme effects may be due to the use of preparations developed for NSP in grain rather than rapeseed. Short testing periods may also explain inconclusive results (Table 9).

**Table 9: Effect of carbohydrase in a broiler ration with 30% rapeseed meal from a yellow variety<sup>1</sup>**

in parameters	Enzyme addition <sup>2</sup>	
	without	with
Viscosity of digesta (mPaos)	1.73	1.66
8-week body weight (kg)	3.68	3.77
FCR (kg feed/kg gain)	2.19	2.08

<sup>1</sup> Zdunczyk *et al.* (2011),

<sup>2</sup> enzyme mix of pectinase, cellulase, xylanase, glucanase, mannanase and galactanase

### Comparison of Rapeseed vs. Soybean

The differences in nutritional value compared to soybean meal have become smaller and are now almost negligible (Table 10). The dominating share of soybean meal in poultry rations could be largely replaced by rapeseed products.

**Table 10: Feed quality comparison of rapeseed meal vs. soybean meal (based on data in previous tables)**

Parameter	Rape seed meal		Soybean meal
	Yellow variety	Dehulled 00-variety	
Crude protein (g/kg DM)	498	450	510
Crude fiber (g/kg DM)	- <sup>1</sup>	65	67
Lignin (g/kg DM)	37	44	
Lys-digestibility (%)	88 (chicks)	82 (chicks)	84 (chicks)
AMEN (MJ/kg DM)	9.18 (chicks)	9.91 (hens)	11.55 (cocks)

<sup>1</sup> not analyzed

Improvements of the nutritive value have also been achieved in other feedstuffs as a result of plant breeding and a variety of feed treatments. Current knowledge should be fully utilized to optimize modern feed formulation (Jeroch *et al.*, 2013).

### Conclusions

Rapeseed meal and its by-products from oil mills are good examples to illustrate the importance and benefit of improving the nutritive value of feed components for poultry rations. Even small inclusion rates of rapeseed products from conventional varieties resulted in compromised thyroid function and reduced productivity forty years ago, but up to 25% of products from improved double and triple varieties can be included in poultry ration today without any problem.

The following possibilities are suggested:

- Reduction of additional antinutritive components using conventional and molecular genetic methods in plant breeding and use of new feed additives
- New generation of carbohydrases for effective degradation of NSP-fraction in protein feedstuffs
- Development of phytase with significantly improved efficacy and consistency
- Application of new protease enzymes in order to improve protein and amino acid utilization from the raw materials – with the final target to reduce crude protein in diets
- Utilization of the NSP-fraction in raw materials as source of energy by enzymatic disintegration within and outside the intestinal tract in order to release additional nutrients for poultry.

## **Zusammenfassung**

### **Möglichkeiten der Futterwertverbesserung beim Geflügel am Beispiel Rapsprodukte**

Im vorliegenden Beitrag werden zunächst Ziele und Methoden der Futterwertverbesserung beim Geflügel vorgestellt. Am Beispiel von Rapssaat und Rapsfuttermitteln wird der Nutzen von verschiedenen Maßnahmen demonstriert. Der Pflanzenzüchtung ist es in wenigen Jahrzehnten gelungen, den Gehalt an antinutritiven und futterwertmindernden Inhaltsstoffen so stark zu reduzieren, dass die Nebenprodukte aus der Rapssaatverarbeitung inzwischen wertvolle Proteinfuttermittel geworden sind und in Geflügelrezepturen in beachtlichen Anteilen eingesetzt werden können. Deutliche Futterwertverbesserungen lassen sich auch durch technische Behandlungen, z.B. durch Schälen der Rapskörner, erreichen. Ein weiteres Potenzial liegt im gezielten Einsatz von Enzympräparaten (Phytasen, Carbohydrasen, Proteasen). Auf diesem Gebiet der Futterwertverbesserung gibt es noch beachtliche Entwicklungsmöglichkeiten und somit auch Forschungsbedarf.

References to the literature cited can be obtained from the corresponding author:

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## Infrared beak treatment – a temporary solution?

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Beak treatment of laying hens is a „hot spot“ issue in animal welfare. In most EU countries, this prophylactic treatment is limited to a 5 year period, requires a special permit from veterinary authorities and has to be applied no later than 10 days of age. As demonstrated in many experiments (e.g. Eißele & Kraft, 1993; Lange, 1997; Damme, 2011), beak treatment of pullets is an effective means of reducing feather loss and cannibalism in laying hens. Even animal welfare organizations and politicians accept that banning beak treatment on short notice would only trade one welfare problem (pain of chicks from beak treatment) for another welfare problem (increased injuries and mortality due to cannibalism in adult hens). Two current field studies in Lower Saxony (11 farms) and Bavaria (15 farms) are designed to improve our understanding of primary causes of picking behavior in order to minimize the risk of cannibalism by optimized management practices, housing environment, nutrition, health and hygiene as well as lighting. A long-term solution should also include genetic selection (Bessei, 2012). This has been recognized as an important challenge by primary breeders, and responsible geneticists of Lohmann Tierzucht and Hendrix Genetics suggest at least 6-8 years as a realistic time window to expect significant improvements in practice from dedicated selection at the pedigree level.

In the meantime, hen welfare can also be improved step by step, e.g. applying beak treatment earlier and with new techniques to minimize pain. In previous years, pullets were commonly beak treated with a hot blade up to 10 days of age on the growing farm. Nowadays, layer chick hatcheries are increasingly using the infrared (IR) technique from Nova-Tech, which had already been used for turkey poults since a number of years.

The present study was designed to study the effects of different methods of beak treatment in commercial laying hens during the growing and laying period.

### Experimental design

A total of 3,600 day-old chicks, representing a popular white-egg (LSL) and brown-egg (LB) variety, were obtained from a commercial hatchery (Gudendorf-Ankum) and divided into 6 experimental groups: 1/3 per strain kept as untreated control, 1/3 beak treated with infrared technique (Nova Tech) in the hatchery, 1/3 beak treated with a hot blade (Lyon Debeaker) on the rearing farm 9 days after housing.

The pullets were reared to 126 days (09.06-13.10. 2010) in a windowless house (30 x 12 m) with thermostatically controlled low pressure ventilation and spray cooling; 600 pullets per strain and treatment in a pen of 42.5 m<sup>2</sup> (8.32 m x 5.11 m); 14.1 birds /m<sup>2</sup>; nipple drinkers, chain feeders and gas brooders. Feed and water were offered ad libitum on floor level and on an elevated platform (Big Dutchman), which was easy to reach with help of an A-frame from week 3. Two thirds of the floor area had litter with heat treated soft wood shavings, one third had perches over plastic grids, with a manure belt underneath. The step-down, step-up lighting program was as described by Urselmans and Damme (2012). Prophylactic health treatment was organized by the Bavarian Poultry Health Service GGD. The following feeding program was used:

Type of feed	Weeks	ME (MJ)	CP (%)	Meth. (%)	Ca (%)
Chick starter	1-8	11.5	18.5	0.40	1.00
Pullets AF	9-18	11.4	16.5	0.35	0.90
Layers AF I	19-56	11.6	18.0	0.42	3.75
Layers AF II	57-72	11.4	17.5	0.40	3.85

During the laying period the hens were kept in two window houses with 12 pens each, for 110 and 138 hens, respectively (8/m<sup>2</sup>). Each strain and treatment was thus tested with 496 hens in 4 replicate pens. Each pen had a litter area with wood shavings, 5 feeder pans and access to a common line of nipple drinkers.

## Data recording

### Rearing:

Body weights were recorded from a sample of 80 birds per pen at 7, 14, 21 and 28 days of age. After 8, 12 and 18 weeks all pullets were weighed and feed consumption per pen determined. Mortality was recorded daily.

### Laying period:

Egg production, mortality and apparent cause of death was recorded daily throughout the 52 week laying period, average egg weight and grading results weekly, and feed consumption at the end of each 28-day period.

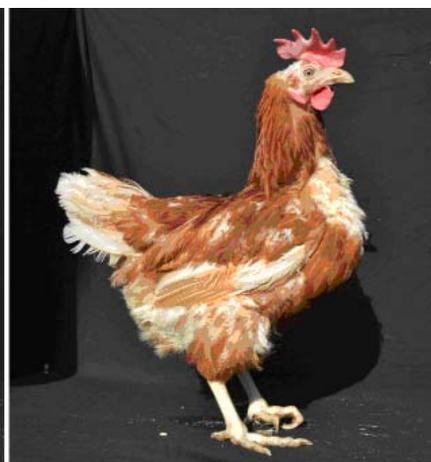
**Plumage condition** was subjectively scored by two persons at 72 weeks of age, when the test ended. The 3 point system used is illustrated below:



## Subjective scoring system for plumage condition

**1**

No feather loss

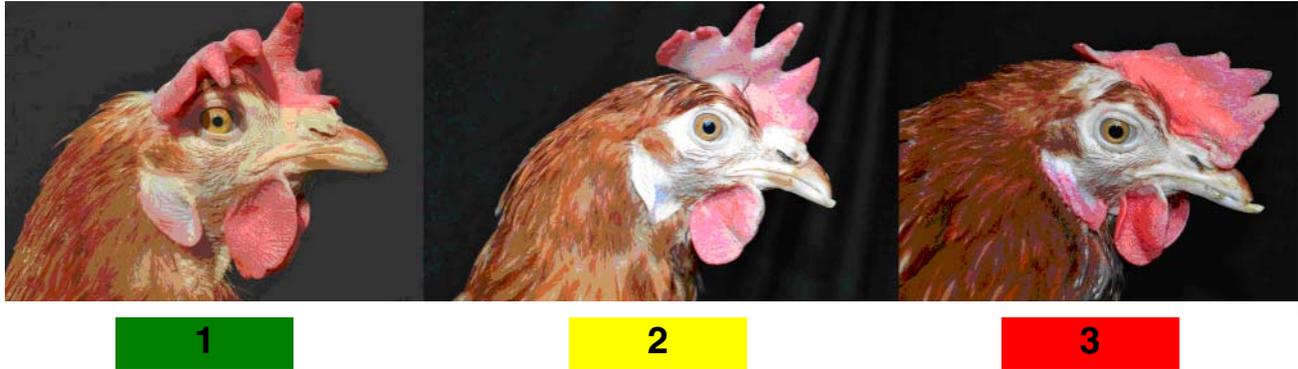
**2**

little feather loss

**3**

severe feather loss

The quality of beak treatment was also scored by two persons at 72 weeks for 474 LSL and 369 LB hens from 8 pens, using a three point classification as illustrated below.



**Score 1:** beak closed (upper and lower beak of same length); rounded point; no growth beyond the edges; little bone material removed.

**Score 2:** length of lower and upper beak differs by 2-3 mm; parts of the growth beyond the edges broken off and/or up to 1/3 of beak bone removed;

**Score 3:** the lower beak is significantly longer than the upper beak (> 4mm) due to regrowth; crossed beak or bony base structure cut by more than 1/3.

## Results

### Rearing

The beak treatment with infrared technique results in heat coagulation in all tissue cultures, and the necrosis involves about 1/3 of the upper beak and 1/4 of the lower beak. An important advantage of this method is that no neuromas develop, which are relevant in connection with phantom pain after amputation (Haider, 2012).

Traditional beak trimming with a hot blade cuts off approximately one third of the upper and lower beak, and cauterization seals the blood vessels and prevents bleeding. Advantages and disadvantages of the two systems are summarized in Table 1. One advantage of IR is application in the hatchery on day one, leaving the beak morphologically intact until the dead tissue drops off at about two weeks of age. Beak trimming with the traditional hot blade technique before 10 days of age on the rearing farm has no effect on feed intake during the first days. This study was intended to show to what extent beak amputation affects feed intake and growth after treatment, based on weekly weighing of samples of 80 birds per strain and treatment.

**Table 1: Advantages and disadvantages of IR beak treatment** (van Niekerk, 2011; Haider, 2012)

Advantages	Disadvantages
No open wounds, no bleeding, no risk of infection, no neuromas	High leasing cost for hatchery
Automated, precise adjustment	Chicks should not be too small; possibly problems with first hatches from young parent flocks
No health risk from outside personnel	Chick size needs to be uniform for batch treatment (individual adjustment is not practical)
Treatment in the hatchery combined with sexing and vaccination, no catching stress on the rearing farm	Additional challenges for logistics; limited experience with different strains and chick size
Usually no effect on feed intake and growth rate	Higher standards of rearing management required; more sensitive beaks during first days

**Table : 2 Effect of beak treatment on body weight of LSL and LB pullets (g)**

Age (days)	No treatment		Infrared Tmt.		Hot blade Tmt.	
	LSL	LB	LSL	LB	LSL	LB
1	39	36	37	36	38	36
7	62	69	61	66	59	67
14	113	127	114	127	109	129
21	178	197	169	186	166	189
28	238	270	240	255	240	257
56	545	580	536	572	550	564
84	869	976	912	981	916	985
126	1112	1339	1116	1316	1132	1353

**Table 3: Effect of beak treatment on feed consumption of LSL and LB pullets**

Age (days)	No treatment		Infrared Tmt.		Hot blade Tmt.	
	LSL	LB	LSL	LB	LSL	LB
1-7	74	77	71	73	73	77
8-14	137	142	142	137	137	137
15-21	151	152	149	147	149	142
22-28	182	170	178	158	176	172
29-56	1039	980	1071	975	1034	1004
57-84	1572	1576	1610	1547	1616	1593
85-126	2469	2552	2401	2467	2458	2505
1-126	5624	5649	5622	5504	5643	5630
127-134	991	1131	1061	1072	1086	1094
1-140	6615	6780	6683	6576	6729	6724

**Table 4: Feed conversion ratio (kg feed/kg weight gain) and mortality during rearing (18 weeks)**

FCR	No treatment	Infrared Tmt.	Hot blade Tmt.
LB	4.218	4.182	4.161
LSL	5.058	5.037	4.984
<b>mortality</b>	%	%	%
LB	1.7 %	3.3 %	2.2 %
LSL	0.5 %	1.1 %	0.5 %

As shown in table 2, the beak treated LSL and LB pullets had a slightly lower body weight at 2-3 weeks, but the difference soon disappeared, and the beak treated groups were actually a little heavier at 18 weeks of age. Differences in feed intake (table 3) reflect not only different rates of growth, but also feeding behavior (including feed wastage) and activity. Table 4 shows larger differences in feed conversion ratio to 18 weeks of age between strains than due to beak treatment.

Mortality was low in all groups, and the differences are not statistically significant due to the small number of replicates.

### Laying period

The analysis of variance showed significant differences between strains and beak treatment for hen-housed egg production and mortality, expressed as production days lost due to mortality (Table 5). IR treated LSL hens laid 7 more eggs to 72 weeks of age than untreated controls. Although total mortality (Table 6) was very low in this test, beak treatment reduced the number of production days lost due to cannibalism (cloaca picking in LB, toe picking in LSL) significantly. The lowest mortality was achieved with IR treatment, and since the mortality in the treated groups started late, the economic loss was only 1/3 compared to the untreated control groups.

Table 6 shows the performance of LSL and LB hens with and without beak treatment. High levels of production and livability indicate good bird management throughout the test. While the white-egg layers LSL were superior to the brown-egg layers in hen-day egg production and egg income over feed cost (IOFC) in all three beak treatment groups, the IR treated groups outperformed the group with conventional beak treatment and the untreated controls. Hens with IR treatment of the beak tip at day old required 25 g (LB) and 48 g (LSL) less feed per kg egg mass than untreated controls. The IR treated groups exceeded the untreated controls by 22 and 49 cents per bird, respectively, or 220 (LB) and 490 (LSL) Euro more profit per 1,000 hens housed.

The advantage of IR treatment compared to traditional beak trimming was 15 (LB) and 38 (LSL) cents per hen housed, indicating that a higher price for IR treated day-old chicks is not only justified as a contribution to hen welfare, but also by elimination of beak treatment cost on the rearing farm and higher egg income over feed cost on the layer farm.

**Table 5: Effect of beak treatment on egg number and mortality**

Beak treatment	egg number per hen housed	production days lost due to mortality
Untreated control	316 <sup>a</sup>	1.57 <sup>a</sup>
Hot blade Tmt.	320 <sup>ab</sup>	0.90 <sup>b</sup>
Infrared Tmt.	323 <sup>b</sup>	0.49 <sup>b</sup>

**Table 6: Effects of beak treatment on livability, egg production and feed efficiency**

Treatment	Livability	Trait					
		No. Eggs	No. Eggs	Egg weight	FC	FCR	IOFC
LB	%	HD	HH	(g)	g/day	kg F/kg EM	€/HH
Control	96.0	314.4	310.2	63.6	117.4	2.138	5.04
hot blade	96.5	314.7	311.1	63.4	116.5	2.126	5.11
IR	98.2	316.9	315.7	63.5	116.7	2.113	5.26
<b>LSL</b>							
Control	96.3	328.7	322.7	62.1	117.0	2.087	5.51
hot blade	97.8	332.0	329.9	62.1	117.8	2.079	5.62
IR	98.1	332.5	330.6	62.9	117.2	2.039	6.00

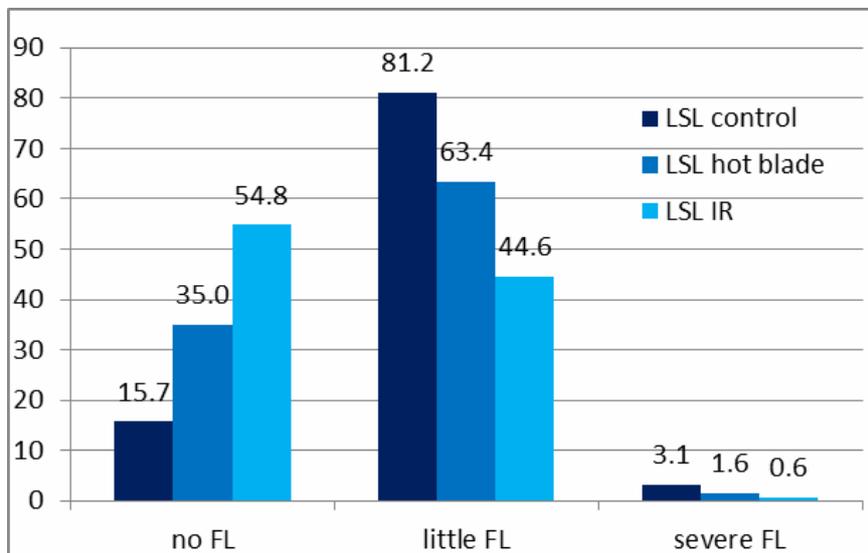
$$\text{IOFC} = \text{EM (kg)} * 1,00 \text{ €} - \text{FC (kg)} * 0.35 \text{ €}$$

### Feather loss during the laying period

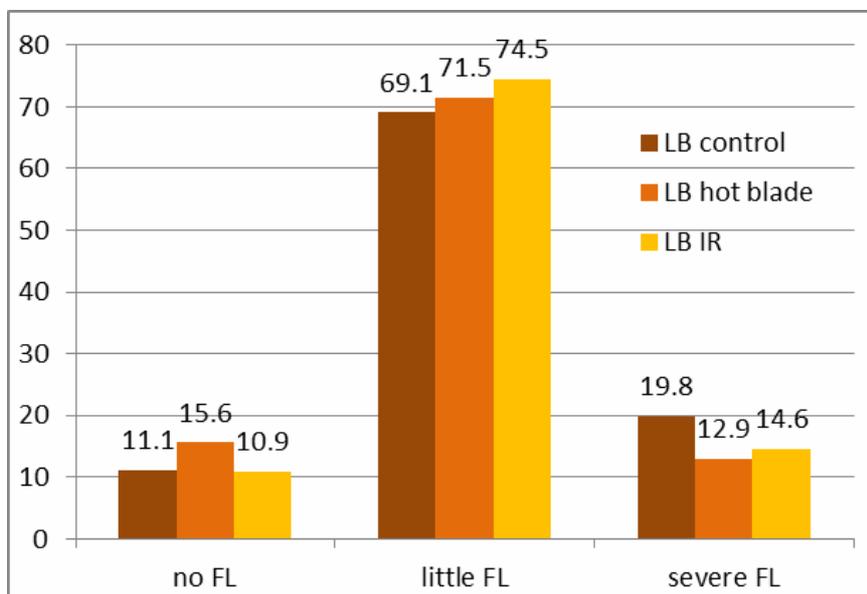
The feather condition was judged for all birds at 72 weeks of age as described above and indicates interesting differences between strains as well as beak treatment (Figures 1 and 2). The LSL hens were scored better than the LB hens, which is probably due to the fact that brown-feathered hens look partially de-feathered as soon as the brown cover feathers are lost, while the White Leghorns keep their natural color until the skin becomes visible. Doubts remain whether our scoring system is a satisfactory measure to compare functional properties of the feather cover between different breeds or gives only a visual impression.

Comparisons between beak treatment groups are consistent across breeds: 54.8% of the IR treated LSL hens had an intact feather cover at the end of test, compared to 35.0 % after traditional beak trimming and only 15.7% of the untreated control. The differences due to beak treatment were less consistent in the LB groups, where conventional beak treatment was apparently more effective than IR to prevent feather damage due to picking, but again the untreated control had the poorest feather condition.

**Figure 1: Effect of beak treatment on feather score of LSL hens**



**Figure 2: Effect of beak treatment on feather score of LB hens**



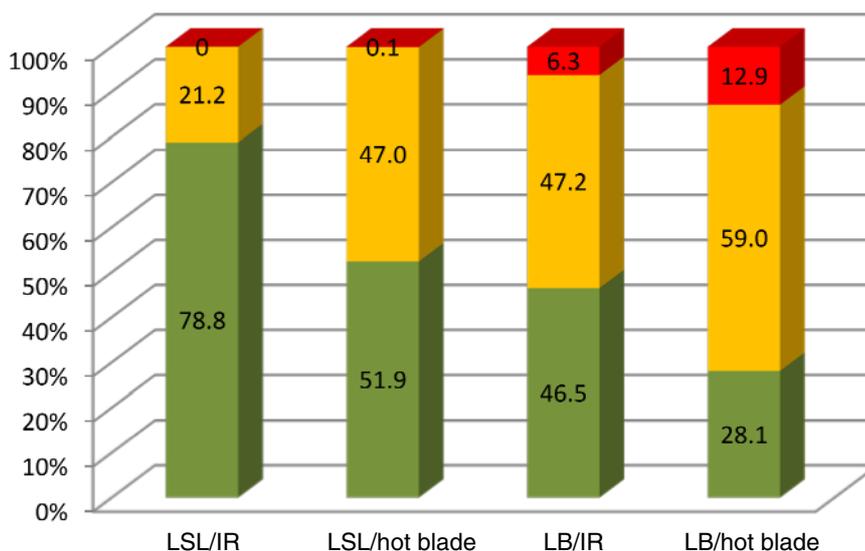
## Quality of beak treatment

An intact beak of chickens is hook-shaped and pointed. The upper beak is longer than the lower beak and enables the hen to pluck and pull, select feed particles and to preen. The organ at the tip of the beak plays an important role for these functions.

Therefore, the goal of any beak treatment must be to destroy as little live nerve tissue as possible and to induce quick recovery, while reducing the frequency of aggressive picking. The treatment should minimize pain and interfere as little as possible with feed selection, feed intake and preening, but prevent the misuse of the beak as a pair of tweezers to pull feathers of other hens. The goal is therefore a well closed beak without sharp or pointed ends. The quality and uniformity of beak treatment with the hot blade depends largely on the experience of the people doing the job. Additional factors to be taken into account with IR treatment are the size and uniformity of the chicks and adjustment of the machine.

The beak quality assessment at the end of the laying period (Figure 3) indicates that different strains may respond different to beak treatment, and these effects can be additive.

**Figure 3: Subjective scores for beak quality at 72 weeks of age**



The benefit of IR beak treatment, compared to conventional hot blade treatment, is more obvious for LSL than for LB hens. To what extent these differences reflect genetic differences in bone formation and potential for re-growth cannot be answered from a comparison of hens from a single hatch day, followed by beak treatment with the same setting of equipment.

As a basis for future refinement of the IR technique, more records on chick size (or age of parent flock) and settings of IR equipment should be collected for breeder flocks of different age and correlated with records on beak quality of adult hens. A realistic short-term target should be more flocks with similar beak quality as the IR treated LSL hens in this test, while geneticists continue to select for reduced picking and non-genetic factors contributing to picking are controlled as much as possible.

## Summary

Effects of infrared (IR) beak treatment of day-old chicks were compared with beak trimming at 9 days with hot blade technique and untreated control groups. For this test, the Bavarian Poultry Research Station in Kitzingen obtained day-old chicks of two strains from a commercial hatchery (1,800 LSL classic and 1,800 LB classic), 1/3 of which had been beak-treated by IR technique in the hatchery. The chicks were reared under commercial conditions in a windowless house with 6 pens of equal size, and one pen per strain was beak treated at 9 days, using a Lyon Debeaker (LD).

Effects of beak treatment on weight gain and feed intake were monitored throughout the rearing period. At 18 weeks, the pullets were transferred to two laying houses with windows and 12 floor pens per house. Data collected during the laying period (20-72 weeks of age) included daily mortality and egg production, weekly egg weight, four-weekly feed intake as well as subjective scores for feather condition and beak quality at the end of test. The following results were obtained:

- Slightly reduced weight gain due to beak trimming at 3 weeks (LSL) and 3-4 weeks (LB), but compensatory growth at 4 weeks (LSL) and 12 weeks (LB).
- No reduction of feed intake due to beak treatment in LSL pullets, but reduced feed intake of beak treated LB pullets throughout the rearing period.
- Somewhat better feed conversion for beak treated birds and a little higher early mortality for IR treated chicks.
- Significantly higher egg production per hen housed (+7 eggs/HH after IR and +4 eggs/HH after LD beak treatment).
- Although mortality was low in the untreated controls, a significant further reduction of mortality, especially due to cannibalism, was achieved with beak treatment. IR reduced cumulative mortality by 50%, and the production days lost due to mortality was only one third (-1.8 days) compared to the control group (-5.7 days).
- Feed conversion ratio and egg income over feed cost were improved by beak treatment, and the best results were found after IR treatment.
- Feather cover at the end of the laying period was better after beak treatment, especially in LSL hens.
- The beak condition at the end of the laying period was significantly better after IR treatment than after conventional beak trimming at 9 days with LD.
- Better beak quality of LSL hens in this test suggests that genetic differences in beak morphology and regrowth of tissue may exist, which should be taken into account while further improving and fine-tuning the IR technique.

IR beak treatment of day-old chicks has significant advantages over conventional beak trimming at 9 days and can be recommended as a contribution to improved bird welfare, without increasing production cost. Combined with optimal management, it can reduce the risk of feather pecking and cannibalism while geneticists try to solve the problem by selecting for improved picking behavior.

## Zusammenfassung

In einer Studie mit 1.800 LSL classic und 1.800 LB classic Hennen wurden am LVFZ für Geflügel in Kitzingen die Auswirkungen der Schnabelbehandlung auf Leistung, Mortalität, Federkleid und Schnabelmorphologie geprüft. Dazu wurden 1/3 der Küken mit dem Nova Tech Verfahren in der Brüterei Infrarot (IR) behandelt, 1/3 mit einem heißen Messer am 9. Tag gebrannt und 1/3 der Tiere diente als unbehandelte Kontrolle. Folgende Ergebnisse wurden erzielt:

- Tendenziell geringere Körpergewichte bei Schnabelbehandlung in der 3. Lebenswoche von LSL Küken und in der 3.-4. LW bei LB Küken mit voller Kompensation der Lebensgewichtsentwicklung ab der 4. LW (LSL) und ab der 12 LW (LB).
- Kein Einbruch der Futteraufnahme bei LSL Küken zum Zeitpunkt der Schnabelhandlung. Tendenziell leicht geringere Futteraufnahme der gebrannten LB Küken in der 2. und 3. LW bei den IR behandelten Tieren über die gesamte 20 wöchige Aufzucht im Vergleich zur unbehandelten Kontrolle.
- Tendenziell eine bessere Futterverwertung der Schnabel behandelten Gruppen bis zur 18. LW. Geringfügig höhere Anfangsverluste bei IR Behandlung.
- Signifikant höhere Eizahl je Anfangshenne und Jahr bei den behandelten Tieren: +7 Eier mit IR und +4 ./AH konventionell gebrannten Tieren.

- Signifikant geringere Verluste (insbesondere durch Kannibalismus bedingt) bei den Schnabel behandelten Gruppen. Durch die IR-Behandlung konnten die kumulierten Verluste halbiert und der Verlust an Produktionstagen auf 1/3 der Kontrolle reduziert werden.
- Tendenziell bessere Futterverwertung und höherer Futterkostenüberschuss in der Legeperiode bei Schnabelbehandlung, wobei die größten Unterschiede zwischen dem IR Verfahren und der Kontrolle beobachtet wurden.
- Besseres Federkleid bei Schnabelbehandlung am Ende der Legeperiode, vor allem bei den LSL Tieren.
- Signifikant besserer Schnabelschluss und Morphologie des Schnabels zu Ende der Legeperiode bei dem IR-Verfahren im Vergleich zum Brennen. Genetische Unterschiede in der subjektiven Beurteilung der Qualität der Schnabelbehandlung zugunsten der LSL Hennen.

Das IR Schnabelbehandlungsverfahren zeigt während der Legeperiode deutliche Vorteile bezüglich Leistung und Mortalität und Federverlust gegenüber den unbehandelten Tieren und zusätzlich einen besseren Schnabelschluss im Vergleich zum Brennen am 9. Tag. Die Differenzen zur Kontrolle sind bei den LSL deutlicher ausgeprägt als bei den LB.

Damit erfüllt das IR- Verfahren, in Kombination mit einem optimalen Management, die Voraussetzungen einer empfehlenswerten Brückentechnologie bis zu einer erhofften endgültigen Lösung durch erfolgreiche Selektion gegen dieses Verhalten.

### **Acknowledgement**

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## **Quantifying the environmental impacts of UK broiler and egg production systems**

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### **Introduction**

UK poultry production, including broilers and eggs, has been identified as being relatively environmentally friendly compared to the production of other animal commodities. However, like all agricultural systems, any current poultry system has scope to improve and reduce its environmental impacts even further. The aim of the work conducted at Newcastle University was to apply the environmental Life Cycle Assessment (LCA) method “from conception to farm gate”, to quantify the environmental burdens of the main broiler and egg production systems in the UK, and hence to identify the main opportunities to reduce these impacts within each system. The broiler systems included in the study were 1) standard indoor, 2) free range and 3) organic production and the egg production systems were 1) conventional cage, 2) barn, 3) free range and 4) organic laying. Although egg production in conventional cages has been banned by the EU and is not used in the UK anymore, it is still in use in some other European countries, and therefore the results for the cage laying system are also presented in this study. Results for enriched cages now used in the UK are expected to be broadly similar.

### **Environmental impact assessment methodology**

The quantification of the environmental impacts of agricultural systems is demanding, since these systems include complicated links between different production sectors. For example, livestock production is closely connected to arable production, as it specifies the demand of the feed crops. Furthermore, livestock produces manure, which in turn is used as fertilizer when producing arable crops. There are also specific interactions and feedbacks within each livestock system, as for example in broiler production the level of productivity specifies the quantity of broiler chicks, breeder birds and so on required to produce a certain level of output (broiler meat). As a result, a consistent, quantitative calculation method is needed to handle the whole livestock production system and related activities. A method called environmental Life Cycle Assessment (LCA) was used in this study to quantify the environmental impacts (caused for example by emissions of greenhouse gases to atmosphere or leaching of nutrients to environment) of broiler and egg production. LCA evaluates the scenarios systematically to account for all inputs and outputs that cross a specified system boundary and relates these to the “functional unit”. In this study, the functional unit was set as either 1000 kg of expected edible broiler carcass or 1000 kg eggs, and the modelled system was defined as “from cradle to farm gate”. The calculation method was based on a modelling framework describing the general structure of the industry, combined with process models and simulation models so that changes in one area of the system caused consistent interactions elsewhere. This approach was applied to both feed crop and animal production.

The structural model for broiler and egg systems combined all the main activities of the industry, and quantified the interactions between them. This framework was used to calculate all of the inputs required to produce the functional unit, taking into account the breeder, broiler, pullet and layer systems, and actual levels of productivity, feed conversion and mortality. It also calculated the outputs, both useful (broilers, eggs and spent hens) and unwanted. Changes in the proportion of any activity resulted in changes to the proportions of others in order to keep producing the desired amount of output.

An animal growth, production and feed intake model, based on actual biological processes, was used in this study in order to calculate the total consumption of each feed ingredient during the whole production cycle, and to calculate the amounts of main nutrients, nitrogen (N), phosphorus (P) and potassium (K) in manure produced by the birds during the production cycle. The model was calibrated to match the real production and feed intake data, provided by the UK poultry industry for different

systems by adjusting the model parameters for growth rate, energy requirement for maintenance and egg production.

The model calculated the N, P and K contents of the manure according to the mass balance principle, i.e. the nutrients retained both in the animal body and eggs were subtracted from the total amount of nutrients obtained from the feed (including the additional nutrients obtained from foraging in free range and organic production). In addition to the nutrients excreted by the birds, nutrients in the spilled feed and uncollected eggs were added to the manure in the calculations. For the purpose of the study, it was assumed that all broiler, pullet, layer and breeder manure was transported for soil improvement, excluding the proportion that was excreted outside in the non-organic free range production systems.

A separate sub-model for arable production was used to quantify the environmental impacts of the main feed ingredients. The greenhouse gas emissions arising from land use change were taken into account according to the principles of the carbon footprinting method PAS 2050 (BSI, 2011). A separate sub-model was also used for manure emissions and the nutrient cycle. In the model, the main nutrients that were applied to the soil in manure were accounted for either as intake by crops or as losses to the environment. The benefits of N, P and K remaining in soil after land application of manure were credited to poultry by offsetting the need to apply synthetic fertilizers, or in the case of organic production, the need of dedicated legume and rock P and K.

When estimating the environmental impacts of any agricultural system, it should be noted that both the methods and the inputs contain uncertainties (e.g. model errors and variations in input data) which should be taken into account. For example, when comparing different production systems, statistical comparison of the outputs of the LCA model is only possible if the uncertainties in the inputs and the resulting overall uncertainty of the outputs are quantified. In this study, the uncertainties of the input variables were based on the data from the industry, and they also included potential errors of the models. The error distributions of the emission factors followed the IPCC (2006) guidelines. As a combination of these, the overall uncertainties of the outputs were estimated and used to determine the possible statistical significance of the differences between the systems.

## **Environmental impacts considered**

The output of the LCA-systems model was the emissions to the environment in different poultry systems. The emissions were aggregated into environmentally functional groups as follows:

Global Warming Potential (GWP) is a measure of the greenhouse gas emissions to the atmosphere, and was calculated here using a timescale of 100 years. The main sources of GWP are carbon dioxide (CO<sub>2</sub>) from fossil fuel and land use changes, nitrous oxide (N<sub>2</sub>O) and methane (CH<sub>4</sub>). GWP was quantified as CO<sub>2</sub> equivalent: with a 100 year timescale 1 kg CH<sub>4</sub> and N<sub>2</sub>O are equivalent to 25 and 298 kg CO<sub>2</sub> respectively. The sum of GWP per functional unit is also known as the “carbon footprint”.

Eutrophication Potential (EP) is used to assess the over-supply of nutrients as a result of nutrients reaching water systems by leaching, run-off or atmospheric deposition. EP was calculated using the method of the Institute of Environmental Sciences (CML) at Leiden University (<http://www.leidenuniv.nl/interfac/cml/ssp/index.html>). The main sources are nitrate (NO<sub>3</sub><sup>-</sup>) and phosphate (PO<sub>4</sub><sup>3-</sup>) leaching to water and ammonia (NH<sub>3</sub>) emissions to air. EP was quantified in terms of phosphate equivalents: 1 kg NO<sub>3</sub>-N and NH<sub>3</sub>-N are equivalent to 0.44 and 0.43 kg PO<sub>4</sub><sup>3-</sup>, respectively.

Acidification Potential (AP) is mainly an indicator of potential reduction of soil pH. AP was also calculated using the method of the Institute of Environmental Sciences (CML) at Leiden University. The main source is ammonia emissions, together with sulphur dioxide (SO<sub>2</sub>) from fossil fuel combustion. AP was quantified in terms of SO<sub>2</sub> equivalents: 1 kg NH<sub>3</sub>-N is equivalent to 2.3 kg SO<sub>2</sub>.

Primary Energy Use was quantified in terms of the primary energy needed for extraction and supply of energy carriers, including gas, oil, coal, nuclear and renewable. There are also other categories of environmental impacts, such as Abiotic Resource Use and Pesticide Use. These categories were not considered here because reliable estimates of the uncertainties of the related inputs were not available, and therefore the comparison between the systems would not have been meaningful.

## Production systems considered

The production systems in this study were considered to represent typical UK broiler and egg production (Table 1 and Table 2 respectively). These figures, together with estimates of farm energy consumption (for heating, lighting, ventilation, feeding and incineration of dead birds) were based on average data from typical farms as provided by the industry.

**Table 1: Typical production and feed intake figures for the different broiler production systems in UK as provided by the industry**

Praxisübliche Mastdauer, Schlachtgewicht, Futterverbrauch und Verlustraten bei Broilermast in verschiedenen Produktionssystemen (Leinonen *et al.*, 2012a)

System Haltungsform	Standard Intensivmast	Free range Freiland	Organic Bio
Final age, days Mastdauer, Tage	39	58	73
Average final weight, kg Endgewicht, kg	1.95 <sup>a</sup>	2.06	2.17
Feed intake, kg/bird Futterverbrauch, kg/Tier	3.36	4.50	5.75
Mortality, % Tierverluste, %	3.5	4.7	4.1

<sup>a</sup> 25% of birds were removed by thinning at bodyweight 1.8 kg. The final weight of remaining birds was 2.0 kg.

**Table 2: Typical production and feed intake figures for the different egg production systems in UK as provided by the industry**

Praxisübliche Legeleistung, durchschnittliches Eigewicht, täglicher Futterverbrauch und Tierverluste in verschiedenen Produktionssystemen (Leinonen *et al.*, 2012b)

System Haltungsform	Cage Käfig	Barn Boden	Free range Freiland	Organic Bio
Eggs collected/hen <sup>a</sup> Eizahl je eingestellte Henne	315	300	293	280
Average egg weight, g Durchschnittliches Eigewicht	62.0	63.5	63.5	63.5
Feed consumption, g/bird/day Futterverbrauch, g/Henne/Tag	115	125	130	131
Mortality, % Tierverluste, %	3.5	6	7	8

<sup>a</sup> based on the initial number of hens

The baseline diets representative of those used in the UK were constructed using information provided by the poultry industry. The broiler diets included four and the layer diets five separate phases, according to common practice. Separate diets for broiler breeders were also specified.

## Estimates of environmental impacts for broilers

The number of broiler birds required to produce the expected edible carcass weight of 1000 kg was higher in the standard indoor system than in the free range and organic systems because the finishing weight was lowest in the standard indoor system. The length of the production cycle was much higher in free range and organic systems than in the standard indoor system, thus the feed consumption per bird was also higher in these systems. This had a major effect on the trends in environmental burdens (Table 3).

**Table 3: Global Warming Potential (GWP), Eutrophication Potential (EP), Acidification Potential (AP) and Primary Energy Use per 1000 kg of expected edible carcass weight in the main broiler production systems in the UK.**

Erderwärmung (GWP), Eutrophierung (EP), Versäuerung von Böden und Gewässern (AP) sowie Primärenergieverbrauch zur Produktion von 1000 kg Broilerfleisch (Leinonen *et al.*, 2012a)

System Haltungssystem	Standard Intensivmast	Free range Freiland	Organic Bio
GWP (t CO <sub>2</sub> e)	4.41 (0.44) <sup>a</sup>	5.13 (0.52) <sup>ab</sup>	5.66 (0.62) <sup>b</sup>
EP (kg PO <sub>4</sub> <sup>3-e</sup> )	20.3 (2.12) <sup>a</sup>	24.3 (2.51) <sup>a</sup>	48.8 (6.69) <sup>b</sup>
AP (kg SO <sub>2</sub> e)	46.8 (4.94) <sup>a</sup>	59.7 (6.11) <sup>b</sup>	91.6 (8.37) <sup>c</sup>
Primary Energy (GJ)	25.4 (2.05) <sup>a</sup>	25.7 (1.74) <sup>a</sup>	40.3 (2.70) <sup>b</sup>

a,b,c The standard deviation is given in the parentheses; different superscript indicates statistically significant difference ( $P < 0.05$ ) between the systems.

In many of the environmental impact categories, feed caused relatively higher impacts than any other materials involved in broiler production, for example 71 - 72% of the total GWP and 65 - 81% of the Primary Energy Use of the system. The GWP was affected by relatively high CO<sub>2</sub> emissions from the production and transport of some feed ingredients (e.g. non-organic soya, palm oil, fish meal and pure amino acids) in the standard and non-organic free range broiler diets. On the other hand, organic feed had generally much higher impact than the non-organic feed in other impact categories, especially EP. Although the emissions per land area are sometimes lower in organic crop production compared to non-organic, the yields are generally much lower as fertility building and cover crops are required, and this makes the emissions higher per unit of the product.

Emissions from manure were the main component of AP in broiler production and had also a relatively high contribution to EP. This was mainly a result of ammonia emissions, which contributed to both these potentials, together with nitrate leaching (affecting only EP). The AP from manure was especially high in the organic system.

### Estimates of environmental impacts for eggs

The production of 1000 kg eggs required 51.2 laying birds in the cage system, 52.6 in the barn system, 53.8 in the free range system and 56.3 in the organic system. This general trend in productivity also affected other aspects of the activity data, such as feed consumption. Furthermore, the average feed consumption per bird was also higher in the alternative systems than in the cage system. Much of the explanation of the trends in environmental burdens that followed resulted from these differences in the efficiency of the systems (Table 4).

As in the broiler systems, feed was the biggest component of GWP in egg production (contributing 64 - 72% to the overall GWP and 54 - 75 % to the overall Primary Energy Use of the systems). Compared with broiler production, the farm electricity use had a higher relative contribution to GWP and Primary Energy Use, especially in barn egg production. Again, manure was a major source of both EP and AP, which were especially high in the organic egg production system.

### Discussion

The results of this study show that the environmental impacts of both broiler and egg production are largely related to the efficiency of resource use of each system. In broilers, the standard indoor system had a shorter production cycle compared to the alternative systems, and therefore also lowest feed consumption and manure production per functional unit. Also in egg production, the alternative systems were generally less efficient than the cage system, and therefore had also higher environmental impacts.

**Table 4: Global Warming Potential (GWP), Eutrophication Potential (EP), Acidification Potential (AP) and Primary Energy Use per 1000 kg of eggs in the main egg production systems in the UK**

Erderwärmung (GWP), Eutrophierung (EP), Versäuerung von Böden und Gewässern (AP) sowie Primärenergieverbrauch zur Produktion von 1000 kg Eimasse (Leinonen *et al.*, 2012b).

System Haltungssystem	Cage Käfig	Barn Boden	Free range Freiland	Organic Bio
GWP (t CO <sub>2</sub> e)	2.92 (0.21) <sup>a</sup>	3.45 (0.26) <sup>b</sup>	3.38 (0.27) <sup>ab</sup>	3.42 (0.34) <sup>b</sup>
EP (kg PO <sub>4</sub> <sup>3-</sup> e)	18.5 (1.57) <sup>a</sup>	20.3 (1.78) <sup>b</sup>	22.0 (2.01) <sup>b</sup>	37.6 (4.21) <sup>c</sup>
AP (kg SO <sub>2</sub> e)	53.1 (5.23) <sup>a</sup>	59.4 (5.99) <sup>b</sup>	64.1 (6.90) <sup>b</sup>	91.6 (8.66) <sup>c</sup>
Primary Energy (GJ)	16.9 (1.01) <sup>a</sup>	22.2 (1.20) <sup>b</sup>	18.8 (1.15) <sup>a</sup>	26.4 (1.62) <sup>c</sup>

a,b,c The standard deviation is given in parentheses; different superscript indicates statistically significant difference ( $P < 0.05$ ) between the systems.

Feed production and processing was the main component of the global warming potential both in broiler and egg production systems. This was partly affected by the fact that some ingredients, most notably soya and palm oil, were considered to be partly produced on land that has been only recently converted from natural vegetation to agricultural use in South America and South Asia. When calculating the land use change effect on GWP, this study applied the guidelines of the carbon footprinting method PAS2050 (BSI, 2011). However, there is not a full international agreement on the method of how to account for land use changes in LCA, and this has potentially a very big effect on the estimate of the environment impact of broiler and layer feed and poultry production in general.

In addition to the general comparison between different broiler and egg production systems, the modelling framework applied in this study provides an opportunity to carry out detailed farm level assessments on how to reduce the environmental impacts of production. Since the analysis is largely based on functional relationships built in the animal and crop production sub-models, it is possible to examine the overall effects of the expected changes within the system by taking into account all relevant interactions between different production sectors. For example, changes in consumption and composition of feed have effects both on the impacts occurring during the crop production and feed processing, and also on the subsequent emissions from poultry manure during housing, storing and field application. Similarly, the differences in the growth rate of broilers affect the amount of feed consumed per functional unit, the amount of manure produced and the amount of energy and buildings needed, among other things.

Future options for reducing the environmental impacts of animal production include genetic selection for better environmental performance. The current results indicate that improving feed efficiency has potential to reduce the environmental impacts. The modelling framework with functional relationships applied in the present study will allow detailed and realistic tools for quantifying the environmental consequences of future genetic progress in animals. Further options for reducing the high environmental impacts from livestock feed include the use of alternative, more environmentally friendly ingredients. For example, it can be expected that reducing the inclusion of imported soya, partly originated from recently converted agricultural land, and replacing it using locally grown protein sources may reduce the high greenhouse gas emissions related to both land use changes and long transport distances.

## Summary and conclusions

Life cycle assessment procedures were applied to compare the environmental impact of producing broiler meat and eggs in different management systems. Input and output parameters used in the calculations were obtained from the UK broiler and egg industry and are summarized in Tables 1 and 2. The environmental impact was expressed in terms of for criteria: (1) global warming potential (GWP), (2) Eutrophication potential (EP), (3) Acidification potential (AP) and (4) Primary energy use per unit broiler meat or eggs. The results are shown in Tables 3 and 4.

There were relatively large differences in many categories of the environmental impacts between different UK broiler and egg production systems and generally these reflected the differences in the efficiency in production, feed consumption (and related production of manure) and material and energy use.

The methodology used in the current study with functional relationships between different activities related to animal production and mechanistic representation of biological processes provides a realistic tool for quantification of environmental impacts of various agricultural systems. This includes the quantifications of the overall uncertainties of the model outputs, which allows systematic comparison between different production systems.

## **Zusammenfassung**

### **Eine quantitative Analyse der Umweltbelastung durch Broilermast und Produktion von Eiern in verschiedenen Haltungssystemen in Großbritannien**

Die Ökobilanz verschiedener Haltungssysteme für die Produktion von Broilerfleisch und Eiern wurde berechnet. Es wurde von typischen Praxisergebnissen in Großbritannien ausgegangen, die in den Tabellen 1 und 2 dargestellt sind. Die Umweltbelastung wurde anhand folgender Kriterien berechnet: (1) Beitrag zur globalen Erwärmung, (2) Beitrag zur Eutrophierung von Gewässern (EP), (3) Versäuerung von Böden (AP) und (4) Primärenergieverbrauch pro Tonne Broilerfleisch und Eier. Die Ergebnisse in den Tabellen 3 und 4 zeigen erhebliche Unterschiede zwischen den Haltungssystemen in vielen Kriterien der Effizienz, hauptsächlich wegen des unterschiedlich hohen Futteraufwands und der entsprechend höheren Ausscheidung von Kot, aber auch Material- und Energiebedarfs.

Die für diese Untersuchung eingesetzte Methode kombiniert funktionale Beziehungen zwischen verschiedenen Abläufen der Tierhaltung mit mechanistischer Beschreibung biologischer Prozesse und führt im Ergebnis zu einem realistischen und quantitativen Vergleich der Ökobilanz verschiedener Haltungssysteme.

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## Short Periods of Incubation During Egg Storage – SPIDES

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### Summary

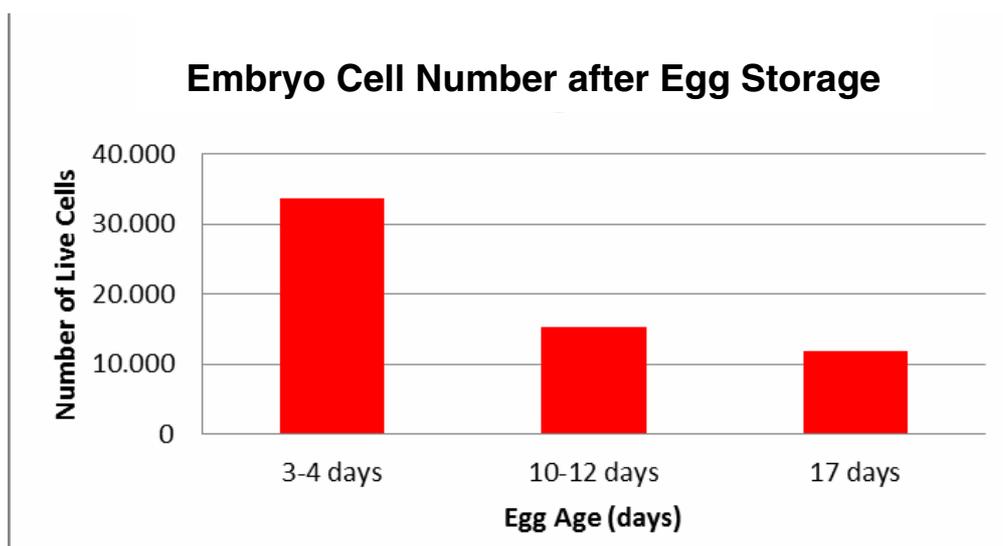
As a broiler breeding company, Aviagen offers advice on the best way to store hatching eggs between oviposition and the start of incubation. Ideally, all eggs should be set while still fresh - within a week of being laid. If longer storage is unavoidable, then storage temperatures should be reduced, and any temperature fluctuations avoided. The advice is safe, and offers minimal opportunities for misunderstanding. In contrast, when hens lay and incubate their own eggs, the eggs laid first will be re-warmed every time the hen returns to the nest to lay another egg. A series of experiments and field tests looking at short periods of incubation during egg storage (SPIDES) have shown that it is possible to recover 60-70% of the hatchability lost when storage has to be prolonged for more than a week. The technique is potentially of value to broiler grand-parent, layer parent and turkey breeder programmes where order patterns may be uneven, and egg storage unavoidable.

### Introduction

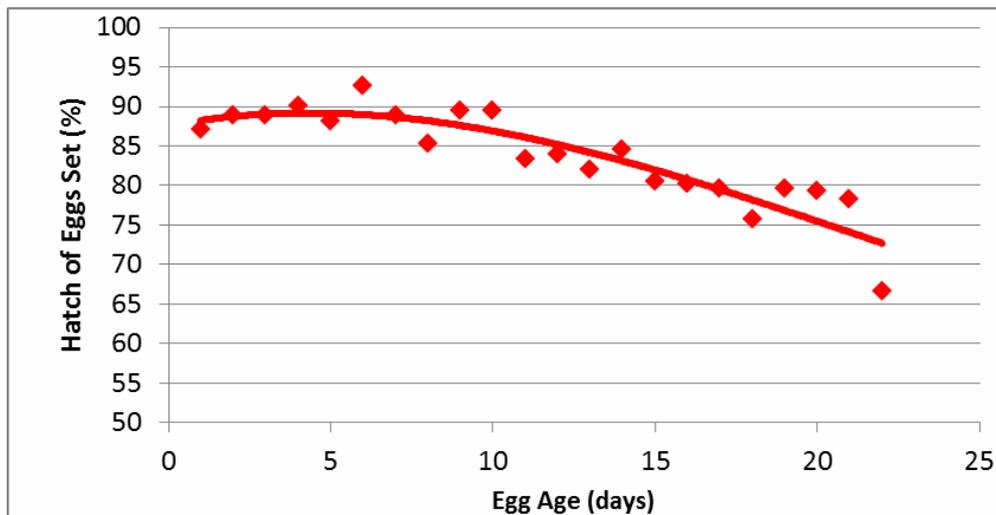
#### Changes during egg storage

During the day it takes for the egg to form in the oviduct, the embryo will be held at body temperature, and normal embryonic development will occur. When the egg is laid, the embryo will contain over 30,000 cells, and will have reached Stage IIX-X of development. Once the eggs are cooled for storage, embryo development will usually stop, provided the eggs are stored below 24 °C (Eyal-Giladi and Kochav 1976). If the eggs are stored for more than a few days, then embryo cells start to die. After 10-12 days of storage, more than half of the cells present at oviposition will have died (Bakst *et al.* 2012; Fig. 1).

**Figure 1: Embryo cell numbers in broiler breeder eggs change with egg age**



As well as changes in the embryo, the egg contents change as the egg gets older. The albumen becomes thinner and lysozyme activity drops. The yolk membranes become much weaker, and will tend to rupture if placed under any stress. All of these changes will tend to increase embryo mortality. As egg age increases, hatchability usually rises slightly after two days of storage, and then starts to fall again around 7 days, as shown for Ross 308 eggs in Figure 2.

**Figure 2: Changes in hatchability over 21 days in Ross 308 parent stock eggs.**

Most of the embryo mortality happens very early in embryonic growth – at the membrane stage of development. In a commercial hatchery this will usually be seen as a higher percentage of candled clears. The chicks that do hatch will need a longer incubation period, because the embryo will have had to grow from the reduced number of live cells at the end of storage. If incubation time is not adjusted, otherwise viable chicks may be lost because they are not yet dry, or are still going through the hatching process. The amount of hatch loss will be variable, depending on how well-controlled egg store temperatures are, and whether there are existing quality issues in the eggs which make them more fragile.

### Heat Treatment Before or During Egg Storage

Heating hatching eggs to incubation temperature before storage is not a new idea. Allowing embryo development to continue a little longer after the egg is laid, so that the embryo reaches a slightly later stage of development is known to improve embryo survival after longer storage. At the University of Alberta, Fasenko *et al.* (2001) showed that giving eggs a single 6 hour period of incubation before the start of egg storage improved the hatch of 14 day stored eggs compared to untreated controls. Eggs vary in their developmental stage when they are laid, but most of them will be at stages IIX-X, just before the formation of the hypoblast. Fasenko and her co-workers suggested that eggs cope better with long egg storage if the formation of the hypoblast is complete but the embryo has not progressed to the stage called the primitive streak.

An alternative hypothesis was proposed by Meir and Ar (1998). They suggested that giving the eggs a short (less than 6 hours) period of incubation at regular intervals during long storage would allow the embryo to carry out cell repair and so reduce the rate of cell death.

Heating eggs to incubation temperatures immediately before cooling them for storage can be difficult to achieve within a commercial farming operation. Most companies transport eggs from the farm to the hatchery twice a week, so eggs are between 1 and 4 days old when they reach the hatchery. For an incubation treatment to be given on the day of lay, either the eggs need to be taken to the hatchery every day, or there needs to be an incubator on every farm. It would not be an easy process to manage. For this reason, our work has focused on incubation during the storage period, which can be imposed at a single location (the hatchery) which is already equipped with suitable equipment.

## Experiments

Four replicated experiments were carried out between July 2010 and June 2011.

The first three experiments were carried out at the Aviagen Product Development Unit at Albertville in Alabama, using Ross 308 broiler hatching eggs from a commercial parent flocks at peak hatchability. Each experiment used eggs from a single flock laid on one day. The fourth experiment was carried out in the UK, in the Aviagen PS hatchery, using Arbor Acre female line eggs.

In the first three experiments, the eggs were held in setter trays on a wheeled buggy, which was moved to the corridor of a Chickmaster fixed-rack multi-stage incubator for each heat treatment. Storage in between treatments was in a controlled environment egg store set at 15.5-18.3°C. A total of 1944 eggs were allocated to each treatment in every experiment, spread over 12 replicate trays of 162 eggs. In each of the experiments there was a positive control, where the eggs were set fresh (3 days old) and a negative control where the eggs were stored without heat treatment for 21 days.

For the final experiment, eggs were heated in setter trays in small Bristol single stage machines, again being restored to the cooled eggs store (17-18°C) between treatments. Eggs were laid by a single small flock over several days, with eggs from each day's production evenly distributed across the treatments; there were 2520 eggs set per treatment, in replicate trays of 132 eggs.

The experiments explored different variables as follows:

Experiment 1 – How many heat treatments were needed to give the best hatchability?

Experiment 2 – What benefit to hatch could be seen in eggs stored for 7, 14 or 21 days?

Experiment 3 – What was the best combination of treatment duration and treatment frequency?

Experiment 4 – Is the speed at which eggs are heated important?

## Experiment 1

### Treatments

In this experiment, all eggs were stored for 21 days except for the positive control. The eggs were held in the setter corridor for 4 hours for each heat treatment, with 3, 4 or 5 repetitions as follows:

- Treatment 1 - Positive control – eggs set when 3 days old
- Treatment 2 - Negative control – eggs set when 21 days old
- Treatment 3 – Eggs placed in setter corridor for 4 hours on days 8 and 15 of storage.  
Set on day 21
- Treatment 4 – Eggs placed in setter corridor for 4 hours on days 5, 10, 15 and 18 of storage.  
Set on day 21
- Treatment 5 – Eggs placed in setter corridor for 4 hours on days 3, 8, 12, 15 and 19 of storage.  
Set on day 21.

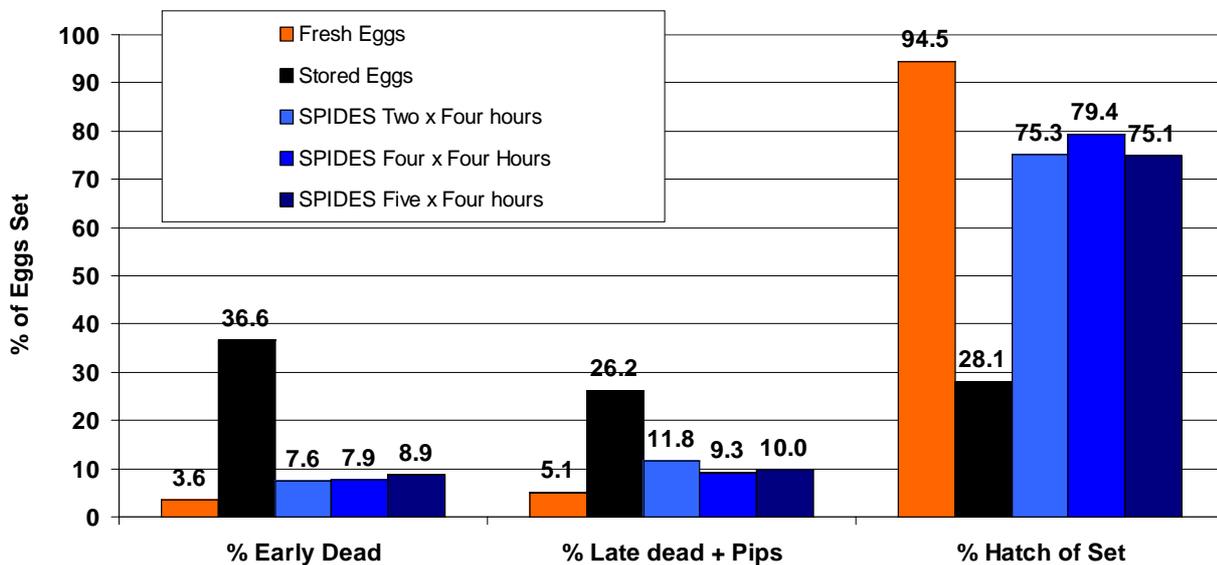
### Results

The results from Experiment 1 are shown in Figure 3.

### Comments

Although the positive control eggs hatched very well (94.5% hatch of eggs set), hatchability was particularly poor after 21 days storage. This was probably due to suboptimal egg storage conditions during exceptionally hot summer weather. All of the SPIDES treatments improved hatch of stored eggs. It was noticeable that both early dead embryos and late dead embryos and live pips were much reduced by SPIDES treatment; hatchery staff also commented that the treated eggs showed much less hatch delay than the untreated ones after the 21 days storage. Applying four treatments gave better results than applying three or five treatments, the difference was statistically significant ( $P < 0.001$ ).

**Figure 3: Early and late embryo mortality and hatch of eggs set in Experiment 1**



## Experiment 2

### Treatments

In this experiment we wanted to confirm the results of the first experiment during cooler weather, and also to look at how long storage needed to be for there to be a benefit from SPIDES treatment

- Treatment 1 – Positive control – eggs set when 3 days old
- Treatment 2 – Negative control 1 – eggs set when 7 days old
- Treatment 3 – Eggs placed in setter corridor for 4 hours on days 4 of storage. Set on day 7
- Treatment 4 – Negative control 2 – eggs set when 14 days old
- Treatment 5 – Eggs placed in setter corridor for 4 hours on days 4, 7 and 11 of storage. Set on day 14
- Treatment 6 – Negative control 3 – eggs set when 21 days old
- Treatment 7 – Eggs placed in setter corridor for 4 hours on days 5, 10, 15 and 19 of storage. Set on day 21.

## Results

### Internal Egg Temperatures

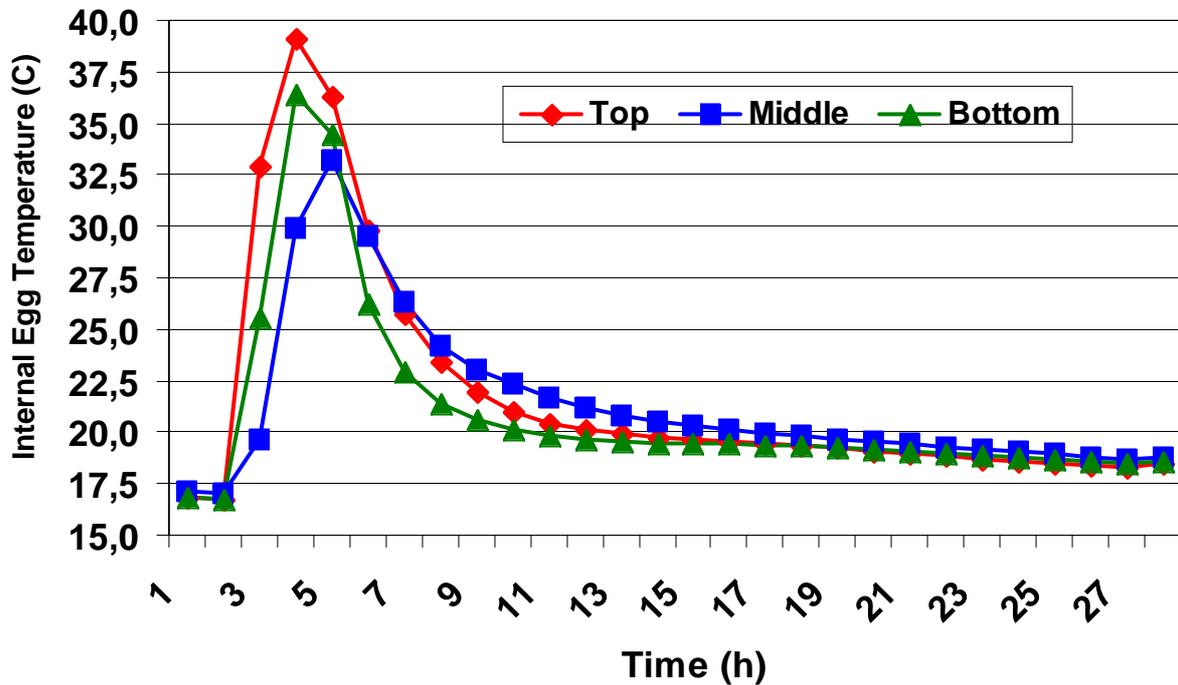
Temperature sensors placed in sample eggs showed that the eggs heated faster at the top of the buggy, nearest the roof-mounted fan, and slowest in the middle of the buggy, which was shielded from air movement by surrounding trays of eggs (Figure 4). It is noticeable that the four hour exposure was not long enough to lift the temperature in the middle trays to incubation temperature. However, inspection of the individual replicate (tray) results showed that the treatment was equally effective in all positions.

### Hatchability and Embryo Mortality Patterns

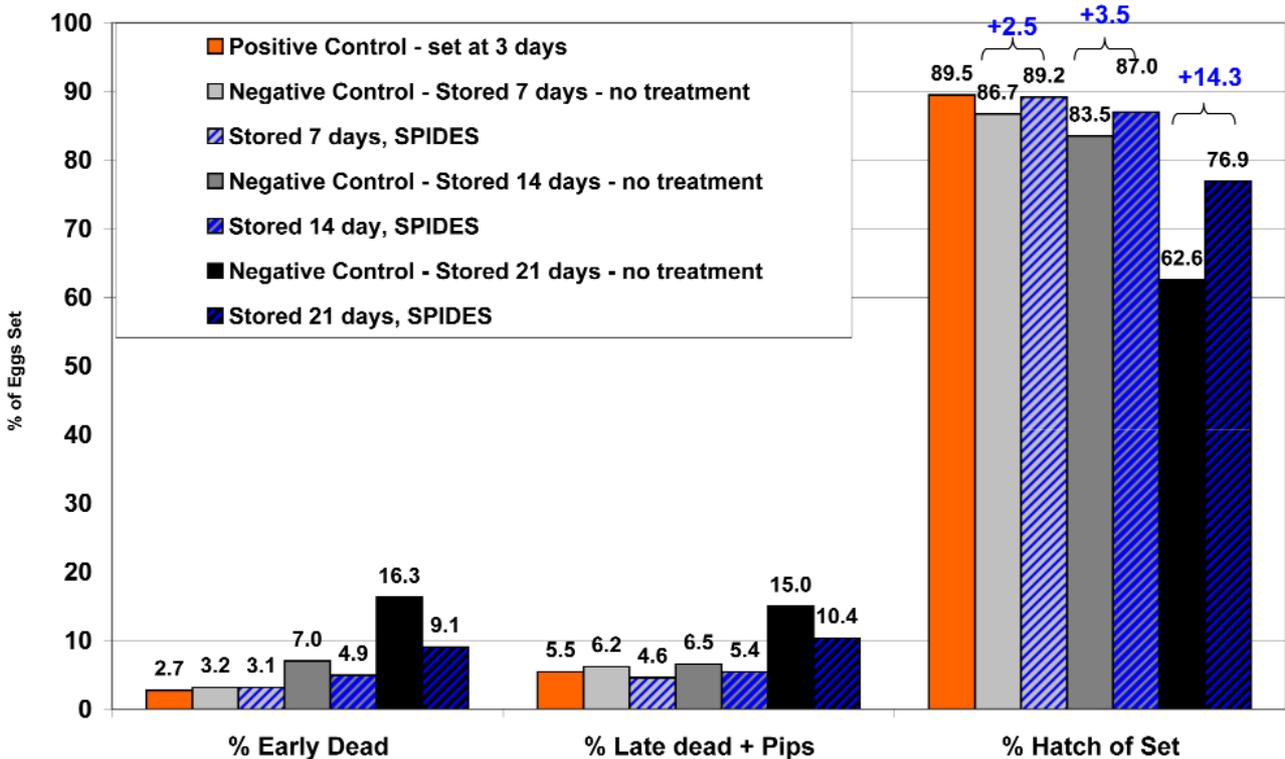
The eggs in this experiment hatched in December, and the hatch loss after 21 days was much closer to normal levels than in Experiment 1. As shown in Figure 5, SPIDES treatment improved hatch at all three egg ages.

Even after only 7 days, where the hatch drop was numerically small, the improvement was statistically significant ( $P < 0.05$ ).

**Figure 4:** Internal egg temperatures measured at 3 locations on the egg store trolley during the application of 4 hours of SPIDES treatment.



**Figure 5:** Early and late embryo mortality and hatch of eggs set in Experiment 2



### Experiment 3

#### Treatments

- Treatment 1 (positive control) – eggs set when 3 days old
- Treatment 2 (negative control) – eggs set when 21 days old without any storage treatment
- Treatments 3-11 – eggs stored 21 days, during which time they were treated 3, 4 or 6 times for either 2, 4 or 6 hours.

Exact timings of heat treatment are given in the table below:

Treatment length (hours)	Which day of storage the eggs were treated		
	3 treatments	4 treatments	6 treatments
2			
4	5,11 & 16	4,8,13 & 17	3,6,9,12,15 & 18
6			

Exact treatment days were adjusted to avoid overloading the setter on any one day, while still having roughly even intervals between each treatment.

### Results

#### Internal Egg Temperatures

**Figure 6: Average Internal egg temperatures during SPIDES treatments of different duration**

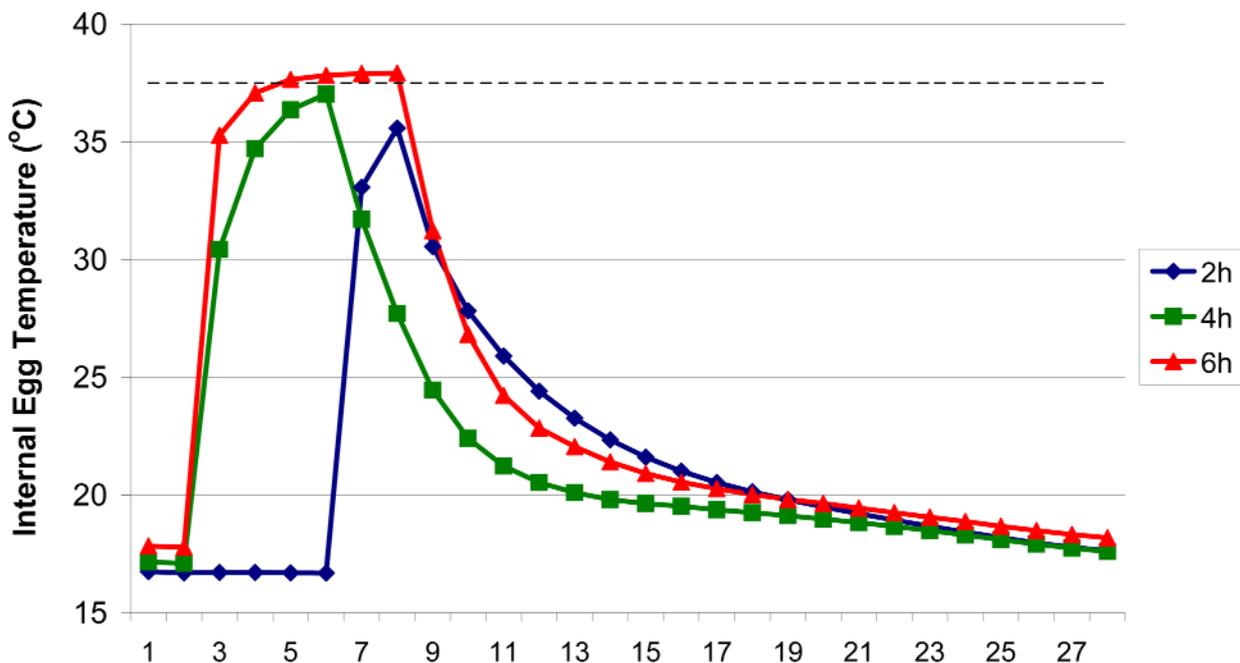
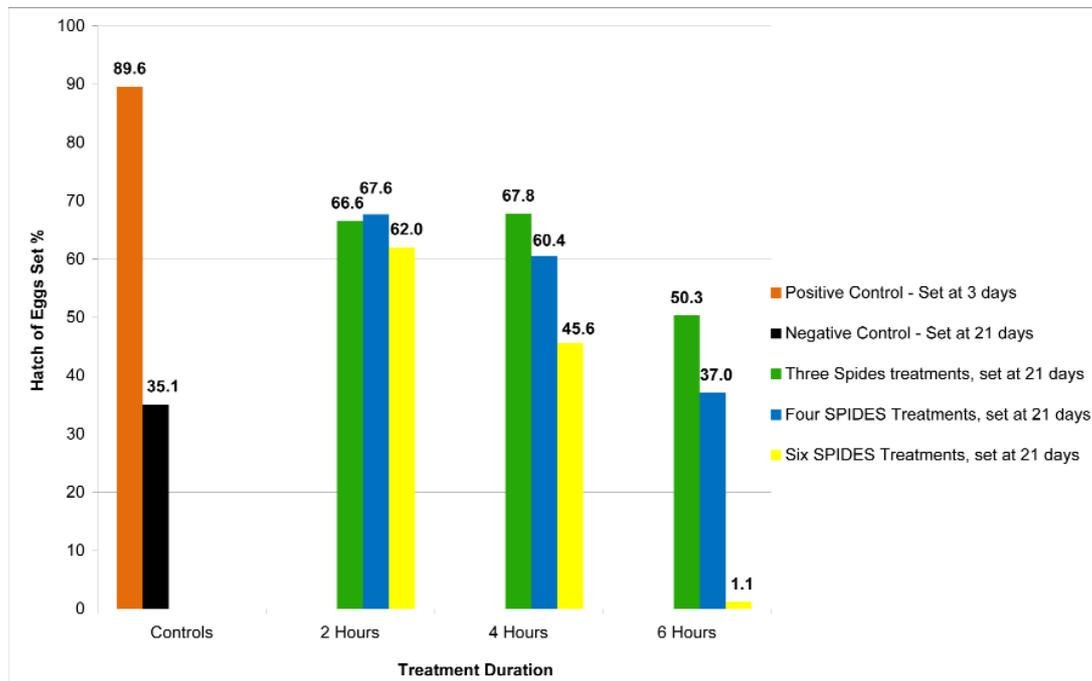


Figure 6 shows the average of top, middle and bottom trays for each treatment duration. It can be seen that after 6 hours exposure, the eggs had received 4 hours at incubation temperature, while after 2 hours they only reached an average of 35°C. Temperature range between top, middle and bottom trays was greatest for the 2 hour treatment, and least for the 6 hour.

**Hatchability and Embryo Mortality Patterns**

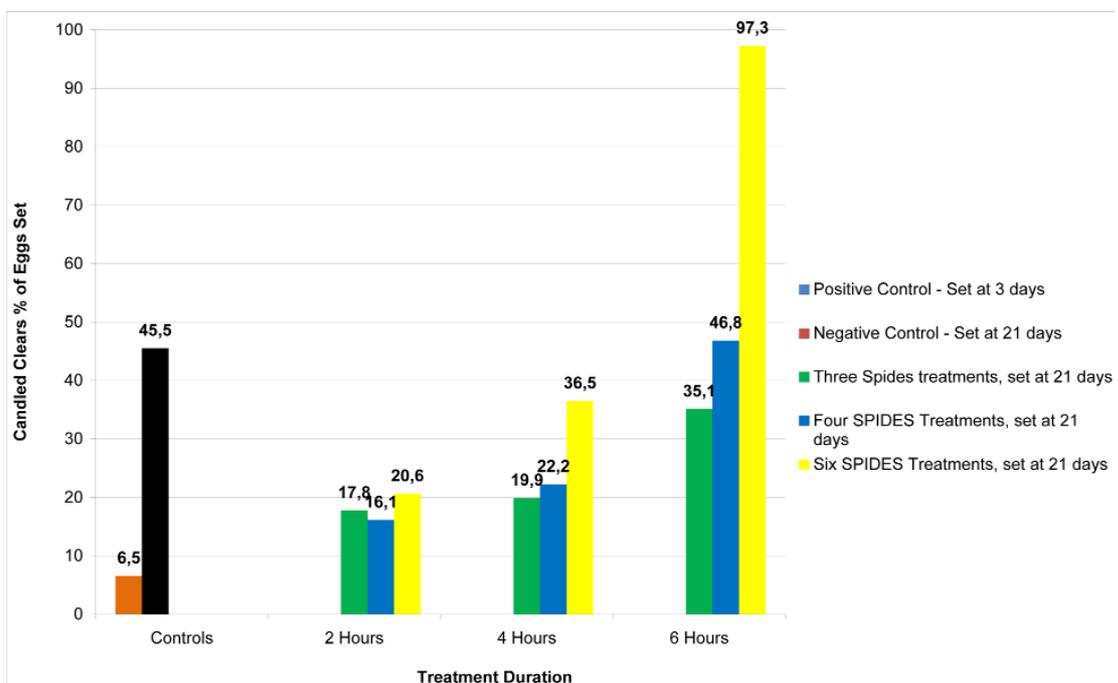
**Figure 7: Hatch of eggs set in Experiment 3**



It can be seen that the shorter exposure times gave the best results, despite reaching a lower and more variable temperature. As shown in Figure 7, six repetitions of a six hour treatment caused almost complete hatch failure.

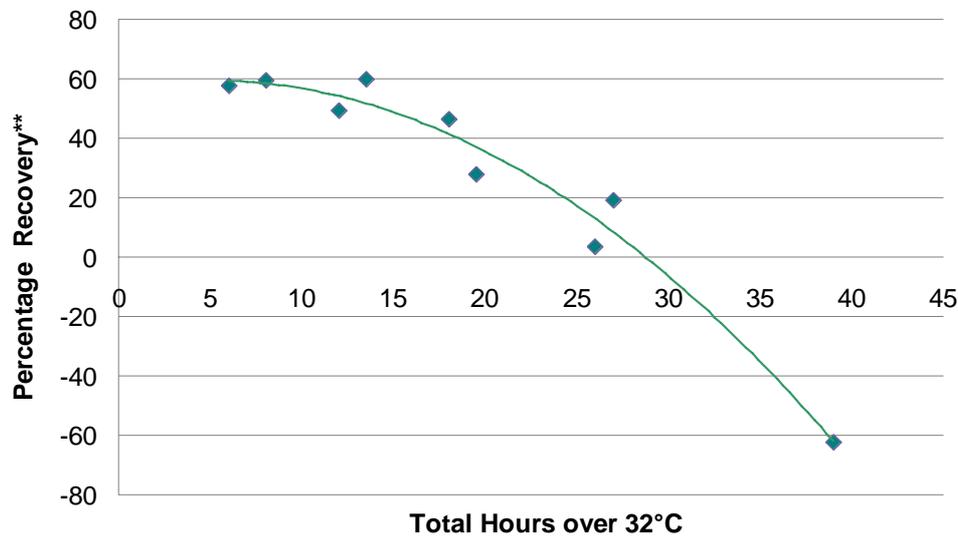
It was not possible to break open un-hatched eggs in this experiment, but Figure 8 shows the candling figures, - candled clears include both infertile and early dead embryos. Six hour treatments gave more clears than 2 or 4 hour treatments. Embryo survival in eggs given four repetitions of six hours was no better than in stored untreated eggs, while six repetitions of six hours caused nearly all the embryos to die early in incubation, showing 97% of the eggs clear at candling.

**Figure 8. Candled Clears Experiment 3 (includes infertile and early dead embryos)**



The temperature traces were re-examined to investigate how long the egg contents remained above 32°C. The observed hatch lift relative to the negative control is plotted against the exposure time in Figure 9.

**Figure 9: Percentage recovery in hatchability after SPIDES vs cumulative time above 32° C**



\*\* Calculated as (improvement in hatch due to SPIDES)/(loss of hatch due to storage without SPIDES)\* 100

This relationship suggests that the eggs should be limited to no more than 15 hours above 32°C for optimal results, so cooling time after treatment is also important.

The three experiments had shown that SPIDES can give a significant improvement in hatchability in eggs stored for 7 days or longer. With prolonged storage, repeated treatments were needed, but long exposure times gave poor results.

## Experiment 4

Experiment 4 was carried out in a commercial parent stock hatchery in the UK, using Arbor Acres female line eggs from a relatively young (28 weeks) flock. It was a small population, and in order to accumulate sufficient eggs for the experiment, eggs were saved over several days' production. We were aware that the speed at which the eggs were warmed up in the Chickmaster machines in the first three experiments was considerably faster than we would be able to achieve in a fully-loaded single stage machine, which would be the norm for commercial implementation. Experiment 4 was set up to compare rapid warming (up to incubation temperature in 4 hours) with the norm for the hatchery, where eggs take 8 hours to reach incubation temperature.

For logistical reasons, the eggs were 8 days old when they reached the hatchery, so the positive control was set immediately on arrival. The rest of the eggs were stored until they were on average 24 days old, receiving fast or slow SPIDES treatments 4 times during the storage period.

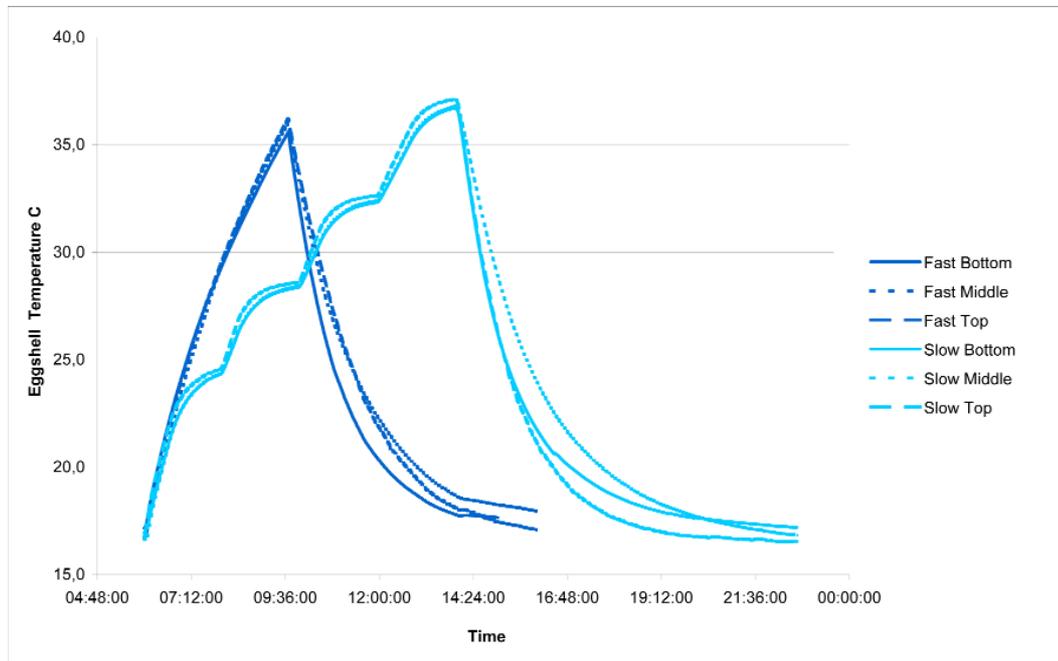
- Treatment 1 (positive control) – eggs set when 7 or 8 days old
- Treatment 2 (negative control) – eggs set when 22, 23, 24 or 25 days old
- Treatment 3 (fast SPIDES) – eggs heated so that they reached incubation temperature in 4 hours, set when 22, 23, 24 or 25 days old.
- Treatment 4 (slow SPIDES) – eggs heated so that they reached incubation temperature in 8 hours, set when 22, 23, 24 or 25 days old.

In both SPIDES treatments, the eggs were returned to the cooled egg store immediately after they reached incubation temperature

**Results**

**Internal Egg Temperatures**

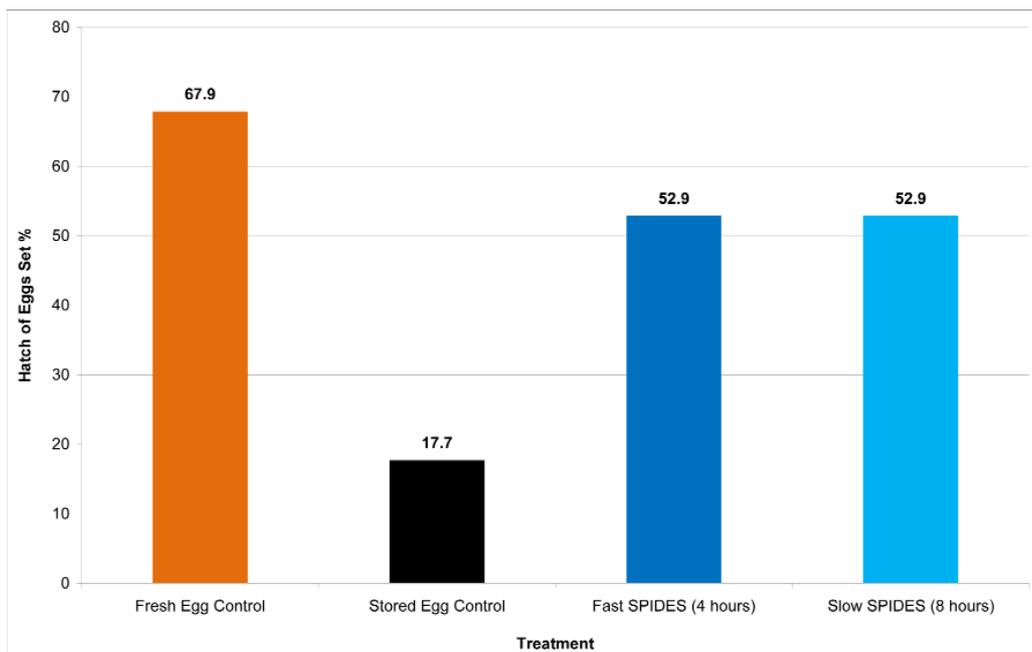
**Figure 10: Egg Shell temperature of eggs heated fast or slowly in Experiment 4**



Good treatment separation was achieved, and both treatments reached incubation temperature.

**Hatchability and Embryo Mortality Patterns**

**Figure 11: Hatchability Experiment 4**



This experiment showed that heating the eggs over 8 hours worked just as well as heating them to the same temperature over 4 hours. So a single-stage setter can be used to give the SPIDES treatments. It also showed that SPIDES treatment improved hatch in eggs laid by high-generation pure line stock – all previous experiments had used Ross 308 broiler hatching eggs.

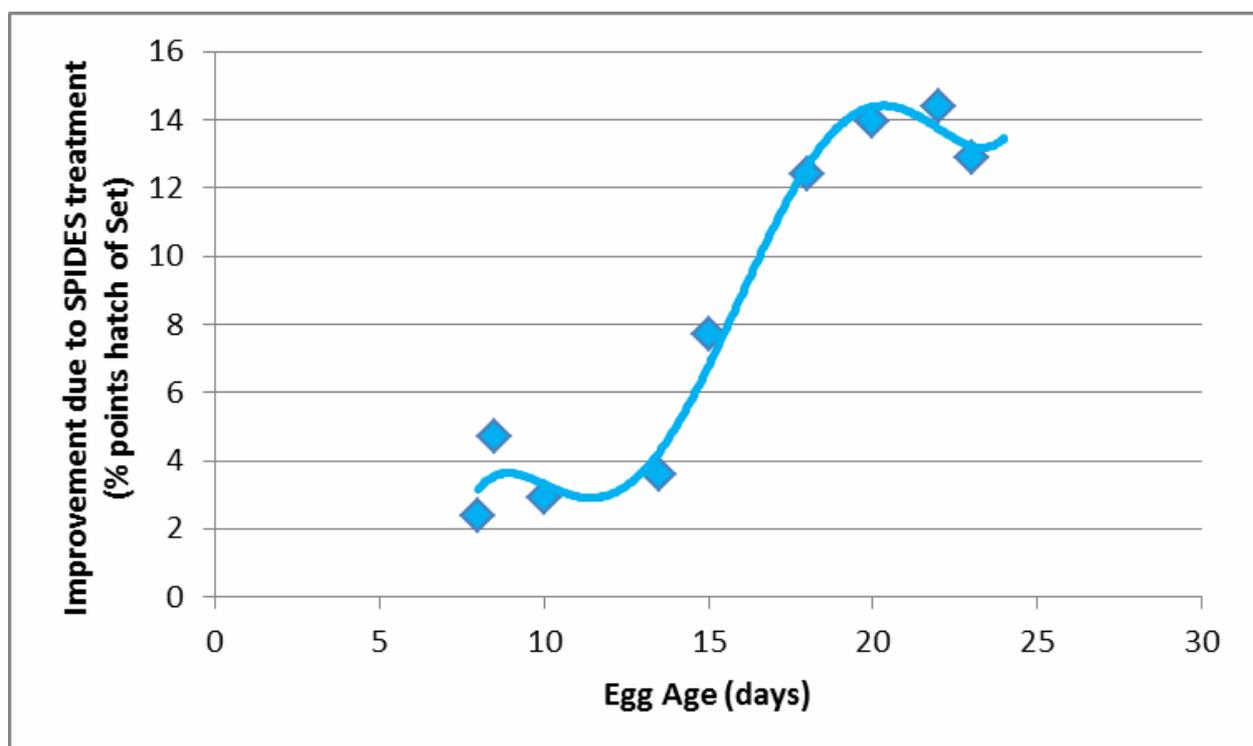
**Field Trials**

Having shown in small-scale experiments that SPIDES could be made to work, we asked the Aviagen hatchery managers around the world to test it in their own hatcheries. Trials were run in New Zealand, the UK, the USA, Turkey, Hungary, Russia, India and Sweden – a total of 17 trials and 57 comparisons over the full range of Aviagen broiler pure lines and crosses.

It soon became apparent that it was not helpful to talk in terms of exposure time – different machines take more or less time to reach incubation temperature, which will be further modified by how fully they are loaded. We also found that provision to cool the eggs made a big difference, and here the big single stage incubators were a big help, because many of them had a pre-warm programme designed to take the eggs up to 26°C, which was equally useful in cooling them down again.

Taking the results of all the trials where temperature management had been within the desirable range, we found that the average improvement after SPIDES treatment followed a very similar pattern to that shown in Experiment 2.

**Figure 12: Average improvement in hatchability after SPIDES treatment vs. egg age. Average of 34 paired comparisons, various Aviagen lines and crosses**



**Commercial Layers and Turkeys**

Having shown in Experiments 1&2 that SPIDES could be made to work, the results were shared with sister companies within the EW Group producing commercial layer stock, vaccine eggs and turkeys. Trials with layer breeders have given very similar results to those seen with the broiler breeder lines. The turkey hatcheries have also seen good results, although the optimal treatments seem to be slightly different in detail. Turkey embryos are less developed when the eggs are laid, which probably explains most of the differences seen.

## Conclusions

The best practice for storing broiler hatching eggs is still to set the eggs within a week of being laid. However, if longer storage is unavoidable, hatchability can be maximised by using appropriate SPIDES treatments during storage. For the best results, the eggs should be treated before hatch starts to fall, with repeat treatments every 6-7 days. While the heating speed and final temperature are both very forgiving, even cooling after treatment will help to maximise the impact. Warming the eggs too often, or for too long will limit the value of using SPIDES, and cumulative time above 32°C should not exceed 15 hours. SPIDES treatment is of potential benefit to broiler and commercial layer breeds, and to turkeys.

## Zusammenfassung

### SPIDES: Kurzzeiterwärmung von Bruteiern während der Lagerung

Als Basiszüchter von Broilern bietet die Firma Aviagen u.a. Brütereien Empfehlungen für die optimale Lagerung von Bruteiern vom Legetag bis zur Einlage. Im Idealfall sollten alle Eier frisch, d.h. innerhalb einer Woche nach der Eiablage, eingelegt werden. Die übliche Empfehlung für längere Lagerung ist, die Lagertemperatur abzusenken und Temperaturschwankungen zu vermeiden. Wenn aber Hennen ein Gelege im eigenen Nest sammeln, wärmen sie alle bisher gelegten Eier jedes Mal kurz auf, wenn sie das Nest aufsuchen, um das nächste Ei zu legen. In einer Serie von gezielten Versuchen und Feldtests konnten wir nachweisen, dass kurzzeitige Erwärmung von Bruteiern bei mehr als einwöchiger Lagerung helfen kann, 60-70% der Schlupfminderung zu vermeiden. Die Technik bietet sich für Großelterntiere und Elterntiere von Broilern, Legehennen und Puten an, bei denen schwankende Liefertermine und Herdengrößen im Aufzuchtbetrieb häufig eine längere Bruteilagerung erfordern.

## Thanks

The experiments and field trials described in this article could not have been done without the help and active support of Aviagen staff in our hatcheries all over the world. Thanks are especially due to Danny Goyne, Jeanette Veal and Vanessa Kretzschmar at the Aviagen Product Development Unit in the USA and to the Andy Hogg and Malcolm Price at Aviagen's Stratford Hatchery in the United Kingdom. Timea Torma, John Sims, Ricky Cates, Roberto Avila, Manickam Ganesan, Birgitta Hakensson, Serap Yasun and Daniel Chapman have all carried out field trials and given invaluable feedback on the problems they had, as well as hard data from the successes.

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