

## Editorial

### Ladies and gentlemen, friends and colleagues:

Since we started publishing **Lohmann Information** in its new online format, the number of subscriptions has steadily grown. This issue will be mailed to more than 2100 addresses in 107 countries. We trust the topics chosen as "food for thought" will find your interest and appreciate your comments. You are also welcome to send us addresses of colleagues who like to receive future issues of our publication.

1. In his review of the book "**Protein - Population - Politics**", **Prof. Gerhard Flachowsky**, FAL, Braunschweig, focuses on sustainable protein supply for the growing world population from the nutritionist's point of view. The questions raised are indeed complex and solutions must be found in a global context. The basic needs of people with different purchasing power have to be met, but animal ecology and environmental concerns will play an increasing role in the search for sustainable solutions.
2. Satisfying basic nutritional needs is not only a question of quantity, but also involves food quality and safety, especially freedom from food-borne agents. In his article "**Preventing Salmonella infections by rationally designed feed additives - the use of organic acids**", **Dr. Filip Van Immerseel**, specialist in Salmonella control in poultry at Ghent University, reviews the use of organic acids in poultry feed and advocates a new approach, aiming at an increase in the butyrate producing flora in the chicken caeca.
3. The improvement of food safety by controlling zoonoses is a major issue in the EU while implementing "Regulation No. 2160/2003 of the European Parliament on the control of salmonella and other specified food-borne zoonotic agents" until 2013. In his article "**Control of Salmonella and other zoonotic agents in the European Community**", **Dr. Matthias Voss**, head of the Veterinary Laboratory of LTZ in Cuxhaven, reviews the current legislation and time frame for the control of salmonella in poultry and pig production.
4. Production of safe food of animal origin starts with healthy animals. Hatcheries and associated parent farms play a key role in this respect, as emphasized in "**Breeder farms and hatchery as integrated operation**" by **Prof. Hafez Mohamed Hafez**, Berlin, an internationally recognized authority on poultry health and currently President of the World's Poultry Veterinary Association.
5. Beyond the challenge to produce poultry meat and eggs at low cost, in high quality and with minimal use of natural resources, producers also have to respect and respond to demands of ethicists to treat animals well. People in the industry may question whether correct beak treatment is an unnecessary "mutilation", but management practices must be optimized based on current knowledge of the bird's physiology and practical experience with less invasive methods. In her paper "**Mutilations in poultry in European production systems**", **Thea Fix - van Niekerk**, Wageningen, member of WPSA Working Group Welfare and Management takes a balanced look at the general subject from a welfare and industry point of view.

With kind regards,

Prof. Dr. Dietmar Flock

## **Protein – Population – Politics: How protein can be supplied sustainably in the 21<sup>st</sup> century**

**G. Flachowsky**  
**Institute of Animal Nutrition of the Federal Research Center (FAL)**  
**Braunschweig, Germany**

### **Introduction**

The satisfaction of hunger and thirst are basic human needs noted in the United Nations Declaration of Human Rights. At the time the declaration was written in 1948, the world population was about 2 billion people. Now there are more than six billion inhabitants, and in the foreseeable future it could easily rise to ten billion.

Hunger is much more than not feeling full. Hunger can also mean a deficit of protein and amino acids as well as other micronutrients, such as macroelements, trace elements and vitamins. These nutritional deficits may cause illnesses and diseases, preventing affected people and populations from performing at their best and adding to the medical bill. Hunger and undernourishment can be seen as the fundamental causes of a spiral of underdevelopment – not tapping physical and mental potential – dissatisfaction and development of local and international tension.

According to United Nations statistics, the WHO and FAO estimate that out of the current 6.5 billion people living on Earth, approximately 0.8 billion are not getting enough calories. Many more suffer from a lack of certain micronutrients, such as vitamin A, iodine and iron. Reducing by half the number of starving (and impoverished) people by 2015 is a primary goal formulated by the Food and Agriculture Organization. At the current rate of increase, the world population will grow to about 8.5 billion within the next 25 years (UN 2003).

In this context, the production of high-quality animal-based food takes on a special meaning, along with more efficient plant production, based on progress in plant breeding, cultivation, pest control, harvest, food preservation and storage.

There is no question that animal-based foods are not absolutely necessary for a carefully balanced diet. Predominantly vegetarian diets could already feed more than ten billion people. But it is easier to eat a balanced diet when animal protein is on the menu. This is due to the fact that in addition to high-quality protein and amino acids, milk, meat, fish and eggs contain essential macroelements and trace elements as well as vitamins. It is estimated that about 20 grams of edible animal-based protein daily would be sufficient for a complete human diet (Waterlow, 2001; Young, 2001). According to FAO estimates, livestock food products globally contributed 17% of energy and 33% of protein to dietary intakes in 2003, and it is predicted that the global average diet will reach the OECD average of 30% of energy and 50% of protein intake (Steinfeld et al., 2006).

At present, an estimated average of 30 grams per day is available per person on Earth, varying from six to 80 grams between countries and even more on an individual basis within countries. The differences in protein consumption are due to many factors, including purchasing power and social standing, but also preferences due to ethnic and religious tradition. In addition to physiological dietary aspects, foods from animal sources have a considerable enjoyment value. Rising incomes in developing countries lead to elevated demand and consumption of meat, fish, milk and eggs (Keyzer et al., 2005). Sufficient feed is the most important prerequisite for efficient animal production. Food producing animals consume about seven times more dry matter than humans all over the world (Table 1).

**Table 1: Estimated dry matter (DM) consumption by humans and farm animals**

Species	Number (billions) FAO Stat 2005	Consumption (DM)	
		(kg/day)	(billion t/year)
Humans	6.3	0.45	1.0
Cattle, buffaloes, horses, camels	1.6	10	5.8
Sheep, goats	1.8	1	0.6
Pigs	0.95	1	0.35
Poultry	17.4	0.7	0.45
Total (animals)			7.2

### Production of edible protein of animal origin

The sizeable conversion losses in food production from animal sources are a main point of criticism. On the one hand, these losses contribute to considerable resource consumption (e.g., 3 - 5 kg grain to produce one kg pork), on the other hand to the excretion of nutrients that pollute the environment (Flachowsky and Lebziern, 2006; Verstegen and Tamminga, 2006).

As shown in Table 2, protein production via milk and eggs is more efficient and more environmentally friendly than via pork and beef. As the feed conversion into food improves, the excretion decreases with higher animal performance.

Lower plant yields and lower animal performance require more land to produce a certain amount of protein of animal origin. The land area needed per inhabitant and year is calculated in Table 3 under consideration of plant and animal performances, the ratio of protein from meat and milk, and the level of consumption of protein of animal origin.

In addition to the traditional competition of land use between production of vegetarian food for human consumption and feed production for animal production, land area is increasingly being used for bioenergy/fuel production in response to the challenge of global warming (Keyzer et al., 2005). Possible strategies to overcome this situation include:

- Continued investments to increase plant yield and animal performance by traditional and innovative biotechnology.
- Improved efficiency of utilizing limited resources (land, water, fertilizer etc.).
- Lower consumption of animal protein by people with current overconsumption

About two thirds of the world's ruminants are kept in tropical and subtropical regions, but these animals account for only about one third of the ruminant protein intended for human consumption. The lion's share of the world's meat supply is produced by a much smaller number of animals in the world's temperate zones. Improvements in efficiency including optimal feeding of animals (e.g. to meet their requirements in energy and protein/amino acids) must be recognized as a top priority for farmers and researchers.

**Table 2: Production of edible protein of animal origin and corresponding N excretion from different animal species with various performance at recommended N supply (Flachowsky, 2002)**

Protein source (average body weight)	Production per day	Edible fraction %	Protein content in the edible fraction (g per kg fresh substance)	Estima- ted food competi- tion to humans (% of feed) <sup>3)</sup>	Edible protein		N-excretion	
					g per day Percent- ages	g per kg body weight	edible protein	kg per kg of intake
Milk Cow  (650 kg bw)	10 kg	95	34	0	323	0.5	0.65	75
	20 kg			(20)	646	0.9	0.44	70
	40 kg			(40)	1292	2.0	0.24	65
Goat  (60 kg bw)	2 kg	95	36	0	68	1.1	0.40	70
	5 kg			(30)	170	2.8	0.23	60
Beef  (400 kg bw)	500 g bwg <sup>1)</sup>	50	190	0	48	0.12	2.5	90
	1000 g bwg			(20)	95	0.24	1.6	84
	1500 g bwg			(40)	143	0.36	1.2	80
Lamb  (40 kg bw)	200 g bwg	50	200	0	20	0.5	1.5	85
	400 g bwg			(30)	40	1.0	1.0	80
Pork  (80 kg bw)	500 g bwg	60	150	(30)	45	0.55	0.8	85
	700 g bwg			(50)	63	0.8	0.7	80
	900 g bwg			(60)	81	1.0	0.6	75
Poultry meat (1.5 kg bw)	40 g bwg	60	200	(40)	4.8	3.2	0.4	70
	60 g bwg			(70)	7.2	4.8	0.3	60
Eggs  (1.8 kg bw)	50 % lp <sup>2)</sup>	95	120	(35)	3.6	2.0	0.6	80
	70 % lp			(50)	5.1	2.8	0.35	65
	90 % lp			(65)	6.6	3.7	0.2	55

<sup>1)</sup> Body weight gain   <sup>2)</sup> Laying performance   <sup>3)</sup> Depends on amount of roughage and by-products in the diets

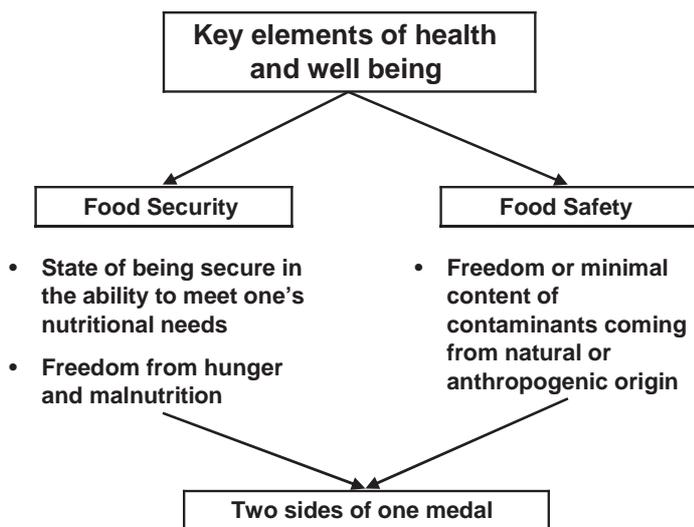
## Food security and food safety

Global food security means far more than providing enough food. The quality and safety of these products are equally important. Getting enough to eat in the immediate future is of primary concern for the starving, but even for these people at lowest end of the distribution food loaded with harmful substances cannot be an option, not to mention the fact that food-deficit countries would be excluded from international trade unless they meet the food quality and food safety standard of importing countries.

**Table 3: Influence of the yield level of plants, the performance level of animals, the ratio of protein from meat and milk, and the level of consumption of protein of animal origin on land area needs (m<sup>2</sup> per capita)**

Consumption (g protein/day) Yield level	10		20		40		60	
	A1)	B2)	A	B	A	B	A	B
Ratio between protein from meat <sup>3)</sup> and milk (% of protein)								
70 : 30	260	105	520	210	1050	420	1560	630
50 : 50	225	95	450	190	900	380	1350	570
30 : 70	190	85	380	170	760	340	1140	510

1) Yield level A per hectare: 4 t DM of cereals, 10 t DM of forage; performance level A per day: 15 kg milk; live weight gain: beef cattle: 600 g; pigs: 400 g; poultry: 30 g  
 2) Yield level B per hectare: 8 t DM of cereals, 15 t DM of forage; performance level B per day: live weight gain: beef cattle: 1200 g; pigs: 800 g; poultry: 60 g  
 3) Ratio between protein from beef, pork and poultry (in %): ≈ 15 : 60 : 25



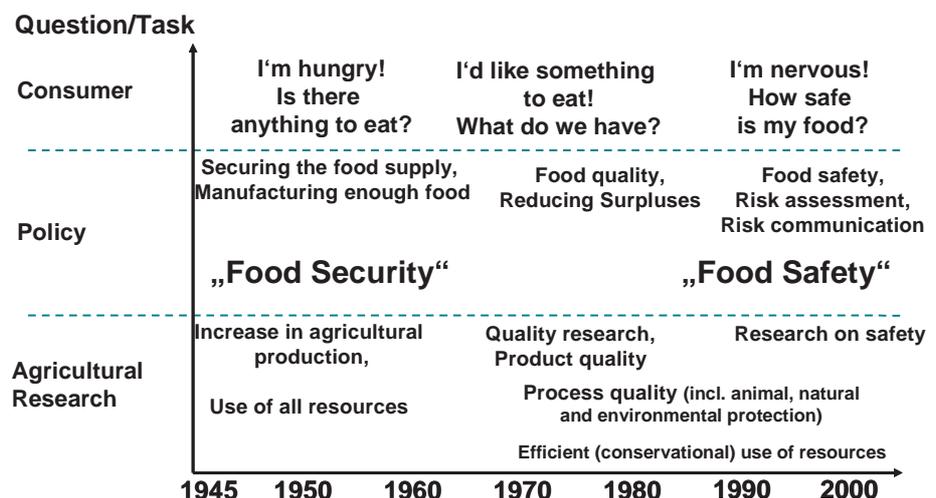
**Figure 1:**

**Food security and food safety as elements of health and well being**

Various consequences for politicians, agricultural producers and also scientists are illustrated in Figure 2. They include fair distribution of available food, increased food production in deficit regions (»helping them to help themselves«), application of current scientific knowledge and investment in research to secure food for a growing world population. Increasing demand for high quality animal-based protein should be taken into account as a reality.

**Figure 2:**

**Main questions related to food as well as tasks for policy and agricultural research (Flachowsky, 2003)**



The quantitative issue of food security and the qualitative issue of food safety are key elements of human health and well being, as shown in Figure 1 (from Flachowsky and Dänicke, 2005).

## **Protein – Population – Politics**

The goals mentioned above can only be achieved through the efficient use of available resources such as water, fossil fuels, soil and raw materials such as phosphorus, a minimization of pollution in food production and effective conversion of available feed into animal-based food including new learning's in animal sciences (e.g., optimal feeding incl. feed additives). The many agricultural research disciplines, beginning with plant cultivation and including everything from genetic modification to agricultural economics, can and must make substantial contributions to ensure long-term, sustainable food security.

These and more were the topics of a large symposium held in Berlin entitled "Protein, Population, Politics". Scientists, farmers and politicians involved in aspects of global nutrition presented interesting papers on these themes.

Two well-known journalists, Dirk Maxeiner and Michael Miersch, moderated the Symposium, structured and illustrated the contributions and helped to put the scientific information into understandable language also for people outside the scientific community. The book, originally published in German, was recently translated into English to make it accessible for more readers (Wennemer et al., 2006). Main aspects of the 10 chapters are:

The 1<sup>st</sup> chapter deals with diet and evolution. The struggle for meat seems to be a driving force in the evolution of humankind. It required everyone to be cooperative within a group and ready to fight those on the outside.

Nearly all cultures favor animal protein over other foodstuffs and pursue meat at a cultural expense. A biological mechanism seems to be behind it all. Also today, meat is the determining part of the meal. For example, when choosing from a menu, most people will name the meat portion of the meal.

The 2<sup>nd</sup> chapter is entitled "The race between the stork and the plow" and deals with the question: How many people can the earth feed? Interesting figures characterize future developments concerning world population, food need and area per capita available in future. In 2050 only around 1750 m<sup>2</sup> of arable land will be available for every person. Much attention has been spent in this chapter to Norman Borlaug and his success story with the green revolution (see also Hesser, 2006). Jaques Diouf, the General Secretary of the FAO stated in an interview about the second "green revolution": "There isn't any more land. We are exploiting the available production factors to a great extent. The environment is becoming more polluted. Increased production has to come from high-yielding farming". New technologies, including biotechnology cannot solve the problems of developing countries alone, but they can lay the essential groundwork for development.

The 3<sup>rd</sup> chapter deals with shepherds and rulers or how breeding animals changed the world. The animal species and their potential to improve the yields or to use feeds more efficiently are analyzed in this chapter. "Perestroika in the chicken coop" is the title of the 4<sup>th</sup> chapter. The human attitude towards animals is changing. More and more countries are experiencing an explosion in the public debate concerning animal welfare and animal husbandry.

The 5<sup>th</sup> chapter, written by Wolfgang Haber, deals with sustainable agriculture: "Accountable to people and the environment". Around 350 000 human generations got their food from hunting and gathering, taking a percentage of biomass that was produced naturally, without being manipulated by humans. But the basic diet became inadequate because of increased population and the humans recognized that they could use certain plant and animal species to their own ends by gardens and fields. Natural processes and systems could be transformed to increase the production of that part of the biomass that was desired as food. In Central Europe, only around 325 generations of farmers were needed to make this fundamental transformation. Humans and their needs are still increasing, which means they need increasing amounts of space and food. Ways for more efficient and sustainable production

of protein of animal origin are discussed. Supplementation of diets of non-ruminants with essential amino acids like lysine and/or methionine may substantially contribute to a more efficient conversion of feed into food of animal origin.

Chapter 6 deals with soybeans on the high seas. Feed, meat and even live animals have become international commodities. Pros and cons of shipping meat or feed are discussed. Chapter 7 is entitled “Competition vs. cooperation”. Does feeding the world’s livestock take food away from the poor? This is a difficult question, and various opinions are offered as tentative answers (e.g. Rifkin, 1992; FAO, 2002; Keyzer et al., 2005). Animal species, their level of production and amounts of roughage and by-products included in animal feed (see Table 2) influence the competition with humans.

Chapters 8 (“From fisherman to farmers of the sea”) and 9 (“Meatless alternatives”) are relatively short (6 and 3 p.) and deal with domesticating fish through aquaculture and alternatives in addition to widely used protein plants like soybeans. Exotic alternatives such as fungi, algae and insects add variety to the human protein palette. Fish farming will play an important role in satisfying the increasing protein demand. In 2020 more than 40 % of all fish on the market will be farmed (Delgado, 2000). Around 80% of the world’s fish will be produced and consumed by developing nations. Today’s fishermen will be tomorrow’s farmers on the sea.

The last chapter (10) deals with the “Brave new swine”. The desire for food of animal origin will continue to increase sharply in the coming decades. Much needs to be done to ensure a sustainable protein supply. More activities to improve plant yields and animal efficiency as well as to utilize local resources and by-products are necessary to produce safe food of animal origin (see Figure 1) with low demand of land (see Table 3) and low excretion of nitrogen (see Table 2), phosphorus, methane and other pollutions (see also v. Weizsäcker et al., 1997).

Finally an afterword on genuine dialogue is presented by the theologian Roger J. Bush. The author looks at modern livestock feeding and sustainable development from an ethical perspective. The main question is: how can the society find common ground on economic, ecological and social issues? The following ethically relevant areas and questions concerning animal feeds must be considered and answered by people working with animals:

- Animal ecology: Does behavioral research take into account describable, fundamental needs of the animals to be fed?
- Environmental ecology: What effects do feed systems have on the environment?
- The relationship between local and national, European and global “solutions” for using protein resources.
- Consideration of the rights of people to participate and to fulfill their potential in economically disadvantaged areas on Earth.

Altogether the authors of the book tried to find answers to the questions “Can the Earth feed everyone in the long term?”; or “Are we making efficient use of the Earth’s natural resources?”; or “What role do animals play in all of this and how should they be treated?”

It is extremely difficult to find clear answers to such complex and difficult questions, but at least the book shows how feed production and feeding of animals can contribute to a more efficient production of protein of animal origin. Hopefully the book will not only be enjoyed by many readers, but also contribute to current public debate and give impulses to overcome deficits in worldwide human nutrition.

Some of the basic issues raised in this book are also discussed in a recent FAO publication, mainly from an environmental angle (Steinfeld et al., 2006). Clearly, environmental concerns will play an increasing role in animal production. Political decisions to use more land for bio-energy production contribute to the pressure to maximize the efficiency of feed conversion in animal production. Synthetic amino acids will play an important role in any plan to improve global human nutrition while minimizing the environmental impact of animal agriculture.

## Summary

The production of edible protein of animal origin is the primary objective of livestock husbandry. The protein intake of people in developed countries is high (more than 50 g per capita daily from animal origin) and rising incomes in developing countries lead to elevated demand and consumption of meat, fish, milk and eggs.

On this basis, the following questions must be answered: “Can the Earth feed everyone in the long term?”, “Are we making efficient use of the Earth’s natural resources?”, “What role do animals play in all of this and how should they be treated?” Some answers are given in this paper.

In the first part, fundamentals are presented such as protein production of various animal species (depending on their performance), need for arable land per capita (depending on plant and animal yields), animal protein consumption per capita and the connection with food security and food safety.

In the second part, the book “Protein – Population – Politics” is reviewed in some detail. This book (10 chapters, 160 pages) was primarily written for people outside the scientific community and shows the responsibility of politicians, scientists and farmers for future developments to satisfy the physiological needs and wishes of humans under consideration of physiological, ecological, economical and ethical aspects. In some cases more questions are arisen than answers could be given. Future challenges for all those involved in animal production are shown.

## Zusammenfassung

### **Protein – Population – Politik: Wege zur nachhaltigen Eiweißversorgung im 21. Jahrhundert**

Die Erzeugung von essbarem Eiweiß tierischer Herkunft ist eine der wichtigsten Aufgaben der Nutztierhaltung. Der Proteinverzehr der Menschen in den sog. entwickelten Ländern ist bereits hoch (> 50 g je Einwohner und Tag als Protein tierischer Herkunft). Mit zunehmendem Einkommen steigt aber auch in den Entwicklungsländern der Verzehr von Fleisch, Fisch, Milch und Eiern weiter an.

Auf der Grundlage dieser Entwicklung sind u.a. folgende Fragen zu beantworten: „Kann die Erde langfristig alle Menschen ernähren?“, „Werden auf der Erde die natürlichen Ressourcen effizient genutzt?“ oder „Welche Rolle spielen Nutztiere in diesem Zusammenhang und wie sind sie zukünftig zu betrachten?“.

Einige Antworten zu diesen komplizierten Fragen werden im Beitrag gegeben. Grundlagen sind im ersten Teil beschrieben, wie z.B. die Proteinerzeugung mit verschiedenen Tierarten und in Abhängigkeit von der Leistungshöhe; der Bedarf der Einwohner an Ackerfläche in Abhängigkeit von Pflanzenertrag und tierischer Leistung sowie dem Verzehr je Einwohner an tierischem Protein oder der enge Zusammenhang zwischen ausreichender Lebensmittelbereitstellung, deren Qualität und Sicherheit.

Der zweite Teil des Beitrages beschäftigt sich mit dem Inhalt des Buches „Protein – Population – Politik“ und beschreibt einige Aussagen der 10 Kapitel des Buches. Das Buch ist primär geschrieben für die allgemeine Öffentlichkeit, um Verständnis für die globale Herausforderung der „Welternährung“ zu wecken. Es zeigt die Verantwortung der Politiker, Wissenschaftler, Landwirte und all jener, die sich mit Ernährung für die zukünftige Befriedigung des ernährungsphysiologischen Bedarfs und der Wünsche der Menschen an Lebensmittel unter Berücksichtigung physiologischer, ökologischer, ökonomischer und ethischer Aspekte beschäftigen. Manchmal entstanden aus einer Frage mehr neue Fragen als Antworten gegeben werden konnten. Herausforderungen für alle Beteiligten werden aufgezeigt.

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Prof. Dr. G. Flachowsky  
Institute of Animal Nutrition  
Federal Agricultural Research Centre  
Bundesallee 50  
38116 Braunschweig, Germany

## Preventing *Salmonella* infections by rationally designed feed additives: the use of organic acids

Filip Van Immerseel  
Ghent University, Merelbeke, Belgium

### Introduction

Poultry meat and eggs are important food products and the poultry-related industry is an economically important component of the agro-industry in the EU. The industry however is under pressure and faces many challenges. Most importantly, there is a demand for eggs and meat of high nutritional value and free of microbiological and chemical hazards. Unfortunately poultry eggs and meat are one of the major sources of food borne bacterial infections in humans. In this respect, *Salmonella* (mainly *Salmonella* Enteritidis) and *Campylobacter* are of particular importance, since these pathogens can colonize the chicken host without causing discernible illness in the infected chickens.

In Europe and the USA, *Salmonella* Enteritidis has become by far the most important egg-associated pathogen resulting in a pandemic of non-typhoidal salmonellosis in humans. Despite the recent decrease in human *Salmonella* Enteritidis infections due to the introduction of control measures in layer flocks, *Salmonella* still is one of the most important bacterial foodborne infections in the world. Serotypes other than Enteritidis are not decreasing in prevalence to the same magnitude as Enteritidis.

While some serotypes, such as Derby and Typhimurium, can be traced back to porcine meat, other serotypes, such as Virchow, Infantis, Hadar and again Typhimurium, can be traced back to poultry meat. Since poultry meat will, due to EU legislation, not be brought as fresh poultry meat on the market from 12/12/2010 if *Salmonella* is detected, the broiler industry needs to take measures to decrease the colonization of the animals and their environment.

These include pre-harvest, harvest and post-harvest measures. All of these are equally important and each type of measure has a more or less important effect on reducing the *Salmonella* incidence, but no measure is successful on its own. Harvest measures are essentially hygienic measures during catching and transport, while post-harvest measures include both hygienic measures and the application of decontaminating treatments on the meat.

However, all carcass disinfectants are prohibited in the European Union at present, thus decontamination is not an option. Therefore, prevention and monitoring/eradication during the live phase (pre-harvest) are of great importance in Europe. Pre-harvest prevention and control measures start in dedicated feed mills and on the grandparent farms of primary breeders (MacLeod, 2002) and include preventive hygienic measures as well as physical and chemical decontamination treatments of feed, drinking water and the environment of the birds down to the commercial farms of broiler growers and egg producers. Since antibiotics are not the method of choice to control *Salmonella* and since vaccination cannot be applied in broilers due to the short life span of the animals, the use of feed additives is more and more accepted as a valuable way to combat bacterial gut infections. Currently however there are numerous products on the market and it is not easy for poultry producers to objectively choose products with proven activity against *Salmonella*, since most of the products are empirically produced.

### The use of organic acids against *Salmonella*

#### A) Effects of short-chain fatty acids on survival and virulence of *Salmonella* in vitro

Medium chain fatty acids (C6 to C12; caproic, caprylic, capric and lauric acid) appear to be much more effective against *Salmonella* than the short-chain fatty acids (formic, acetic, propionic and butyric acid). As little as 25mM C6 to C10 acids were bacteriostatic to a *Salmonella* Enteritidis strain, while the same strain tolerated 100mM of short-chain fatty acids (Van Immerseel et al., 2003, 2004a). When *Salmonella* Enteritidis and Typhimurium were incubated with low concentrations of monocaprin (5 mM)

that had been combined with an emulsifier, the bacteria did not survive (Thormar et al., 2006). In general, these data indicate that medium chain fatty acids have the greatest antibacterial activity against *Salmonella*, but large scale studies are lacking.

*Salmonella* is an opportunistic intracellular pathogen that has an elaborate set of virulence genes. These genes enable the bacterium to adapt to the environment and move between various micro-niches within a host. An early step in the pathogenesis of *Salmonella* is the penetration of intestinal epithelium.

This activity is promoted by invasion genes that are located on a pathogenicity island (SPI-1), but several pathogenicity islands are required for full virulence. SPI-1 has genes encoding regulatory proteins, structural components of a needle complex and additional effector proteins. Bacterial effector proteins facilitate the entry of *Salmonella* into the cytosol of epithelial cells, by inducing actin rearrangements that lead to uptake of the bacteria. SPI-1 is in turn activated by HilA, and this latter protein is environmentally regulated. When *Salmonella* Typhimurium was pre-incubated in growth media supplemented with various concentrations of butyrate and propionate, epithelial cell invasion was suppressed (Van Immerseel et al., 2003). However, if the bacteria were preincubated in media supplemented with acetate invasion was still observed.

Similar results were obtained with *Salmonella* Enteritidis when primary caecal epithelial cells of the chicken were employed. The effects of organic acids on epithelial invasion can be explained by changes in SPI-1 expression. When *HilA* and *invF* (major activators of SPI-1) expression was measured in *S. Typhimurium* after exposure to acetate, butyrate and propionate at pH 6, exposure of the bacteria to acetate increased the expression of these genes, but similar effects were not observed with propionate or butyrate (Durant et al., 2000).

More recently, it was shown that butyrate and propionate, but not acetate led to a decrease in *hilA*, *invF* and *sipC* expression (Lawhon et al., 2005) DNA microarrays of both *Salmonella* Typhimurium and *Salmonella* Enteritidis indicated that low doses of butyric acid downregulated SPI-1, but it did not alter metabolic gene expression (Gantois et al., 2006). The primary target of butyrate in the bacterial cell is still unknown but butyrate could interfere with HilA dependent regulation of SPI1 by altering the regulation of *hilD* transcription. These data indicate that short-chain fatty acids can regulate the invasive phenotype of *Salmonella*.

## **B) Effects of short-chain fatty acids in *Salmonella* control in vivo**

The use of acidic compounds to control *Salmonella* first appeared in the late 1960's, and mainly focused on decontamination of bone meal. Evaluation of the efficacy of 32 different acid preparations to decontaminate bone meal showed that low molecular weight volatile fatty acids were the most promising (Khan and Katamay, 1969). These results were a basis for the development of non-toxic, naturally occurring acidic compounds to control *Salmonella*. More than 35 years later, it is clear that their thoughts were prophetic. These acids have been added to feed, drinking water, and other matrices, in order to prevent *Salmonella* colonization of animal tissue and transmission through the food chain.

Poultry feed is a major source for *Salmonella* introduction to the farm. When chickens are given artificially contaminated feed, the gut is colonized and *Salmonella* are shed into the environment. The original concept of incorporating acids into feed was based on the notion that the acids would decontaminate the feed itself and prevent *Salmonella* uptake by the chickens.

When Iba and Berchieri (1995) inoculated feed with high doses of a *S. Typhimurium* strain, a commercial mixture of formic and propionic acid decreased the viability more than 1000-fold over 7 days. When broiler chicks were given the acid treated feed that had been mixed with either *Salmonella* Enteritidis, Typhimurium or Agona, the caecal *Salmonella* numbers were 7 logs lower than control animals at day 5 ( $10^2$  versus  $10^9$  cfu/g). Mixtures of formic and propionic acid were also effective when feed was artificially inoculated with low doses of *S. kedougou*, and the decrease was most obvious after several weeks of storage. In a large scale study, the number of *Salmonella* positive breeder feed samples decreased from 4.1 to 1.1% after the feed was supplemented with 0.5% formic acid. The

antibacterial activities of organic acids were dependent on the temperature and moisture. Since the water content of poultry feed is generally low, the action of the acids is not always optimal, and it is not clear whether effects of the acids in the feed itself or effects of the acids in the gastrointestinal tract of the animals were the major reason of protection.

In the 1980's, it became clear that the acid concentrations were also increased in the crop, and this antibacterial action could aid in controlling infection caused by horizontal transmission. Indeed, when the acid treated feed is eaten by the chickens, it is both warmed and moistened and the activity of the short chain fatty acids should increase. It appears that supplemental acids are most apt to affect in the crop and gizzard rather than in the intestine. This point is illustrated in a study of Thompson and Hinton (1997), who fed laying hens a feed supplemented with a commercial mixture of formic and propionic acid. In these animals, pH values of the crop, gizzard, jejunum, caecum and colon were not altered relative to control animals, but formic and propionic acid concentrations in crop and gizzard were significantly increased. At the same time, the lactic acid concentration in the crop decreased significantly, suggesting that lactobacilli were either inhibited or killed.

Later in the 1980's, many studies examined the effects of supplementing acids on *Salmonella* colonization of chicken tissues. Mainly the action of formic and propionic acids was tested. In a small-scale field trial, formic acid controlled shedding and caecal colonization by *Salmonella* serovars in naturally infected the animals. Indeed, 50% of all control animals had *Salmonella* positive cloacal swabs and caecal content samples, but *Salmonella* could not be detected in animals that consumed significant concentrations of formic acid (Hinton et al., 1985).

In a 3 year study, the cumulative number of infections of newly hatched chicks with *Salmonella* decreased after breeder stocks were given formic acid treated feed (Humphrey and Lanning, 1988). Breeders that received acidified feed shed lower numbers of *Salmonella* in the litter (4.3 versus 1.4%), hatchery waste (15.3 versus 1.2%) and insert paper samples (4.6 versus 1.4%). These decreases were evident from the moment the breeders received acidified feed and illustrate the effects of vertical transmission.

The most striking proof of the efficacy of formic and propionic acid as feed additives to control *Salmonella* was given by Hinton and Linton (1988). In three independent experiments, only natural infections were monitored. Formic and acetic acid supplemented feed, given from the day of hatch, decreased the number of positive faeces and caecal content samples dramatically. The control groups had 25, 27 and 60% *Salmonella* positive faecal samples, but the treatment groups were 3, 0 and 0%. When the formic acid treated feed was given at later age (16 or 32 days), no differences were detected between control and treated groups. This illustrates that preventing initial colonization of *Salmonella* is most important. Once an infection is established, it is very difficult to counteract by using acid treated feed, at least in the same production round.

Recently, researchers have attempted to transport the organic acids further down in the gastrointestinal tract by micro-encapsulation, which should prevent absorption of the acids in the upper tract and ensure release further down in the gastro-intestinal tract. Van Immerseel et al. (2004b) examined the effect of microbeads containing formic, acetic, propionic and butyric acid on colonization of *S. Enteritidis* in caeca, liver and spleen. Animals were infected (day 5 post-hatch) with  $5 \times 10^3$  cfu *S. Enteritidis* and samples were taken 3 days post-infection. Cecal colonization was significantly increased when acetic acid was added to the feed, but decreased when butyric acid was added (Table 1). Internal organ colonization was increased if either formic or acetic acid were added to the feed, and this result is consistent with the idea that acids can enhance the virulence of *Salmonella*.

When powder and coated butyric acid additives (0.63 g/kg butyric acid) were compared using the same infection protocol, the coated form decreased colonization of the caeca, but the powdered form did not (Table 2). The inability of the powdered form to give a positive response may have been due to the short time interval between infection and sampling. In an infection study using a seeder model in which 10 broilers were infected at day 5 post-hatch with  $10^5$  cfu *S. Enteritidis* and housed together with 40 non-inoculated broilers, 0.63 g/kg coated butyric acid in the feed significantly reduced shedding of *S. Enteritidis* in broilers until slaughter age (Van Immerseel et al., 2005, (Figure 1)). The effect of the acids on other members of the microbial community was not determined.

**Table 1. Colonization of the caeca at day 8 of life (inoculation with  $10^3$  cfu *S. Enteritidis* 76Sa88 on day 5 and 6) in chickens fed a diet supplemented with formic, acetic, propionic and butyric acid or no feed additives.**

	CTRL <sup>A</sup> (n=20)	FOR <sup>A</sup> (n=20)	ACE <sup>B</sup> (n=20)	PROP <sup>AC</sup> (n=20)	BUT <sup>C</sup> (n=20)
Negative	0*	0	0	0	0
Positive after enrichment	6	1	1	8	11
$10^2 < x < 10^3$ cfu/g	0	1	1	1	2
$10^3 < x < 10^4$ cfu/g	0	4	0	1	1
$10^4 < x < 10^5$ cfu/g	3	2	0	2	3
$10^5 < x < 10^6$ cfu/g	2	2	2	1	3
$10^6 < x < 10^7$ cfu/g	8	7	3	3	0
More than $10^7$ cfu/g	1	3	13	4	0

\* Number of chickens in a group of 20 that has a given amount of *Salmonella* bacteria in the caeca.

A,B,C Groups with different superscripts are significantly different.

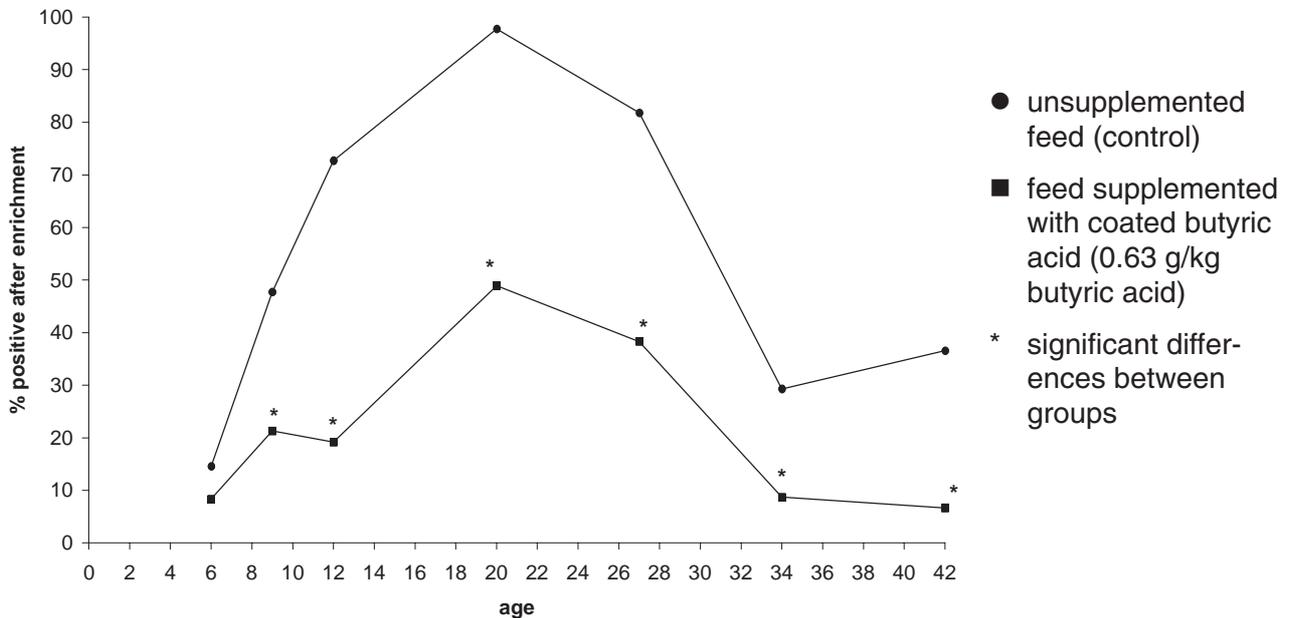
**Table 2. Colonization of the caeca at day 8 of life (inoculation with  $10^6$  cfu *S. Enteritidis* 76Sa88 on day 5) in chickens fed a diet supplemented with butyric acid in powder form, coated form, a combination of half doses of powder and coated form (COMBI), or no feed additives (CTRL). Concentration of the active product butyric acid was 0.63 g/kg feed in each group.**

	CTRL <sup>A</sup> (n=25)	POWDER <sup>A</sup> (n=25)	COATED <sup>B</sup> (n=25)	COMBI <sup>B</sup> (n=25)
Negative	0*	0	2	1
Positive after enrichment	6	8	12	8
$x < 10^4$ cfu/g	2	1	3	2
$10^4 < x < 10^5$ cfu/g	3	1	0	6
$10^5 < x < 10^6$ cfu/g	4	3	4	5
More than $10^6$ cfu/g	10	12	4	3

### Increasing caecal butyrate concentration to control *Salmonella*: use of pre- and probiotic approaches?

Feed and drinking water sanitation, and the addition of acids to the crop appears to prevent pathogen colonization in the live animals, but the type of acid and its concentration can be very important. *Salmonella* colonization of the caeca and internal organs is not always affected by these treatments, especially if the infection pressure is high. Acids from feed or drinking water are not effective further down in the intestinal tract because *Salmonella* colonization is mainly in the caeca. Because the caecum is the main fermentation site, the concentrations of short-chain fatty acids are already higher there than in other intestinal segments. It has already been shown in various animal species that

**Figure 1.** Number of positive cloacal swabs over time (age) following infection of 10 chicks with  $10^5$  cfu *S. Enteritidis* 76Sa88 at day 5 post-hatch and housing them with 40 non-inoculated broilers



*Salmonella* colonization of the gut is decreased when the bifidobacterial population is increased, either by administration of bifidobacteria as probiotic strains, or by addition of certain types of oligosaccharides that stimulate proliferation of these bacteria in the gut (Asahara et al., 2001; Buddington et al., 2002; Bovee-Oudenhoven et al., 2003; Silva et al., 2004; Thitaram et al., 2005).

Increases in lactic acid bacterial counts in the gut are correlated with increases in butyric acid concentrations, and *Salmonella* colonization is decreased when butyric acid levels in the gut are increased. Bifidobacteria increase butyric acid concentrations, but these bacteria do not produce butyric acid themselves. Lactic acid bacteria, such as lactobacilli and bifidobacteria, stimulate proliferation of butyric acid producing bacteria. This mechanism is called cross-feeding.

It has been shown that lactic acid, produced in vitro by *Bifidobacterium adolescentis* with starch as sole carbon source, is used by *Anaerostipes caccae* and *Eubacterium hallii* (in co-culture) for the production of high concentrations of butyric acid. Another approach would be a direct stimulation of butyric acid producing bacteria. In human gut samples, butyric acid producers are anaerobic bacteria belonging to the phylogenetic *Clostridium* clusters IV and XIVa, and species related to *Roseburia*, *Eubacterium*, *Faecalibacterium* and *Coprococcus* can also produce butyrate (Pryde et al., 2002; Duncan et al., 2004). Many of the butyrate producing microbiota that are identified are net consumers of acetate. Random cloning and sequencing of 16SrDNA sequences isolated from chicken caeca revealed more than 85% of the clones belonging to eubacteria and clostridia (Bjerrum et al., 2006). Approximately 10% of the clones had high similarity with *Faecalibacterium prausnitzii*, a species that produces butyric acid in the human gut.

When butyric acid producing bacteria from poultry caeca were isolated (producing more than 10mM in culture) in the author's lab, 16SrDNA sequence analysis showed that these isolates were rather closely related to the butyrate producing species described for humans. The isolates consumed acetate and produced butyrate, and are most likely also cross-fed by lactate. These data can open new opportunities for *Salmonella* control. Indeed, increasing the concentration of these butyrate producers in poultry caeca using nutritional strategies and addition of pro- or prebiotics would be an efficient way to combat *Salmonella* infections.

Chances of success are increased, since these bacteria produce butyric acid at the site where *Salmonella* is predominantly colonizing and invading. Furthermore, after years of adding feed additives

to poultry feed in an empirical way, the approach could finally be more rational, aiming at an increase in the butyrate producing flora in chicken caeca, finally providing a way to measure the action of the additives. The criteria for *Salmonella* control thus would be: do the additives increase the amount and distribution of butyrate producing bacteria in chicken caeca?

Currently tests are being developed to quantify the presence of the key enzyme involved in butyrate synthesis in the anaerobic butyrate producing flora at the DNA level, in order to produce a simple and reliable test to evaluate the effect of additives and *Salmonella* control, even without performing large scale infection trials. This would enable us to rationally design feed additives in the future.

## Summary

Acidification of drinking water and feed has been used for years in poultry to control *Salmonella*. For drinking water and powder form feed supplementation, the choice of the acids used is dependent on its ability to kill bacteria. Currently also coated or impregnated acid products are on the market, aiming to bring the acids further down in the gastro-intestinal tract. It was shown that supplementation of acetic acid coated products to the feed increases colonization of the chicken gut by *Salmonella*, and that coated propionic and butyric acid products decreases colonization of the chicken intestinal tract. Moreover, it was shown that coated butyric acid decreased faecal shedding in a seeder model in broilers throughout the rearing period up to slaughter age. This can be linked with effects of the acids on *Salmonella* virulence, since exposure of *Salmonella* to low concentrations of acetic acid increases invasion of *Salmonella* in intestinal epithelial cells, while propionic and butyric acid decrease invasion at non growth inhibitory concentrations. This is due to changes in virulence gene expression of the genes of the *Salmonella* Pathogenicity Island I, necessary for invasion. Currently the approach of increasing butyric acid producing gut microbiota is under research in different institutes. Increasing the concentration of butyrate producers in poultry caeca using nutritional strategies and addition of pro- or prebiotics would be an efficient way to combat *Salmonella* infections. Indeed, these bacteria produce butyric acid at the site where *Salmonella* is predominantly colonizing and invading, i.e. the caeca. Furthermore, after years of adding feed additives to poultry feed in an empirical way, the approach could finally be more rational, aiming at an increase in the butyrate producing flora in chicken caeca.

## Zusammenfassung

### Kontrolle von *Salmonella* Infektionen durch gezielten Einsatz von organischen Säuren im Futter

Seit Jahren wird versucht, mit einer Erhöhung des Säuregrades im Trinkwasser oder Futter *Salmonella* in den Griff zu bekommen. Für Trinkwasser und Futterzusätze in Pulverform kommt es auf die richtige Wahl der Säuren nach ihrer bakteriziden Wirksamkeit an. Gegenwärtig sind auch beschichtete bzw. imprägnierte Produkte auf dem Markt, die die Säure möglichst tief in den Darmtrakt bringen sollen.

Es konnte gezeigt werden, dass der Futterzusätze mit beschichteter Essigsäure die Darmbesiedlung von Broilern mit *Salmonella* fördert, während der Zusatz von Produkten mit Propionsäure und Buttersäure die Besiedlung hemmt. Weiterhin konnte nachgewiesen werden, dass der Zusatz von geschützter Buttersäure nach gezielter Infektion die Ausscheidung von *Salmonella* im Kot während der gesamten Mastdauer bis zur Schlachtung signifikant verringerte.

Das kann damit zusammenhängen, dass verschiedene Säuren die Virulenz von *Salmonella* unterschiedlich beeinflussen, denn geringe Konzentration von Essigsäure fördert das Eindringen von *Salmonella* in die Epithelzellen des Darms, während Propionsäure und Buttersäure in nicht wachstumshemmender Konzentration die Besiedlung hemmt. Dies erklärt sich aus Veränderungen der Virulenzexpression der Gene der *Salmonella* Pathogenitätsinsel I, die für die Invasion notwendig sind.

Gegenwärtig beschäftigen sich verschiedene Institute mit der Frage, wie die Produktion von Buttersäure in der Darmflora erhöht werden kann. Wenn es gelänge, durch Futterzusätze von Pro- bzw. Präbiotica

die Konzentration von Buttersäure produzierenden Bakterien im Blinddarm zu erhöhen, dann wäre das ein effizienter Beitrag werden, um *Salmonella* Infektionen über das Futter zu bekämpfen. Diese Bakterien produzieren Buttersäure da, wo *Salmonella* überwiegend kolonisieren and eindringen, nämlich im Blinddarm. Nachdem jahrelang Futterzusätze empirisch verabreicht wurden, könnte sich die gezielte Vermehrung von Buttersäure produzierenden Bakterien im Blinddarm von Hühnern als rationeller erweisen.

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Filip Van Immerseel  
Ghent University  
Faculty of Veterinary Medicine  
Department of Pathology, Bacteriology and Avian Diseases  
Salisburylaan 133  
9820 Merelbeke  
Belgium

## **Control of Salmonella and other zoonotic agents in the European Community – current status of legislation –**

**Matthias Voss**

**Veterinary Laboratory, Lohmann Tierzucht, Cuxhaven**

### **Introduction**

For the Commission of the European Communities the protection of human health against diseases and infections transmissible directly or indirectly between animals and humans (zoonoses) is of paramount importance. This will be a major issue until complete implementation of „Regulation (EC) No 2160/2003 of the European Parliament and of the Council of 17 November 2003 on the control of salmonella and other specified food-borne zoonotic agents“ in 2013.

In 1992, the European Union adopted a directive to monitor and control Salmonella infections in breeding flocks of domestic fowl (Council Directive 92/117/EC of 17 December 1992 concerning measures for protection against specified zoonoses and specified zoonotic agents in animals and products of animal origin in order to prevent outbreaks of food-borne infections and intoxication's provided for the establishment of monitoring systems for certain zoonoses and controls on salmonella in certain poultry flocks.) This directive laid down specific minimum measures to control infections with Salmonella enteritidis and Salmonella typhimurium in breeding stocks of Gallus gallus.

In recent years, the Scientific Committee on Veterinary Measures relating to Public Health considered that the measures in place to control food-borne zoonotic infections were insufficient at that time. It further considered that the epidemiological data collected by Member States were incomplete and not fully comparable.

As a consequence, the Committee recommended to improve the existing control systems for specific zoonotic agents and in November 2003 the following legislation was implemented for

- the monitoring of zoonoses: Directive 2003/99/EC of the European Parliament and of the Council of 17 November 2003 on the monitoring of zoonoses and zoonotic agents, amending Council Decision 90/424/EEC and repealing Council Directive 92/117/EEC, replacing the monitoring and data collection systems established by Directive 92/117/EEC, and
- for the control of zoonoses: Regulation (EC) No 2160/2003 of the European Parliament and of the Council of 17 November 2003 on the control of salmonella and other specified food-borne zoonotic agents. This Regulation covers the adoption of targets for the reduction of the prevalence of specified zoonoses in animal populations at the level of primary production.

The purpose of Regulation (EC) No 2160/2003 is to ensure that proper and effective measures are taken to detect and control salmonella and other zoonotic agents at all relevant stages of production, processing and distribution, particularly at the level of primary production, in order to reduce their prevalence and the risk they pose to public health. The Regulation covers:

- the adoption of targets for the reduction of the prevalence of specified zoonoses
- the approval of specific control programs established by Member States
- the adoption of specific rules concerning certain control methods
- the adoption of rules concerning intra-Community trade and imports

Therefore, Regulation (EC) No 2160/2003 and especially it's Annex I, is the basis for the co-ordinated control of zoonoses (Salmonella at the moment) within the European Community and gives a detailed time frame in which the prevalence have to be evaluated, targets have to be established and national control programs have to be prepared, approved by the European Community and implemented (see Table 1).

**Table 1: Annex 1 of Regulation (EC) No 2160/2003, specified with dates and targets already defined**

Zoonosis or zoonotic agent	Animal population	Stage of food chain	Date by with target must be established	Date from which testing must take place
All Salmonella serotypes with public health significance	Breeding flocks of Gallus gallus	Primary production	Com. Reg. (EC) 1003/2005 Target less than 1%	01.01.2007
All Salmonella serotypes with public health significance	Laying hens	Primary production	Com. Reg. (EC) 1168/2005 Reduction by 10-40% dep. on prevalence	01.02.2008
All Salmonella serotypes with public health significance	Broilers	Primary production	March/April 2007	Presumably: 01.01.2009
All Salmonella serotypes with public health significance	Turkeys	Primary production	March/April 2008	Presumably: 01.01.2010
All Salmonella serotypes with public health significance	Herds of slaughter pigs	Slaughter	March/April 2008	Presumably: 01.01.2010
All Salmonella serotypes with public health significance	Breeding herds of pigs	Primary production	March/April 2009	Presumably: 01.01.2011

## 1. Breeding flocks of Gallus gallus:

### 1.1 Prevalence study

Based on the data collection in accordance with Council Directive 92/117/EC the data from the year 2004 have been used as a basis for the prevalence estimation of Salmonella in breeding flocks of Gallus gallus in the member states. The report on results of monitoring / control of salmonella in breeding flocks of Gallus gallus in the European Union and Norway (Sanco/1143/2005) shows, that over all production lines and age groups the prevalence of Salmonella enteritidis in the European Union is 1,5 % and for Salmonella typhimurium is 0,2 %. For the "Top 5" (S. enteritidis, S. hadar, S. infantis, S. typhimurium and S. virchow) the prevalence is 2.8 %, varying from 0.0 to 28.0 %. The prevalence rates of the Top 5 Salmonella serotypes in the Member States is shown in Figure 1.

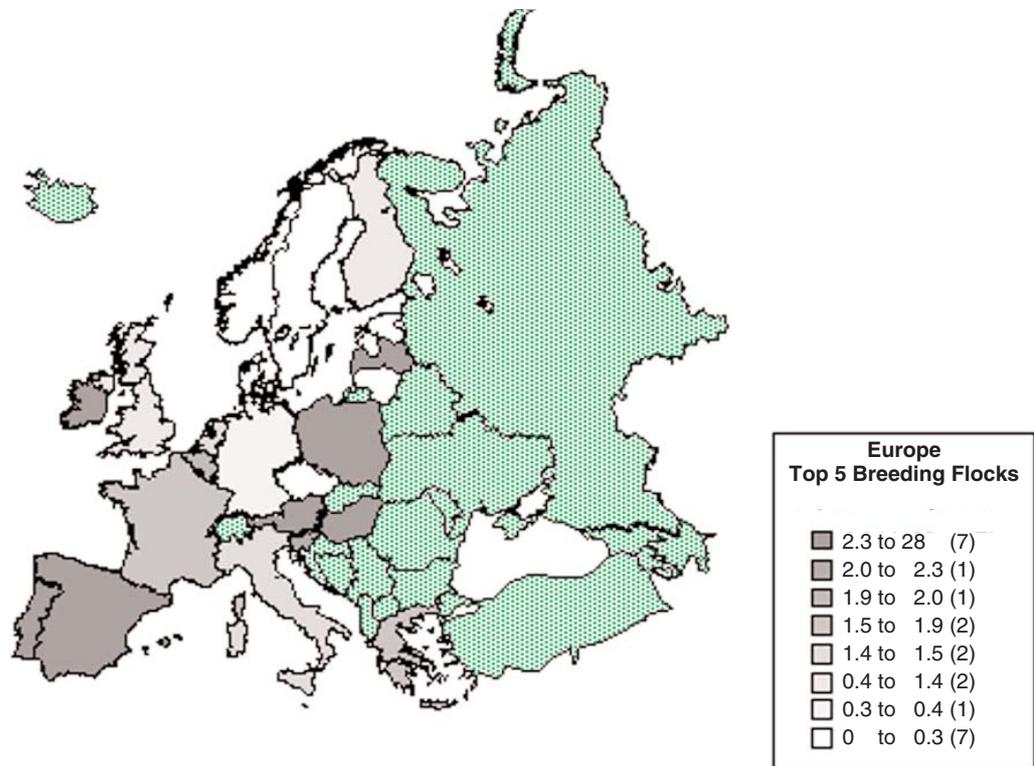
### 1.2 Targets for the reduction of Salmonella

On 30 June 2005 Commission Regulation (EC) No 1003/2005 implementing Regulation (EC) No 2160/2003 as regards a Community target for the reduction of the prevalence of certain salmonella serotypes in breeding flocks of Gallus gallus and amending Regulation (EC) No 2160/2003 was implemented. For a transitional period of three years the Community target for breeding flocks of Gallus gallus covers the five most frequent salmonella serotypes in humans, which are Salmonella enteritidis, Salmonella hadar, Salmonella infantis, Salmonella typhimurium and Salmonella virchow. The target in breeding flocks of Gallus gallus shall be a reduction of the maximum percentage of adult breeding flocks remaining positive to 1 % or less by 31 December 2009.

The testing scheme to verify the achievement of the Community target is set out in Annex I of the Regulation. This includes Monitoring in breeding flocks by sampling at the initiative of the operator and official control sampling. At the initiative of the operator sampling shall take place every two weeks in agreement with the competent authority either at the hatchery or the holding.

The Official control sampling depends on the sampling of the operator. If sampling at the initiative of the operator takes place at the hatchery:

**Figure 1: Prevalence of the Top 5 Salmonella serotypes in Gallus gallus breeding flocks**



- routine sampling every 16 weeks at the hatchery, which shall on that occasion replace the corresponding sampling at the initiative of the operator;
- routine sampling at the holding on two occasions during the production cycle, the first one being within four weeks following moving to laying phase or laying unit and the second one being towards the end of the laying phase, not earlier than eight weeks before the end of the production cycle;
- confirmatory sampling at the holding, following detection of relevant salmonella from sampling at the hatchery.

If sampling at the initiative of the operator takes place at the holding, routine sampling shall be carried out on three occasions during the production cycle:

- within four weeks following moving to laying phase or laying unit;
- towards the end of the laying phase, not earlier than eight weeks before the end of the production cycle;
- during the production, at any time sufficiently distant from the samples referred to in points (a) and (b).

Type and size of sampling material, examination methods to be used and reporting of results are also specified in the Annex.

According to Council Regulation 2160/2003/EC specific requirements concerning breeding flocks of *Gallus gallus* must be followed. In case results of examined samples indicate the presence of *Salmonella* Enteritidis or *Salmonella* Typhimurium in a breeding flock of *Gallus gallus* non-incubated eggs from the flock must be destroyed. However, such eggs may be used for human consumption if they are treated in a manner that guarantees the elimination of *Salmonella* Enteritidis and *Salmonella* Typhimurium in accordance with Community legislation on food hygiene. All birds, including day-old chicks, in the flock must be slaughtered or destroyed so as to reduce as much as possible the risk of spreading salmonella. Slaughtering must be carried out in accordance with Community legislation on food

hygiene. Products derived from such birds may be placed on the market for human consumption in accordance with Community legislation on food hygiene. If not destined for human consumption, such products must be used or disposed of in accordance with Regulation No 1774/2002 of the European Parliament and of the Council of 3 October 2002 laying down health rules concerning animal by-products not intended for human consumption. Where eggs for hatching from flocks in which *Salmonella* Enteritidis or *Salmonella* Typhimurium is present are still present in a hatchery, they must be destroyed or treated in accordance with Regulation No 1774/2002.

### **1.3 Rules for certain control methods**

According to

*Commission Regulation (EC) No 1091/2005 of 12 July 2005 implementing Regulation (EC) No 2160/2003 of the European Parliament and of the Council as regards requirements for the use of specific control methods in the framework of the national programs for the control of Salmonella* antimicrobials shall not be used as a specific method to control salmonella in breeding flocks of *Gallus gallus*.

However, the use of antimicrobials might be permitted in animals presenting salmonella infection with clinical signs in a way likely to cause undue suffering of the animals, salvaging of valuable genetic material in breeding flocks or on a case by-case basis for purposes other than salmonella control in a flock suspect of salmonella infection.

Live *Salmonella* vaccines should not be used if the manufacturer does not provide appropriate methods to distinguish bacteriologically wild-type strains of salmonella from vaccine strains.

Commission Regulation (EC) No 1091/2005 was later repealed and replaced by Commission Regulation (EC) No 1177/2006 (see under II. Laying hens).

### **1.4 National control program**

In order to achieve the Community target established by Commission Regulation (EC) No 1003/2005 for the reduction of the prevalence of the top 5 salmonella serotypes in breeding flocks of *Gallus gallus* each Member State had to establish national control programs for the control of salmonella in breeding flocks of *Gallus gallus*. These control programs have been approved by Commission Decision 2006/759/EC of 8 November 2006 approving certain national programs for the control of salmonella in breeding flocks of *Gallus gallus* and have to be applied since 1 January 2007.

## **2. Laying hens**

### **2.1 Prevalence study**

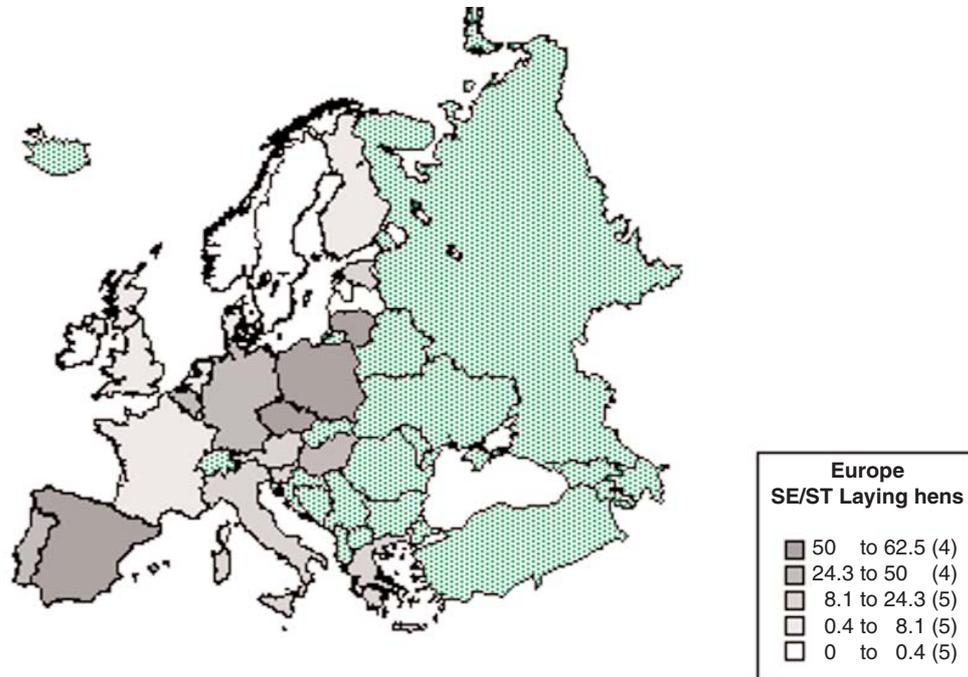
In order to set the target, comparable data on the prevalence of salmonella in the populations of laying hens in Member States should be available. Such information was not available and therefore a special study was carried out in order to monitor the prevalence of salmonella in laying hens during an appropriate period of time in order to take into account possible seasonal variations (Commission Decision 2004/665/EC of 22 September 2004 concerning a baseline study on the prevalence of salmonella in laying flocks of *Gallus gallus*).

The Preliminary Report was published on 7 April 2006 by the European Food Safety Authority (EFSA). The study was conducted on large-scale laying hen holdings with at least 1.000 laying hens per flock in a period of 1 October 2004 to 30 September 2005. From each flock five pooled samples of faeces and two pooled samples of dust were collected during the last nine weeks of the production period.

The EU weighted prevalence for salmonella spp. in laying hens was 30.7 %, ranging from 0.0 % in countries like Sweden and Luxembourg to 79.5 % in Portugal. The EU weighted prevalence of laying flocks being positive for *S. enteritidis* or *S. typhimurium* or both was 20.3 % ranging again from countries with 0.0 % to 62.5 % in Czech Republic. This demonstrates that in most Member States

the majority of laying hens is infected with either *S. enteritidis* or *S. typhimurium* or both. The prevalence rates of *S. enteritidis*/*S. typhimurium* in the Member States is shown in Figure 2.

**Figure 2: Prevalence of the S.e./S.tm. in Laying hens**



## 2.2 Targets for the reduction

On 31 July 2006 Commission Regulation (EC) No 1168/2006 implementing Regulation (EC) No 2160/2003 as regards a Community target for the reduction of the prevalence of certain salmonella serotypes in laying hens of *Gallus gallus* and amending Regulation (EC) No 1003/2005 was implemented.

The Community target for the reduction of *S. enteritidis* and *S. typhimurium* in adult Laying hens of *Gallus gallus* depend on the prevalence in the preceding year:

An annual minimum percentage of reduction of positive flocks of adult laying hens equal to at least:

- (i) 10 % if the prevalence in the preceding year was less than 10 %;
- (ii) 20 % if the prevalence in the preceding year was between 10 and 19 %;
- (iii) 30 % if the prevalence in the preceding year was between 20 and 39 %;
- (iv) 40 % if the prevalence in the preceding year was 40 % or more;

This target should be achieved in 2008 and is based on the results of the prevalence study carried out under Decision 2004/665/EC.

The sampling scheme including frequency, sampling protocols, transport of samples, examination methods and reporting are specified in the Annex. Sampling shall take place at the initiative of the operator at least every 15 weeks, starting at the age of  $24 \pm 2$  weeks. Sampling by the competent authorities shall take place at least in one flock per year per holding or in case of suspicion or detection of *S. enteritidis* or *S. typhimurium*.

## **2.3 Rules for certain control methods**

According to

*Commission Regulation (EC) No 1177/2006 of 1 August 2006 implementing Regulation (EC) No 2160/2003 of the European Parliament and of the Council as regards requirements for the use of specific control methods in the framework of the national programs for the control of Salmonella in poultry*

antimicrobials shall not be used as a specific method to control salmonella in poultry.

However, the use of antimicrobials might be permitted in animals presenting salmonella infection with clinical signs in a way likely to cause undue suffering of the animals, salvaging of valuable genetic material in breeding flocks or on a case by-case basis for purposes other than salmonella control in a flock suspect of salmonella infection.

Live Salmonella vaccines should not be used if the manufacturer does not provide appropriate methods to distinguish bacteriologically wild-type strains of salmonella from vaccine strains. They also should not be used in laying hens during production unless the safety of the use has been demonstrated. At the latest from 1 January 2008 in Member States which did not demonstrate a prevalence below 10 % vaccination programs against Salmonella enteritidis shall be applied at least during the rearing period to all laying hens.

## **2.4 National control program**

In order to achieve the Community target established by Commission Regulation (EC) No 1168/2006 for the reduction of the prevalence of Salmonella enteritidis and Salmonella typhimurium in laying flocks of Gallus gallus each Member State had to establish national control programs for the control of salmonella. Those had to be submitted to the European Union until 31 December 2006 and should be approved by the mid of 2007. They have to be applied from 1 February 2008.

## **2.5 Intra-Community Trade, export and import of eggs**

The Commission of the European Communities is working on a Commission Regulation introducing restrictions on the intra-community trade, export and import of eggs from salmonella infected flocks of laying hens (SANCO/1188/).

According to the draft of this Commission Regulation, intra-community trade and export of eggs, classed A in accordance to Regulation (EEC) No 1907/1990 shall only be authorized if derived from holdings applying the monitoring laid down in the Annex of Regulation (EC) 1168/2006 and which are not suspected of having or infected by Salmonella enteritidis or Salmonella typhimurium. Imported eggs shall only be authorized if derived from flocks providing similar guarantees as stated in a declaration by the competent authority of the country of production.

These restrictions for trade will be implemented either from 1 February 2008 or 1 January 2009. Eggs from laying hens infected with Salmonella enteritidis or Salmonella typhimurium will result in class B eggs with a "S" identification.

## **3. Broilers**

### **3.1 Prevalence study**

In order to provide the scientific basis for setting of the Community target for reduction of the prevalence of salmonella in boiler flocks of Gallus gallus, an European-Union wide study was carried out in order to determine the prevalence of salmonella in boiler flocks (Commission Decision 2005/636/EC of 1 September 2005 concerning a baseline survey on the prevalence of Salmonella spp. in broiler flocks of Gallus gallus).

The Report was published on 28 March 2007 by the European Food Safety Authority (EFSA). The study was conducted on commercial holdings with at least 5.000 birds on the holding in a period of 1

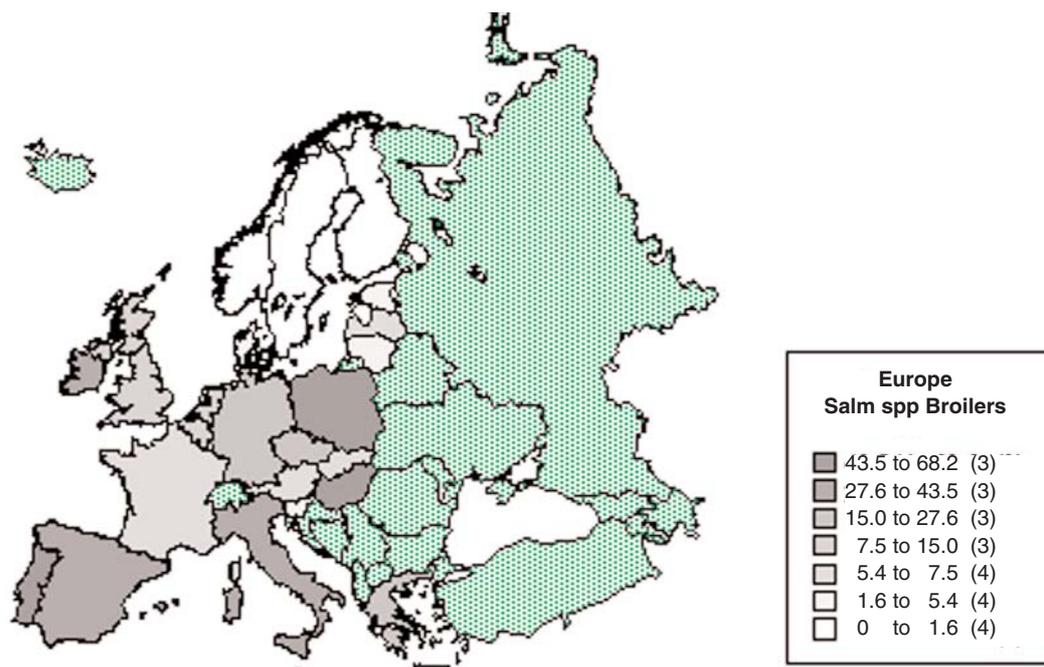
October 2005 to 30 September 2006. From each flock five pooled samples of faeces (by boot swabs or sock samples).

The EU weighted prevalence for salmonella spp. in broilers was 23.7 %, ranging from 0.0 % in Sweden to 68.2 % in Hungary. The EU weighted prevalence of broiler flocks being positive for *S. enteritidis* or *S. typhimurium* or both was 11.0 % ranging again from countries with 0.0 % to 39.3 % in Portugal. The Community weighted observed flock prevalence for the 5 most frequently reported Salmonella serotypes was:

- *S. enteritidis* 10.9 %
- *S. typhimurium* 0.5 %
- *S. infantis* 2.2 %
- *S. mbandaka* 0.4 %
- *S. hadar* 1.1 %
- Others 6.5 %

The prevalence rates of *S. spp.* and *S. enteritidis/S. typhimurium* in broiler flocks in the Member States is shown in Figure 3 and 4.

**Figure 3: Prevalence of the *S. spp.* in broiler flocks**



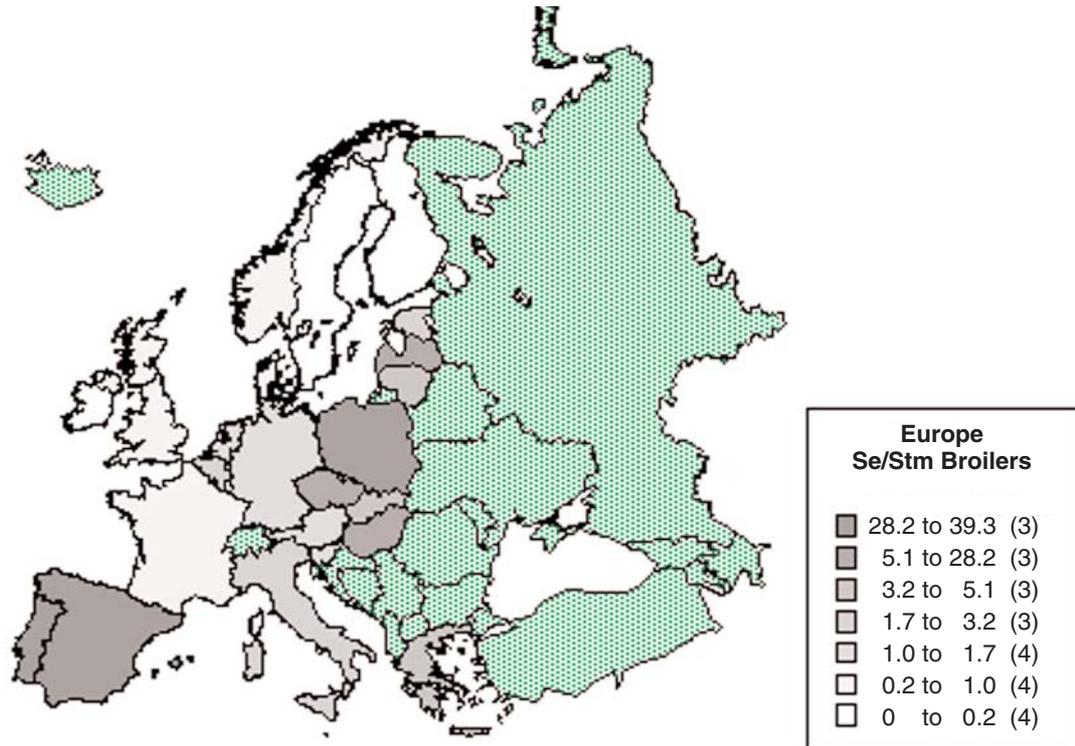
### 3.2 Targets for the reduction

For a transitional 3 year period Regulation (EC) No 2160/2003 foresees the reduction of the Salmonella prevalence in broiler flocks only for two Salmonella serotypes, *S. enteritidis* and *S. typhimurium*, the two most frequently reported Salmonella serotypes in human salmonellosis.

As the prevalence study in broilers demonstrates that in most Member States Salmonella serotypes others than *Salmonella enteritidis* and *Salmonella typhimurium* are more frequently found, the targets might be extended to other serotypes either by the Member States within their national control programs or after risk-benefit analysis also later by the European Union.

Targets for the reduction of the prevalence of *Salmonella enteritidis* and *Salmonella typhimurium* in broiler flocks are still to be established in April 2007.

**Figure 4: Prevalence of the S.e./S.tm. in boiler flocks**



### 3.3 National control programs

In order to achieve the Community target for the reduction of the prevalence of Salmonella enteritidis and Salmonella typhimurium in broiler flocks of Gallus gallus each Member State have to establish national control programs for the control of salmonella. Those have to be sent to the European Union until 31 December 2007 and should be approved by the mid of 2008. Presumably they have to be applied from 1 January 2009.

### 4. Turkeys

In turkeys, both breeder and meat turkey flocks, the prevalence study started on 1 October 2006 and will end on 30 September 2007 (Commission Decision 2006/622/EC of 29 September 2006 concerning a financial contribution from the Community towards a baseline survey on the prevalence of Salmonella in turkeys to be carried out in the Member States). The report on this study is expected in the beginning of 2008. Targets for the reduction of Salmonella in turkey flocks have to be established until March / April 2008 and the national control programs have to be sent to the European Union until 31 December 2008.

The approval of national control programs is expected in early 2009 and they should be applied as of 1 January 2010, focusing on Salmonella enteritidis and Salmonella typhimurium.

### 5. Slaughter Pigs

Also in herds of slaughter pigs, the prevalence study started on 1 October 2006 and will end on 30 September 2007. The report on this study is expected in the beginning of 2008. Targets for the reduction of Salmonella in slaughter pigs have to be established until March / April 2008 and the national control programs have to be sent to the European Union until 31 December 2008.

The approval of national control programs is expected again in early 2009 and they should be applied as of 1 January 2010. On which Salmonella serotypes these control programs have to focus is not clear at the moment and depend on risk-benefit analysis done by the European Union.

## 6. Breeding pigs

In breeding pigs, the prevalence study will start on 1 October 2007 and will end on 30 September 2008. The report on this study is expected in the beginning of 2009. Targets for the reduction of Salmonella in pig breeding herds have to be established until March / April 2009 and the national control programs have to be sent to the European Union until 31 December 2009.

The approval of national control programs is expected in early 2010 and they should be applied as of 1 January 2011. On which Salmonella serotypes these control programs have to focus is again not clear at the moment and depend on risk-benefit analysis done by the European Union.

## 7. Discussion

The complete implementation of Regulation (EC) No 2160/2003 of the European Parliament and of the Council of 17 November 2003 on the control of salmonella and other specified food-borne zoonotic agents will only be achieved in 2013 at the earliest. The current status of implementing this regulation in the primary poultry production is summarized in Table 2.

In breeding flocks of *Gallus gallus* the Community target for the Top 5 salmonella serotypes shall be a reduction of the maximum percentage of adult breeding flocks remaining positive to 1 % or less by 31 December 2009. According to the prevalence evaluation in 2004, this Community target was reached under the conditions of Council Directive 92/117/EC already by 9 Member States. This may change to some degree as the sampling frame under Commission Regulation (EC) No 1003/2005 is much more sensitive as compared to sampling under Council Directive 92/117/EC.

In several countries, this Community target might only be achieved with the intensive use of vaccines against Salmonella. For those Salmonella serotypes of the Top 5, where licensed vaccines are not available, the use of autogenous vaccines might be necessary.

In Laying flocks of *Gallus gallus* the prevalence study has shown that the EU weighted prevalence of laying flocks being positive for *S. enteritidis* or *S. typhimurium* or both was 20.3 %. This means that every 5th flock of laying hens in the Community will be affected at the latest when the Commission Regulation regarding intra-community trade and export of eggs will be in place. These restrictions will be implemented either from 1 February 2008 or 1 January 2009. Latest from this date, eggs from laying hens infected with Salmonella enteritidis or Salmonella typhimurium could not be marketed anymore. This will have dramatic effects for the egg industry.

The prevalence study in broilers has demonstrated that the EU weighted prevalence for salmonella spp. was 23.7 % and for Salmonella enteritidis or Salmonella typhimurium or both was 11.0 %. As the Community target in broilers focus only on Salmonella enteritidis and Salmonella typhimurium, in most Member states the targets to be achieved under Regulation (EC) No 2160/2003 will affect the prevalence only partially. But on the other hand Annex II (E) of Regulation (EC) No 2160/2003 defines specific requirements concerning fresh meat. With the effect from 84 months after entry into force of this Regulation (e.g. in 2010), fresh poultry meat from animals listed in Annex I (see table 1) may not be placed on the market for human consumption unless it meets the following criterion: 'Salmonella: absence in 25 grams'. By this criterion the poultry meat industry is not only challenged in regard to the presence of Salmonella enteritidis and Salmonella typhimurium, but must focus on any type of Salmonella.

Although measure related to the implementation of Regulation (EC) No 2160/2003 might be co-financed with 50% by the European Commission this most probably will be handled by the Member States completely different depending on methods of compensation and reserve-funds available. Therefore, implementation of Regulation (EC) No 2160/2003 remains and continues to be of major economic importance for the poultry industry in the European Community.

And simultaneous to the control of Salmonella Directive 2003/99/EC of the European Parliament and of the Council of 17 November 2003 on the monitoring of zoonoses and zoonotic agents foresees also the control of Campylobacter in poultry. A prevalence study for broilers is in preparation and may still begin in 2007.

**Table 2: REGULATION (EC) No 2160/2203 on the control of Salmonella and other specified food-borne zoonotic agents**

Animal population	Prevalence Study	Community Target	Rules for Control Methods	National control programs
Breeding flocks of Gallus gallus	<b>Council Decision 92/117/EC</b> 01.01.2004-31.12.2004 Report: SANCO/1143/2005 Top 5: EU weighted prevalence <b>2.8%</b>	<b>Comm. Reg.(EC) No 1003/2005</b> Target: Top 5 1 % or less by 31 December 2009	<b>Comm. Reg.(EC) No 1091/2005</b> • No use of antimicrobials • Requirements for use of live vaccines	<b>Comm. Decision 2006/759/EC</b> • Approval of national control programs for breeding flocks of Gallus gallus • To be applied from <b>1 January 2007</b>
Laying hens	<b>Council Decision 2004/665/EC</b> 01.10.2004-30.09.2005 Report: EFSA preliminary report 'Analysis of Salmonella in laying hens', 7 April 2006 Se/Stm: EU weighted prevalence <b>20.3 %</b>	<b>Comm. Reg.(EC) No 1177/2006</b> An annual minimum percentage of reduction of positive flocks of adult laying hens equal to at least: (v) 10 % if the prevalence was less than 10 %; (vi) 20 % if the prevalence was between 10 and 19 %; (vii) 30 % if the prevalence was between 20 and 39 %; (viii) 40 % if the prevalence was 40 % or more;	<b>Comm. Reg.(EC) No 1168/2006</b> • No use of antimicrobials • Requirements for use of live vaccines • From 1 January 2008 vaccination against S. enteritidis in countries with prevalence > 10 %	• Submitted since 31 December 2007 • Approval expected mid 2007 • To be applied from <b>1 February 2008</b>
Broilers	<b>Council Decision 2005/636/EC</b> 01.10.2005-30.09.2006 Report: EFSA baseline study on the prevalence of Salmonella in broiler flocks Se/Stm: EU weighted prevalence <b>11.0 %</b>	To be established in April 2007	To be established	• To be submitted until 31 December 2007 • Presumably to be applied from <b>1 January 2009</b>
Turkeys	<b>Council Decision 2006/622/EC</b> 01.10.2006-30.09.2007	To be established in March/April 2008	To be established	• To be submitted until 31 December 2008 • Presumably to be applied from <b>1 January 2010</b>

## Zusammenfassung

### Stand der EU-Gesetze zur Kontrolle von Salmonellen und anderen Zoonosen

Die Europäische Kommission hat sich zum Ziel gesetzt, die Lebensmittelsicherheit in den Mitgliedstaaten durch entsprechende einheitliche Gesetzgebung zu verbessern. Einen hohen Stellenwert hat dabei die systematische Verringerung von Zoonosen. In einem ersten Schritt wurden 1992 Maßnahmen zur Kontrolle der beiden wichtigsten Salmonellenarten, *S. enteritidis* und *S. typhimurium*, in Zuchtbeständen festgelegt.

Inzwischen wurde der Katalog zu treffender Maßnahmen erheblich erweitert, konkrete Ziele wurden für verschiedene Tierarten festgelegt. Derzeit bestehen jedoch noch Unterschiede zwischen Mitgliedstaaten in der Prävalenz verschiedener Erregertypen und Maßnahmen zu ihrer Kontrolle. In dieser Übersicht werden die Vorgaben der EU für Hühner, Puten und Schweine (getrennt für Zucht- bzw. Elterntierbestände und Endprodukte) beschrieben. Durch Kennzeichnung der Produkte und Handelsbeschränkungen soll in den kommenden Jahren das Risiko von Salmonellen in der EU systematisch reduziert werden. Über die verabschiedeten Maßnahmen zur Kontrolle von Salmonellen hinaus werden entsprechende Maßnahmen gegen Campylobakter vorbereitet.

## References

- Council Directive 92/117/EC of 17 December 1992 concerning measures for protection against specified zoonoses and specified zoonotic agents in animals and products of animal origin in order to prevent outbreaks of food-borne infections and intoxication's provided for the establishment of monitoring systems for certain zoonoses and controls on salmonella in certain poultry flocks *Official Journal of the European Union* 1993, L 062/38, 15.03.1993
- Directive 2003/99/EC of the European Parliament and of the Council of 17 November 2003 on the monitoring of zoonoses and zoonotic agents, amending Council Decision 90/424/EEC and repealing Council Directive 92/117/EEC *Official Journal of the European Union* 2003, L 325/31, 12.12.2003
- Regulation (EC) No 2160/2003 of the European Parliament and of the Council of 17 November 2003 on the control of salmonella and other specified food-borne zoonotic agents *Official Journal of the European Union* 2003, L 325/1, 12.12.2003
- Commission Decision 2004/665/EC of 22 September 2004 concerning a baseline study on the prevalence of salmonella in laying flocks of Gallus gallus *Official Journal of the European Union* 2004, L 303/30, 30.09.2004
- Commission Regulation (EC) No 1003/2005 implementing Regulation (EC) No 2160/2003 as regards a Community target for the reduction of the prevalence of certain salmonella serotypes in breeding flocks of Gallus gallus and amending Regulation (EC) No 2160/2003 *Official Journal of the European Union* 2005, L 170/12, 01.07.2005
- Commission Regulation (EC) No 1091/2005 of 12 July 2005 implementing Regulation (EC) No 2160/2003 of the European Parliament and of the Council as regards requirements for the use of specific control methods in the framework of the national programs for the control of Salmonella *Official Journal of the European Union* 2005, L 182/3, 13.07.2005
- Commission Decision 2005/636/EC of 1 September 2005 concerning a financial contribution from the Community towards a baseline survey on the prevalence of Salmonella spp. in broiler flocks of Gallus gallus to be carried out in the Member States *Official Journal of the European Union* 2005, L 228/14, 03.09.2005
- EFSA preliminary report 'Analysis of Salmonella in laying hens', 7 April 2006
- Commission Regulation (EC) No 1168/2006 implementing Regulation (EC) No 2160/2003 as regards a Community target for the reduction of the prevalence of certain salmonella serotypes in laying hens of Gallus gallus and amending Regulation (EC) No 1003/2005 *Official Journal of the European Union* 2006, L 211/4, 01.08.2006
- Commission Regulation (EC) No 1177/2006 of 1 August 2006 implementing Regulation (EC) No 2160/2003 of the European Parliament and of the Council as regards requirements for the use of specific control methods in the framework of the national programs for the control of Salmonella in poultry *Official Journal of the European Union* 2006, L 212/3, 02.08.2006
- Commission Decision 2006/622/EC of 29 September 2006 concerning a financial contribution from the Community towards a baseline survey on the prevalence of Salmonella in turkeys to be carried out in the Member States *Official Journal of the European Union* 2006, L 272/22, 03.10.2006
- Commission Decision 2006/759/EC of 8 November 2006 approving certain national programs for the control of salmonella in breeding flocks of Gallus gallus *Official Journal of the European Union* 2006, L 311/46, 10.11.2006
- Commission Regulation introducing restrictions on the intra-community trade, export and import of eggs from salmonella infected flocks of laying hens (SANCO/1188/2006)
- Report of the Task Force on Zoonoses Data Collection on the Analysis of the baseline study on the prevalence of Salmonella in broiler flocks of Gallus gallus, Part A, *The EFSA Journal* (2007) 98, 1-85

## Breeder farms and hatchery as integrated operation

Hafez Mohamed Hafez

Institute of Poultry Diseases, Faculty of Veterinary Medicine  
Free University Berlin, Königsweg 63, 14163 Berlin, Germany

### Introduction

The goal of the poultry breeding industry is the production of healthy chicks, which will be viable from both an immunological and nutritional perspective, when placed in the production setting. Communication and shared responsibility between the breeder farms and hatchery are essential to minimize the risk and consequences of health problems.

Good rearing management is the starting point for healthy, productive and profitable hatching egg production. Rearing management means all factors which influence the birds' health and includes several factors such as house structure, climatic conditions (ventilation, temperature, litter condition), stocking density, feed and water supply, hygienic conditions as well as the qualification and knowledge of the stockman. These factors affect each other and can promote or inhibit the health condition of the flock. In aiming to achieve desired performance results, managers of breeder flocks should integrate good environment, husbandry, nutrition and disease control programs. The rearing management must be directed to satisfy the bird's requirements, to promote the production and to prevent diseases. Any disturbance can cause stress, which will reduce the resistance of the birds, increase their susceptibility to infections and reduce their immune response to vaccines.

Infectious poultry diseases are often associated with severe economic losses. Many of these diseases, re-emerging or introduced into a geographic area, can explode into an epidemic and may significantly affect international trade. Early recognition and monitoring programs are essential in managing infections and minimizing their economic impact.

Infectious agents can be introduced into and spread among breeder farms by different routes. All infectious agents are transmitted **horizontally** (laterally) by direct contact between infected and non-infected susceptible birds or through indirect contact with contaminated environments through ingestion or inhalation of organisms. In addition, infections can occur via contamination of feed, water, equipment, environment and dust. Significant reservoirs for micro-organisms are man, farm animals, pigeons, water fowl and wild birds. Rodents, pets and insects are also potential reservoirs and transmit the infection between houses and can contaminate stored feed. Further sources are "carrier" birds who continue to excrete the infectious agent after they recover and no longer show clinical symptoms of the disease. Contaminated material can also be picked up on shoes and clothing and carried from an infected to a healthy flock. The disease can be spread by vaccination and beaking trimming crews, manure haulers, drivers of rendering trucks and feed delivery personnel.

**Vertical transmission** occurs primarily by systemic ovarian transmission, by passage through the oviduct or by contact with infected peritoneum or air sacs. Secondly, vertical transmission happens by contamination of the egg content as a result of faecal contamination of the eggshell in contaminated nests, floor or incubators with subsequent penetration into the eggs (Fig. 1).

### Disease prevention and control

Control measures to prevent introduction and spread of infections in breeder flocks should be concentrated on high standards of poultry husbandry with bacteriological and serological monitoring of breeding birds. These measures must be coupled with meticulous attention at all stages of hatching egg production. The general strategy to control infectious poultry diseases includes:

- (1) eradication of vertically transmitted diseases from top to bottom – initiated by the primary breeder and pursued down to the commercial producer;

**Figure 1: Some possible egg-borne infections**

Systemic		Contamination of the eggshell
Avian encephalomyelitis Lymphoid leucosis Egg drop Syndrome Chicken Infectious Anaemia Salmonella Mycoplasma Reovirus?		Salmonella Staphylococcus E. coli Pseudomonads Proteus spp. Aspergillus spp.

- (2) hatchery hygiene and hatching egg sanitation as well as education programs and regular bacteriological examination of employees; and
- (3) hygienic measures throughout the production chain, vaccination, therapy, decontamination of feed. Efforts of the industry to improve bio-security may be supported or even enforced by legislation.

**Hygienic measures**

It is vital that hygienic standards in the breeder house are impeccable to avoid infection entering the hatchery either within or on the shell of the eggs. The design of the house is usually based on the production objective and focused on efficient production of hatching eggs. The design and construction of breeder houses should also focus on easy management, maintenance, and application of effective hygienic measures. Poultry houses should be kept locked and no visitors allowed to enter. Further precautions related to staff should be taken. Regular bacteriological examinations must be performed to identify carriers and to prevent transmission and cross contamination on the farm. Cleaning, disinfection and vector control must be integrated in a comprehensive disease control program. The procedure should be tailored to meet the particular needs. The cleaning and disinfection program should include time schedule, type of disinfectant and concentration as well as microbiological monitoring of the procedures. The procedures should be established not only for cleaning and disinfecting the house and surfaces but also for cleaning and disinfection of the equipment, which is itself used for cleaning. Rodents, especially rats and mice, are particularly important sources of salmonella contamination of poultry houses. An intensive and sustained rodent control is essential and needs to be well planned and routinely performed and its effectiveness should be monitored. Household pets also constitute a serious hazard. Buildings therefore should be pet proof.

In breeder farms it is important to optimize the production of clean fertile hatching eggs, by keeping egg laying areas as clean as possible, including the nest litter or pads. In addition, the breeder house nesting should be inspected on a regular basis. Hatching eggs should be collected frequently (3-4 times daily) to minimize the time that they are exposed to a contaminated environment. All substandard eggs with misshapen, cracked or thin shells should be removed. The egg shells should be disinfected soon after collection on the farm, since the penetration of the shell by micro-organisms is particularly rapid. If the bacteria penetrate the shell before the egg reaches the hatchery, it is difficult to find an effective method to counteract such contamination.

Two methods are used to disinfect hatching eggs under field conditions, namely fumigation or dipping in a solution of detergent or disinfectant. Fumigation is done best with formaldehyde gas for at least 20 minutes with a concentration of 35 ml formalin mixed with 17.5 g potassium permanganate and 20 ml water per m<sup>3</sup> space. Temperature during fumigation must be maintained at a minimum of 20-24°C and relative humidity at 70%. The eggs should be placed in trays that will permit the fumigant to contact as much of the shell surface as possible. Because of the unpleasant nature of formaldehyde gas and its possible health hazards to the operator, some owners elect to use wet treatments. Different

sanitizing solutions are used and most of them are based on chlorine, glutaraldehyde or quaternary ammonium compounds. Egg dipping in detergents or in disinfectants is highly effective in greatly reducing or eliminating the bacteria from the shell when performed correctly. However, there is little or no effect on those bacteria that have already penetrated the shell. Manufacturer's instruction for the chemicals used should be followed, particularly those concerning the number of eggs that may be dipped per liter solution and how often fresh solution has to be provided. Attention also must be directed to the temperature of the detergent which must be higher than the egg temperature. After hatching egg sanitation, hygienic measures should be followed to preclude recontamination. Farm egg rooms should have a guideline for cleanliness and standard operation. Bacterial contamination in the egg room should be routinely monitored.

## **Vaccination**

Vaccination is one of the most effective tools to prevent specific diseases. Several factors are dictating the choice of the vaccine, vaccination route and frequency of vaccination. These factors are: the epidemiological situation, the type of production, management practices on the farm, the goal of vaccination, availability of the vaccine, cost benefit analysis, general health status of the flock and governmental regulations. Vaccination programs for breeder flocks should be tailored to induce long lasting high antibody levels during the entire production cycle in order to protect against possible field challenge, to maintain acceptable standards of egg production, egg quality and hatchability, and to transfer the desired maternal antibodies to the offspring.

## **Treatment**

In spite of all precautions, poultry may become sick. In such cases rapid medication is essential. Several drugs have been found useful for reducing clinical signs and shedding of some bacterial diseases in infected flocks. Treatment should reduce losses, but in some cases relapses may occur when treatment is discontinued. No drugs should be given until a diagnosis has been obtained; giving the wrong drug can be a waste of money. Drugs should be used very carefully: the correct dose level and duration is important. It should also be kept in mind that residues of drugs in fertile eggs from treated breeders may occasionally cause abnormalities in some embryos.

## **Eradication by industry and legislation**

### ***Salmonella***

Salmonellosis and salmonella infections in poultry are distributed world-wide and result in severe economic losses when no effort is made to control them. The large economic losses are caused by high mortality during the first four weeks of age, high medication costs, and reductions in egg production in breeder flocks, poor chick quality and high costs for eradication and control measures. The most important aspect, however, is the effect of salmonella contaminated eggs, poultry meat and meat-products on public health.

Salmonella belong to the family Enterobacteriaceae and all members are Gram-negative, non-sporing rods without capsules. The genus Salmonella includes about 2500 serovars. Some serovars may be predominant for a number of years in a region or country, then disappear to be replaced by another serovar. The course of the infection and the prevalence of salmonellosis in poultry depend on several factors such as: salmonella serovar involved, age of birds, infectious dose and route of infection. Further, stress-producing circumstances such as bad management, poor ventilation, high stocking density or concurrent diseases may also contribute to the development of a systemic infection with possibly heavy losses among young birds. After recovery, birds continue to excrete salmonella in their faeces. Such birds must be considered as a potential source for transmission of the micro-organisms. The incubation periods range between 2 and 5 days. Mortality in young birds varies from negligible to 10 - 20% and in severe outbreaks may reach 80% or higher. The severity of an outbreak

in young chicks depends on the serovar involved, virulence, degree of exposure, age of birds, environmental conditions and presence of concurrent infections.

If infection was egg transmitted or occurred in the incubator, there will be a lot of unpipped and pipped eggs with dead embryos. Symptoms usually seen in young birds are somnolence, weakness, drooping wings, ruffled feathers and huddling together near heat sources. Many birds that survive for several days will become emaciated, and the feathers around the vent will be matted with faecal material. Furthermore respiratory distress as well as lameness as a result of arthritis may be present. Adult birds serve mostly as intestinal or internal organ carriers over longer periods with little or no evidence of the infection.

In general the main strategy for control of microbial food borne hazards should include: Cleaning the production pyramid from the top (in the case of invasive salmonella) by destroying infected flocks, hatching egg sanitation and limiting introduction and spread at the farm through Good Animal Husbandry Practices. Reducing salmonella colonization by using feed additives, competitive exclusion treatment or vaccines offer additional possibilities.

In 1992, the European Union adopted a directive to monitor and control Salmonella infections (Directive 92/117/EEC) in breeding flocks of domestic fowl. This directive laid down specific minimum measures to control the infection. Those focused on monitoring and controlling salmonella in breeding flocks of the species *Gallus gallus*, when serotypes *Salmonella Enteritidis* or *Salmonella Typhimurium* were detected and confirmed in samples taken.

The Scientific Committee on Veterinary Measures relating to Public Health considered that the measures in place to control food-borne zoonotic infections were insufficient at that time. It further considered that the epidemiological data collected by Member States were incomplete and not fully comparable. As a consequence, the Committee recommended to improve the existing control systems for specific zoonotic agents. Simultaneously, the rules laid down in Directive 2003/99/EC of the European Parliament and of the Council of 17 November 2003 on the monitoring of zoonoses and zoonotic agents, amending Council Decision 90/424/EEC and repealing Council Directive 92/117/EEC, replaced the monitoring and data collection systems established by Directive 92/117/EEC and Council Regulation 2160/2003/EC on the control of salmonella and other specified food-borne zoonotic agents was adopted. This Regulation covers the adoption of targets for the reduction of the prevalence of specified zoonoses in animal populations at the level of primary production. After the relevant control program has been approved, food business operators must have samples taken and analyzed to test for the zoonoses and zoonotic agents. On 30 June 2005 the Commission issued Regulation (EC) No 1003/2005 implementing Regulation (EC) No 2160/2003 as regards a Community target for the reduction of the prevalence of certain salmonella serotypes in breeding flocks of *Gallus gallus* and amending Regulation (EC) No 2160/2003. The Community target for the reduction of *Salmonella Enteritidis*, *Salmonella Hadar*, *Salmonella Infantis*, *Salmonella Typhimurium* and *Salmonella Virchow* in breeding flocks of *Gallus gallus* shall be a reduction of the maximum percentage of adult breeding flocks remaining positive to 1 % or less by 31 December 2009. Details of European legislation to control Salmonella and other zoonotic diseases is dealt with in detail by Voss (2007) in this issue of Lohmann Information.

## **Avian Mycoplasmosis**

Mycoplasmas have affected the industry for many years and effective control of Mycoplasma infection has been a fundamental stepping-stone to improved performance and productivity. However, infections appear to be making a comeback. Numerous species of mycoplasmas have been isolated from avian sources. Two species are recognized as predominantly pathogenic to chickens and turkeys. *Mycoplasma gallisepticum* (MG) affects the respiratory system and is referred to as chronic respiratory disease (CRD) in chickens, and infectious sinusitis in turkeys. *Mycoplasma synoviae* (MS) may cause respiratory and/or joint diseases. Two additional species are known to be pathogenic to turkeys. *Mycoplasma meleagridis* (MM) causes airsacculitis, and *Mycoplasma iowae* (MI) causes decreased hatchability.

The disease spreads from flock to flock by vertical transmission through infected eggs. Infected progeny then transmit the agent horizontally either by direct bird-to-bird contact or by indirect contact through contaminated feed, water and equipment. Concerning vertical transmission, hens which become infected before the onset of lay tend to transmit at a lower rate than hens initially infected during egg production. Generally egg transmission is intermittent and the rate is variable (1-10%) and very low. The spread of infection from bird to bird within one pen is usually rapid, but it is rarely transmitted from one house to another. However, in continuous production complexes (multiple-age) with chronic apparently healthy carriers the spread of infection is difficult to control since the cycle of infection can not be broken without complete depopulation. The agent also can be transmitted by other species of birds as well as mechanically by other animals and man.

The clinical signs and the course of the disease are influenced by several factors such as the presence of concurrent micro-organisms (such as TRT, Influenza, Reo and E.coli) and/ or improper management (increased dust and ammonia levels in the environment).

Eradication of Mycoplasma in breeder flocks through testing and slaughter is the preferred method to clean the production chain from the top and to prevent mycoplasma introduction through primary and commercial breeder flocks. However, in places with intensive continuous poultry production it has been determined that this method is too expensive and impractical. Hatching egg treatments with antibiotics for the control of egg transmitted bacterial pathogens has been widely investigated and seems to be of great value. Different methods of egg treatment have been used such **egg dipping** in antibiotics using pressure differential dipping or temperature differential dipping. These methods greatly reduce the mycoplasma egg transmission, but do not always completely eliminate it.

Dipping solutions can become excessively contaminated with resistant micro-organisms such as pseudomonads and organic material. To prevent bacterial contamination of the solution filtering with subsequent cool storage and/or addition of disinfectants is the most effective method. Thorough and continuous bacteriological monitoring of dip solution is also required. The concentration of the antibiotics must be examined regularly and renewed routinely. By using enrofloxacin the pH-value of the dipping solution can be corrected during storage. The use of egg dipping in antimicrobials should be critically evaluated, because of the irregular uptake of dip solution, uneven distribution of active substance in the egg compartments and lack of standardization in dipping technique. Additionally, it is known that different disinfectants used for washing can influence negatively the antibiotic uptake of hatching eggs. Therefore it is recommended that the compatibility of different disinfectants used for egg washing and/or used in dipping solution has to be examined before application. As the uptake of active substance by the hatching egg can be very irregular during dipping, individual egg injection with accurate delivery of the proper dose is preferred in elite and grandparent breeding stock. Automated systems for in ovo drug disposition before hatch can also be used.

## **Hatchery management**

Hatcheries must be designed to permit only a one way flow of traffic from the egg entry room through egg trays, incubation, hatching and holding rooms to the van loading area. The ventilation system must prevent recirculation of contaminated air. Hatching eggs should be from known sources to ensure that the origin of eggs is traceable. Do not incubate floor eggs, cracked eggs or eggs with hairlines. All eggs should be sanitized on arrival at the hatchery (pre-setting treatment) using fumigation. Additionally fumigation can be carried out after setting. This provides a final disinfection following handling, transport and risks of environmental contamination during storage of hatching eggs.

Good hatchery practice includes the analysis of unhatched eggs on a regular basis and, if infections are found, to trace the sources. Sanitation in the hatchery is paramount to the future health of chicks and poults. Cleaning and disinfection of machines and rooms must be carried out regularly. Hatchery equipment must be free of all organic matter before disinfection to ensure that the hatchery sanitation program is fully effective. Fluff samples from various surfaces in the hatchery should be cultured to detect microbial populations in hatchery air. The health status of all employees, including chick sexers, should be regularly monitored.

## Education programs

The success of any disease control program depends on all people with direct or indirect contact with hatching eggs or chicks. It is essential to incorporate education programs about micro-organisms, modes of transmission as well as awareness of the reasons behind such control programs by people involved in poultry production.

## Conclusions

Disease conditions are mostly accompanied with heavy economic losses in the poultry industry. Breeder farms and hatchery should be considered as an integrated operation. Close communication between breeder farm and hatchery is essential in sharing responsibility. Goals set for hatching egg production, hatchability and chick quality can only be reached with healthy breeder flocks. Good management practices, focused on the health and performance potential of day-old chicks, includes monitoring all breeder flocks and hatchery hygiene on a regular basis. Traceability of each batch of chicks or poults from hatchery to breeder farm helps to detect the source of problems and their elimination.

## References

can be obtained from the author.

## Zusammenfassung

### **Bruteierlieferbetriebe und Brüterei in gemeinsamer Verantwortung für die Produktion von Qualitätsküken**

Wirtschaftliche Produktion von Geflügelfleisch und Eiern beginnt mit gesunden und leistungsfähigen Eintagsküken. Auf deren Produktion sind Brütereien spezialisiert, die wiederum von der Lieferung von Bruteiern definierter Qualität aus spezialisierten Bruteierlieferbetrieben abhängen. Die hierarchische Gliederung in der modernen Geflügelproduktion in Zucht-, Vermehrungs- und Produktionsbetriebe erleichtert die systematische Eradikation vertikal übertragbarer Krankheitserreger und den Informationsaustausch über die jeweils verfügbaren Möglichkeiten, die horizontale Übertragung von Erregern zu unterbinden.

Die Geflügelindustrie hat aus eigenem Interesse erhebliche Fortschritte in der Kontrolle von Geflügelkrankheiten erreicht. Gesetzliche Vorgaben der EU sollen dazu beitragen, die Lebensmittelsicherheit in den Mitgliedsstaaten weiter zu verbessern und insbesondere das Risiko von Salmonellen und anderen Zoonosen zu begrenzen. Integrationen bzw. feste Verträge zwischen Brütereien und Bruteierlieferbetrieben helfen, die Verantwortung zu definieren und konsequent auf vereinbarte Ziele hinzuarbeiten.

## Mutilations in poultry in European poultry production systems

Thea Fiks - van Niekerk and Ingrid de Jong  
Animal Sciences Group, Wageningen University and Research Centre, NL  
thea.fiks@wur.nl

### Introduction

Mutilations of animals, like beak trimming, have been subject of discussion for many years. Opponents point out the lack of respect for animal integrity and the stress and pain it causes to the animal. Although supporters argue that omitting mutilations can lead to harmful pecking behaviour causing pain and stress as well, there is a growing societal plea for adapting husbandry systems according to the behavioural needs of animals instead of mutilate animals to fit them into current husbandry practices. Better management can contribute to reducing the propensity of feather pecking in laying hens, for example, and increasing knowledge on this issue results in more success for farmers that keep non-mutilated poultry. It is often questioned, however, whether intensive poultry production systems will ever be able to do without mutilations.

Many studies have been conducted aiming at finding alternatives for mutilations, but the final solution is still lacking. Some countries have put a ban on specific mutilations. Farmers in these countries have found ways to deal with this, but the discussion on the actual improvement of welfare of birds continuous in those countries as well as discussions on the applicability of those solutions in other countries.

Besides finding alternatives for mutilations, research focused on refining the mutilating technology thus minimizing its adverse effects on the animals. Mutilations are performed at younger age or with more refined equipment or less tissue has been removed, making the mutilation less stressful and less painful for the bird. This discussion is focussing on „how strenuous is the mutilation for the animal?“ and „what are possible permanent consequences of the mutilation?“.

In the light of these two approaches, i.e. alternatives for mutilations and refinement of methods, the most common mutilations for poultry will be discussed. In this review we address beak treatments, toe clipping, de-spurring and dubbing in laying hens, parent flocks and turkeys. Finally the situation in Europe with regards to legislation and current practices will be presented.

### Mutilations

Not all cutting, clipping or trimming parts of the animal's body is a mutilation. In this paper a mutilation is defined as removing or damaging a part or parts of the body, not being the horny dead body tissue and feathers. A mutilation therefore is directed on living tissue and often involves nervous and circulatory tissue. Although it is very hard to prove, it is very likely to cause acute pain. Not all tissues, however, are equally innervated and thus it is plausible that the impact will vary for the various mutilations.

Cutting a part of the beak is usually referred to as beak trimming. However, as more gentle methods are developed that are less strenuous for the animal, this should be reflected in the terminology. Therefore in this paper the more gentle measures are called beak treatments. Also, if beak trimming and beak treatments are discussed in general, they are referred to as beak treatments. Beak trimming will only be used for treatments of birds of 5 weeks or older.

Not all mutilations are applied to all species. In table 1 an overview is given of the mutilations that will be discussed in this paper and the species that are involved. As this paper addresses only mutilations applied in the Netherlands, some mutilations common in other countries are not discussed. An example is beak trimming in Ducks, which is not allowed in the Netherlands.

**Table 1 Overview of most common mutilations per species (the indicated mutilations are not always applied on all genotypes)**

Species	Beak treatment	Despurring	Dubbing	Toe clipping	Identification <sup>1</sup>
Laying hens	X				
Broiler breeding					
Parent stock		X		X	X
(Grand)grandparents	X	X		X	X
Broiler breeders	X	X		X	
Layer breeding					
Parent stock	X		X		X
(Grand)grandparents	X		X		X
Layer breeders	X		X		
Layers for vaccine production	X	X	X	X	
Turkeys <sup>2</sup>					
Breeder stock	X				
Meat turkeys	X				

<sup>1</sup> For broiler breeders this implies removing the nail of the inner toe (to distinguish the various male lines) and cutting the toe membrane (toe slit) or applying a wing band to identify individuals of genetically different groups, for layer breeders this implies the application of a wing band for identification of individuals on pure line level and toe slits for distinguishing the various genetic Lines.

<sup>2</sup> Desnooding of turkeys is not applied in the Netherlands anymore and therefore is not discussed.

## Anatomy of the beak

The beak of a chicken is a very specialised organ. It contains many glands and senses, that help the animal in performing vital tasks, like:

- food seeking, pecking, where the tactile sense of the beak is used to find and select food particles,
- preening, where the beak is used to smooth feathers and to spread feather fat from the tail gland over them,
- nest building behaviour, where the beak is used to collect and arrange the nesting material,
- as weapon for offensive and defensive behaviour.

The centre of the beak consists of bone. The tissue around this bone is well innervated. The outer layer of the beak consists of horny tissue. This layer is thicker towards the tip of the beak.

In selecting food particles the beak is an important tool. It contains several receptors:

- Thermo receptors: these can detect differences in temperature;
- Mechano receptors: these can detect mechanical differences, thus differences in structure;
- Nociceptors, these detect damage of the beak, like for instance beak treatments.

In both chickens and turkeys the receptors are mainly located in the tip of the beak. Further to the basis the number of receptors decreases (Gentle et al., 1997).

In the tip of the beak the beak tip organ is located. In geese and ducks this organ can be found in both the upper and lower beak. In poultry this organ is only found in the lower beak. In the beak tip organ many nerves, blood vessels and sensory receptors are concentrated. The beak tip organ improves the tactile sense of the beak, supporting the bird in selecting food particles.

In the beak nerves often end into a receptor, but they can also have free nerve ends. These can mainly be found in the tip of the upper beak and in the beak tip organ in the lower beak. These free nerve ends have similar morphological characteristics as nociceptors in mammals. Therefore it is assumed that these nerve ends in birds act as nociceptors and will register damage of the beak (Gentle and Breward, 1986).

## **Consequences of beak treatments**

### **Anatomical consequences of beak treatments**

The traditional way of beak trimming consists of removing parts of both upper and lower beak. This is done by cutting through living tissue, removing or touching blood vessels, bone, skin and horny tissue. In case of severe beak trimming also salivary glands may be touched.

Directly after the beak treatment the wound covers with scar tissue and blood coagulates. Within a few days a thin skin layer is formed over the wound. This is replaced by horny tissue after some time (Gentle et al, 1995). The capacity of the beak to re-grow depends on the type of bird, the age the beak is treated, the amount of tissue that is removed and the extent of damage the treatment has caused in adjacent tissue. In turkey poults that were beak treated at 0, 6 and 21 days of age, Gentle et al. (1997) found re-growth of bone, cartilage, blood vessels and nerves. However the treated beaks did not contain sensory receptors and no afferent nerve vessels (nerves that send information from the beak to the brain), whereas these were abundantly present in untreated beaks. From these findings it can be concluded that the tactile senses of treated beaks are affected.

In beaks treated at 5 weeks of age Gentle (1986b) found little regeneration. Along the cutting line he found excessive scar tissue. This can prevent nerve ends from growing into the beak tip, leading to a fairly insensitive beak tip. Instead he found many neuromas right next to the scar tissue. Neuromas are uncontrolled growing nerve ends. At the point where the nerve has been cut, many nerve branches are formed. If the main nerve re-grows, these branches and thus neuromas can disappear. In severe cases of neuromas this will not happen and the branches will stay permanently (Lunam, 2005). Neuromas are not studied in detail in birds, but in mammals it is known that they can spontaneously fire many signals to the brain (Breward and Gentle, 1985 in Hughes and Gentle, 1995). These signals can be interpreted as pain signals by the central nerve centre.

### **Behaviour after beak treatments**

In behavioural studies Gentle et al. (1990) found indications for chronic pain in laying hens whose beaks were trimmed at 16 weeks of age. The pullets showed clear changes in behaviour, directed on preventing tactile stimuli on the beak tip (less pecking to objects in the environment, less bill wiping, drinking of hot or cold water). They avoided those behaviours up to 6 weeks after beak trimming, whereas the beaks were healed after 3 weeks. According to Gentle et al. (1990) this indicates possible chronic pain in the beak. Duncan et al. (1989) also found clear differences in behaviour between

trimmed (at 16 weeks) and untrimmed hens. Especially preening behaviour and pecking to the environment was reduced and inactivity increased. Also feed and water use decreased, but this came back to normal levels quite soon, whereas the earlier mentioned behaviours only reached the original level 5 weeks after the treatment.

### **Age of beak trimming**

Gentle et al. (1997) did not find any neuromas in beaks of turkeys that were treated at young age (0, 6 and 21 days of age) whereas he did find them in beaks of layers trimmed at 5 weeks of age. Hughes and Gentle et al. (1995) concluded that neuromas do not develop in fast growing beaks of very young birds. This was confirmed in a study of Gentle et al. (1997), where they treated the beaks of layer chicks at 1 or 10 days of age (1/3 of the beak was removed, measured from the tip to the nostril). None of the chicks developed scar tissue. The beaks had a fast regrowth, but the afferent nerves and sensory receptors were missing. The findings of Lunam and Glatz (1997) do not match this. They treated the beaks of layer chicks in the hatchery. The group that was treated moderately (1/2 of beak removed) had neuromas at 10 weeks of age, but not at 70 weeks of age. In contrast to the findings of Gentle et al. (1997) these beaks did contain sensory receptors with normal nerve ends, although the number was lower than in untreated beaks. The beaks that were trimmed severely (2/3 of beak removed) developed a lot of scar tissue and neuromas, that were still present at 70 weeks of age. In these beaks no sensory receptors were found.

From the above it can be concluded that early treatment of beaks causes the least problems with scar tissue and neuromas, but will result in more re-growth (Gentle et al. 1997, Lunam and Glatz, 1997). Glatz (2000) reviewed beak trimming at various ages and possible consequences. His findings are in short:

**Beak treatment in the hatchery at 0 days of age:** Provided the beaks are not cut too short this will result in beaks without neuromas and a partly regained tactile sense. Body weight of these birds may be slightly lower, but no consequences for egg weight and egg production are recorded. Some authors report a higher mortality in the first week of age. Other authors could not confirm this. If performed precisely higher mortality is not necessary. Although treatment at this age is not ideal as it falls together with other treatments (vaccinations and moving to rearing house), there are good possibilities to combine treatments (e.g. robot that combines beak treatment and vaccination). In some occasions the pullets are beak treated a second time at a later age because of too much re-growth of the beaks. In large countries with long distances between farms it may not be convenient to perform beak treatment at the farms and treatment in the hatchery is preferred.

**Beak treatment at 5 - 10 days of age:** At this age no neuromas are expected provided beak treatments are not too severe. The beaks will re-grow partly, but less than in the situation of treatment at 0 days of age. Effects on body weight of the pullets are not expected. Several authors didn't find an effect on growth, but found better egg production compared to birds that are treated at a later age. Re-growth is in some (non-European) countries the reason to treat again at 10-14 weeks of age.

**Beak trimming at 4 - 8 weeks of age:** Little re-growth is expected. Neuromas may occur, but it is unclear if these are permanent or will disappear. Van Rooijen and Van der Haar (1990) found better beaks (less abnormalities) when treated at 3 weeks of age compared to treatment at 6 weeks of age. In the rearing period the treatment may lead to temporarily lower feed and water intake and thus a short stagnation in growth. Before the EU-Directive 1999/74 was adopted beak trimming usually was performed at 6 weeks of age, leading to adequate control of feather pecking and cannibalism.

**Beak trimming at 8 - 16 weeks of age:** The risk for permanent neuromas is higher if birds are beak treated at an older age. Regrowth hardly occurs, but mortality as a result of the treatment may be higher compared to treatments at younger age. Van de Haar and van Rooijen (1991) found more beak abnormalities in birds trimmed at a later age.

## How much is removed

Various researches indicate that the extent to which the beak has been trimmed or treated has a major influence on the final result. Shorter beaks result in better feather quality than longer beaks. Also mortality due to feather pecking and cannibalism is lower when beaks are trimmed shorter. In contrast, birds with shorter beaks have more problems with their feed intake compared with birds with longer beaks (Kuo et al, 1990; Andrade and Carson 1975; Craig and Lee, 1990; Gentle, 1986a; Cunningham, 1992; Hughes en Gentle, 1995). This difference is more pronounced when feed is provided in larger particles, due to more sensitive beaks (Gentle, 1986a; Hughes en Gentle, 1995).

Van Rooijen and Stufken (1991) studied shorter and longer beaks (after trimming) and concluded that longer beaks are preferable, because:

- the wound on the beak will heal better with a horny layer over it and without scar tissue
- less abnormalities occur in beaks that are cut less severely
- beak treatments are less strenuous, because less tissue is removed.

Lunam (2005) stated that the amount of tissue that is removed has a major influence on the results. She came to the following categories:

- mild treatment: 1/3 of upper and lower beak of layers and turkeys
- moderate treatment: 1/2 of upper beak and 1/3 of lower beak
- severe treatment: 2/3 of upper beak and 1/2 of lower beak

When applied at day-old, the mild treatment did not result in any neuromas. The moderate treatment resulted in neuromas that disappeared at later age. In the severe treatments neuromas were still present at an older age. Schwean-Lardner et al. (2004) removed up to 50% of the beak at 0, 10 and 35 days of age without any neuromas as result, which is in accordance with the findings of Lunam (2005) for the moderate treatment.

Lunam and Glatz (1997) concluded that there is a critical amount of beak tissue that can be removed. If more is cut off, the beak will not be able to recover and scar tissue and neuromas will be present permanently. In those cases the tactile abilities of the beak will not recover. If less of the beak is removed, the beak will recover without scar tissue and no permanent neuromas will develop. Also the tactile senses will recover to some extent. Beak treatments will always lead to a reduction of tactile senses of the beak.

## Methods of beak treatments

For a beak treatment of laying hens, layer parents, layer type pure lines and broiler breeders a part of both upper and lower beak is removed. This is done during the rearing period. Usually a device is used that cuts part of the beak off with a hot blade that sears up the wound. There are several varieties on this concept. For pullets of 6 weeks of age and older a cross bar is used as beak support. The beaks are positioned on the support, and the blade cuts through the beak with the cutting edge striking the beak support. This method demands high skills to realise a uniform result. For young chicks usually a gauge plate is used. This is a thin, stainless steel plate with a hole through which the beak is inserted. The blade moves behind the plate thereby cutting the beak. The extent that is cut off depends on the chosen size of the hole in the gauge plate (that can be varied). Although with this method it seems easier to obtain a uniform result, this will also depend on the skills of the worker. Small chicks should not be pushed in as far as larger chicks. The method with the gauge plate is available in a normal straight shape and in a V-shape. In the straight version the knife is straight and moves vertically, in the V-shaped version the knife is V-shaped and moves horizontally. The advantage is said to be the fact that the beaks are shorter in the middle compared to the upper and lower points. This would reduce their ability to pull feathers. Although this may result in difficulties in eating (Andrade and Carson, 1975), a skilful application of this method should not give this problem. Reuvekamp and Van Niekerk (1997) did several tests with straight and V-shaped blades. They let skilful workers use

either a straight blade or V-shaped blade (both with a gauge plate) to treat 7 day old chicks. No differences were found between the two methods, beak shapes did not differ, no beak abnormalities occurred and production results were good (Van Emous, 1999; Van Emous et. al, 1998, 1999b, 2000; Van Emous and Van Niekerk, 1999c; Reuvekamp and Van Niekerk, 1999a; Van Niekerk, 1998).

For turkeys only the upper beak is treated during the rearing period. Nowadays this is done with a high energy infrared source that treats the beak tissue without any bleeding. The infrared source doesn't cut or burn, but treats the horny outside layer and the underlying basal tissue. Within a week the outer layers of the beak turn soft and after two weeks the sharp tip has eroded away. Although the method is not painless, the use of infrared seems to cause less acute and less chronic pain and one can conclude that the method is more welfare friendly to treat beaks than traditional methods. The method is already being used for turkeys. First research results indicate that the method is applicable also for layers and broiler breeders (Marchante-Forde and Cheng, 2006a, b, c)

### **Advantages of beak treatments**

Beak treated birds perform less effective feather pecking and feather pulling, leading to less damage to the pecked bird. Beak treatments reduce the risk for cannibalism (Van Emous et al., 2001a; EFSA, 2005), that clearly reduces suffering of a part of the animals.

Many publications report a better feather cover, lower mortality, lower feed intake and lower feed conversion ratio in flocks that are beak treated (Blokhuis et al., 1987; Craig and Lee, 1990, Hughes and Michie, 1982; Van Rooijen and v.d. Haar, 1990). The better feed conversion ratio can mostly be led back to better feather quality (Blokhuis et al., 1987) and less feed spillage (Hughes and Gentle, 1995). The lower mortality and better feed conversion ratio will also lead to economic advantages. Several researchers showed that higher mortality due to the treatment will not occur provided the treatment is applied skilfully (Andrade and Carson, 1975; Van Rooijen and Van der Haar, 1990; Craig and Lee, 1990, Carey, 1990; Struwe et al., 1992).

### **Disadvantages of beak treatments**

First and foremost any mutilation, including beak treatments, will affect the integrity of the animal involved. The animal is being adapted to the environment, whereas from the point of view of safeguarding the integrity of animals the environment should be adapted to the animal. From this point of view, performing mutilations may be seen as defending the symptoms of inadequate husbandry systems, rather than solving the problems associated with these systems. Another disadvantage is the acute pain associated with beak treatment itself. Grigor et al. (1995) recorded resistance and vocalisation of chicks that were treated, especially when using a hot blade. Following the period of acute pain a period follows in which the animal is probably fairly pain-free (Duncan et al. 1989, Gentle et al. 1991 in Hughes and Gentle, 1995). The reason is that the nociceptors (that register pain) are cut off and the nerve endings themselves do not send any extra signals. This pain-free period sometimes can last for 26 hours, after which the pain sensation will return, probably as a result of regained ability of the nerve end to send signals. Thereafter a period of chronic pain follows, that depending on the extent of the mutilation may last up to 6 weeks after treatment (Gentle et al. 1990). In case of permanent neuromas there are strong indications that at least part of the animals will experience chronic pain during the rest of their life.

Apart from neuromas, also abnormalities may occur as a consequence of beak treatments, causing pain or distress to the animal. Van Rooijen and Stufken (1990) have described several abnormalities. When the horny layer is not recovering well, the result will be a fairly soft beak tip, which is very sensitive and easily wounded. Other abnormalities are excessive scar tissue, too long lower beaks, odd beaks, too short beaks, etc. Abnormal beaks can hinder hens in their feed intake leading to reduced production.

A severe beak treatment may (temporarily) reduce feed intake so much that growth and development of the bird is reduced. This results in lower body weight at the start of the laying period and later onset of lay.

Other disadvantages focus on the image of the poultry industry, which finds it difficult to convince welfare-oriented consumers that beak treatments may be necessary and on balance beneficial if done correctly. As a minor disadvantage, the cost of beak treatments should be mentioned, which is easily recovered by reduced feed intake of better feathered hens.

## Other mutilations

Although beak treatment is the most widely applied mutilation, involving various species, there are more mutilations applied to poultry. Although there are always specific reasons why mutilations are applied, they do affect the integrity of the animal involved and thus efforts should be undertaken to make them redundant.

## De-spurring

Spurs of broiler breeder cockerels (parent or grandparent males) can cause deep wounds on the thighs of the hens. Therefore spurs are mostly treated directly after hatch. By pushing the spurs briefly against a hot spot, growth of the spurs is stopped. Usually no or almost no mortality is caused by this measure. As far as known no research has been conducted to investigate if de-spurring can cause acute and/or chronic pain. Gentle and Tilston (2000) found many nociceptors and nerves in the skin of poultry legs. It is therefore reasonable to expect acute stress and pain caused by de-spurring. It is unknown whether any long-term effects occur, but considering the age and extent of the mutilation it is not likely that permanent neuromas will be formed.

Some breeds don't have to be de-spurred, as males tend to have short spurs. However, a small percentage of these males will develop long spurs later in life, making a treatment of adult males necessary. This usually is done with a pair of scissors. Although no research has been done on this, de-spurring of adult cocks will be painful. Whether neuromas will occur in that situation is not known.

Cockerels of egg-type chickens also develop spurs, but they are not de-spurred. Due to their lower body weight, they are unlikely to harm the females at mating. Individual hens of some strains can be observed to develop spurs at later age, which presents a risk of getting caught in poorly designed cage floors, but prophylactic de-spurring of hens is seldom practiced.

## Dubbing

Dubbing (cutting of single combs) is widely practiced in day-old parent, grandparent and pureline cockerels of egg-type and meat-type chickens for two main reasons: to identify sexing errors during rearing and to avoid losses due to excessive comb growth during adult life. If broiler breeders are reared sex-separate, dubbing to detect "sex slips" is not necessary, and intact combs may even be advantageous for sex-separate feeding. In brown-egg layer parents, dubbing may not be necessary, because the sex is identified by colour and the combs are smaller than in White Leghorns.

Undubbed White Leghorn males would have reduced vision, abnormal feed intake, physical difficulties to mate and resulting poor fertility. Also these too large combs prevent males from eating, causing higher mortality. In case parents are kept in cages, e.g. for producing vaccine eggs, dubbing is also considered necessary to prevent injuries. Dubbing is practiced in the hatchery with small (not heated) scissors. If dubbing is performed correctly there is no bleeding afterwards. To our knowledge, no research has been done to investigate possible acute or chronic pain of this measure.

Removing (parts of) the comb may have an effect on communication between birds. As one of the secondary sexual characteristics the size of the comb may influence the acceptance of the male by the female and the social status of the male (Johnsen et al., 2001; Parker and Ligon, 2002). Impaired sexual signals may hamper sexual behaviour and reduce successful matings with lower production as a result (Jones and Prescott, 2000). Research however did not find an effect of dubbing on percentage fertile eggs, production and sexual activity of males (Long and Godfrey, 1952; Fairfull et al., 1985).

A possible disadvantage of dubbing may be a reduced ability for thermal regulation (Khan and Johnson, 1970). In hot climate areas with open housing, we therefore often find broiler breeders and brown-egg layer parents with undubbed combs.

### **Toe clipping**

The clipping of toes of broiler breeder cockerels is done to prevent injuries of the hens caused during mating. At this moment only the hind toe of all cockerels of various meat types is clipped. The same applies to meat type grand parents. In several other countries (also in Europe) it is common practice to also clip the inner toes (forwardly directed) to prevent hens being injured during mating. Also cockerels used for the production of vaccine eggs are toe clipped (hind toe).

For layer type cockerels that are weighing less, this measure is not necessary and thus not applied.

Like desprunning and dubbing toe clipping is carried out directly after hatch with a device with a hot blade or hot wire. Research showed that, like in beak trimming, toe clipping may cause neuromas, although these are smaller and the effects seem to be less severe than in beaks (Gentle and Hunter, 1988). Nevertheless the procedure itself is stressful and causes pain and there is a risk for neuroma formation and thus for chronic pain.

Patterson et al. (2001) did use a microwave device to remove toe nails of chicks. Within 24 hours the basis of the treated toe nails turned white and the nail fell off in 3 days. Although the method worked, negative results were recorded for growth and the goal (less body scratches) was not reached. No other alternative methods are known.

### **Identification**

Mutilations to identify birds are applied to distinguish pure lines, prevent sex failures and to identify individual birds in breeding programs. If other mutilations are already applied, these often are also used for identification. If no other mutilations are applied, various measures are possible to secure identification of single birds. For broiler breeders this implies removing the nail of the inner toe (to distinguish the various male lines) and cutting the toe membrane or applying a wing band to identify individuals of genetically different groups; for layer breeders this implies the application of a wing band for identification of individuals on pure line level and cutting the toe membrane for distinguishing the various genetic lines. Removing the nail of the inner toe can be regarded in the same way as toe clipping. In both applying a wing band and cutting the toe membrane the mutilation is directed to not much more than skin tissue. Although no information on these measures is available it is not likely that they cause significant pain.

### **Situation for various poultry species**

#### **Laying hens**

In certain situations (or under certain conditions) it is possible to omit beak treatments in laying hens. In cages with small groups (up to about 10 birds) the risk for outbreaks of pecking and cannibalism is not very high. By reducing the light level pecking can be restricted and hens with intact beaks can be kept.

In cages the risk for pecking increases with group size (Fiks et al., 2003; Tauson et al., 2005). Measures to reduce pecking, like provision of distraction materials, do not completely prevent the development of feather pecking but only reduce the risk to some extent. Although recent findings indicate that mortality can be low in flocks with intact beaks in large group cages, this requires high management skills and optimization of all factors contributing to the risk for cannibalism (EFSA, 2005; Blokhuis et al., 2007).

In non-cage systems there are more possibilities to prevent feather pecking and cannibalism. These measures however do cost labour and money and are often not easy to apply on larger farms with

more houses and large flocks. The group sizes of these large flocks also mean a higher risk compared to small non-cage flocks. Finally in non-organic farming stocking densities are probably higher than required for successful keeping of non-treated hens. Applying state-of-the-art husbandry, it does not seem advisable to house large groups of laying hens without beak treatments (EFSA, 2005).

To prevent problems with pecking various management measures are advised. None of these measures can exclude damage by pecking behaviour, but research on more promising methods continues and is certainly important. In a pilot study with laying hens it was shown that sandpaper on the bottom of the feed trough blunted the beak tip. The group with sandpaper had a lower mortality than the control, but feather cover did not differ (Fiks and Elson, 2005).

Fundamental research should address a better understanding of the causes of feather pecking and cannibalism. Areas of interest are behaviour, genetics, nutrition, neurobiology and physiology.

### **Breeder flocks**

Not de-spurring broiler breeder cockerels may be possible in lines with relatively small spurs. Cutting of the hind toes and beak treatments in broiler breeders seem to be necessary measures for the near future. Cockerels with intact beaks and non-clipped toes cause high mortality and a lot of injured hens, and up to now no solutions for these problems have been found (Van der Haar et al., 2002). Dubbing and cutting of the inner toes is not performed anymore in broiler breeders in various countries.

For broiler breeders aggressive mating behaviour seems to be an important cause of injured hens (Jones and Prescott, 2000). Research to the cause of this behaviour may lead to possibilities to reduce or omit mutilations of broiler breeders.

Dubbing of cockerels of layer parents may not be necessary in brown-egg lines, because they have smaller combs and sex failures can be recognized. For White Leghorns, grandparents and purelines, this measure is still considered as necessary. Toe clipping and de-spurring is not performed on layer type of birds. At the moment there are insufficient alternatives to omit beak treatments (see layers). For parents used for vaccine production, de-spurring and toe clipping may not be necessary, but dubbing is.

### **Turkeys**

Mutilations applied in turkeys are beak treatments and desnooding. The latter is more and more omitted. Beak treatments used to be done with a laser burning a small hole in the upper beak at the hatchery. The beak tip then would fall off in about 5 days. Nowadays mostly the microwave method is used in the hatchery, resulting in erosion of the beak tip within a few days.

From extensive research in the Netherlands and other countries in meat type turkey production, no satisfactory solution has been found to reduce pecking in untreated turkeys to an acceptable level (Fiks et al., 2006, Frackenpohl, 2004; Veldkamp, 1998). All forms of enrichment of the environment only have a short-term effect on the behaviour of turkeys. To keep the enrichments attractive, materials should be modified frequently. Reducing light intensity to very low levels has an effect, but from the point of view of natural explorative behaviour it is a less desired measure. On commercial farms at this moment pilot studies are running where turkeys are offered a covered veranda. In the veranda itself and through the outlet openings the turkeys are exposed to large amounts of light, so dimming of lights is not an option.

### **The current situation in Europe**

There is a lot of variation between European countries with regards to legislation and practice of mutilations in poultry. Only beak treatments of laying hens are regulated on a European level. In Council Directive 1999/74/EC of 19 July 1999 laying down minimum standards for the protection of laying hens, beak trimming is regulated in the annex, which states under no. 8: „ Without prejudice

to the provisions of point 19 of the Annex to Directive 98/58/EC, all mutilation shall be prohibited. In order to prevent feather pecking and cannibalism, however, the Member States may authorise beak trimming provided it is carried out by qualified staff on chickens that are less than 10 days old and intended for laying.“ For the other mutilations no European legislation is available and countries differ in regulations and in practice.

The strictest legislation regarding mutilations is in force in Scandinavian countries (Norway, Sweden, Finland), where beak treatments are not allowed and other mutilations are also banned or strictly regulated (Table 2). In Denmark, Germany and Belgium beak treatments are only allowed if there is proof or a strong indication that in the given situation omitting beak treatments will cause serious welfare problems. In practice this means that on almost all farms, and certainly in non-cage systems, beak treatments are applied. In the UK, the Netherlands and Austria beak treatments are still allowed, but National legislation is in force to regulate the application of mutilations. In the UK and the Netherlands a date for banning beak treatments is already set, although new insights may lead to modification of the (proposed) legislation. In Austria over 95 % of the hens are not beak trimmed.

With regards to other mutilations, many countries don't have any regulations. In general countries with strict National legislation regarding beak treatments also have regulated other mutilations. Most Southern and Eastern European countries have no other legislation regarding mutilations except the Council Directive 1999/74.

The actual situation in European countries is not much different from what their legislation prescribes. In some situations exemptions on a ban on beak trimming are made routinely, but in other countries beak treatments are not performed anymore, whereas there still are legal possibilities.

**Table 2: Legislation regarding mutilations in Europe**

BEAK TRIMMING			OTHER MUTILATIONS		
Allowed (EU-Directive)	Strictly regulated	Not allowed	Not regulated, allowed	Regulated, but (mostly) allowed	Regulated and (mostly) not allowed
Czech Rep. France Hungary Ireland Italy Poland Spain	Austria Belgium Denmark Germany Netherlands Switzerland UK	Finland Norway Sweden	Czech Rep. France Ireland Italy Poland Spain	Austria Belgium Denmark Finland Hungary Netherlands Switzerland UK	Norway Sweden

No information: Bosnia, Croatia, Romania, Serbia-Montenegro, Slovenia, Slovakia

## Summary

In commercially kept poultry, mutilations are applied to prevent behaviour of individuals which may become harmful for group members. Mutilations discussed in this paper are: beak trimming (in laying hens, breeders of egg-type and meat-type chickens and turkeys), de-spurring (in meat-type parent males), toe clipping (in broiler parent and grandparent males), dubbing (in egg-type and meat-type parent males) and various ways for individual identification (of egg-type and meat-type parents and grand parents).

Mutilations cause acute pain when applied and can in some situations cause chronic pain sensation, depending on the age and extent of the mutilation. Although mutilations are mostly done to prevent injuries to birds, opponents feel there should be alternative management strategies to safeguard the

animals from injurious behaviour. However, for the current intensive as well as organic poultry husbandry systems these alternatives do not provide a satisfactory control of injuries and mortality.

A European Directive is in force only for beak trimming of laying hens. Local governments may already have or plan to impose more strict regulations and in some countries even ban mutilations. In general the situation in practice is in accordance with the legislation.

## Zusammenfassung

### Eingriffe beim Geflügel in europäischen Haltungssystemen

Eingriffe beim Geflügel werden vor allem praktiziert, um einem Verhalten von Tieren vorzubeugen, das sich nachteilig für die Tiere selbst und andere Tiere im Bestand auswirken kann. Im Einzelnen werden in dieser Übersicht folgende Eingriffe behandelt: Schnabelstutzen (bei Legehennen, Elterntieren und Puten), Sporenbrennen und Zehenschneiden (bei Broilerelterntierhähnen), Kämmeschneiden (bei Lege- und Mastelterntieren) und verschiedene Arten individueller Kennzeichnung.

Eingriffe können bei ihrer Anwendung akute Schmerzen und je nach Alter der Küken und Intensität der Eingriffe chronische Schmerzen verursachen. Obwohl die Eingriffe vor allem Leiden anderer Tiere durch Pickverletzungen verhindern sollen, fordern Gegner dieser Praktiken andere Lösungen. Nach heutigem Wissensstand lässt sich jedoch eine befriedigende Vorbeuge gegen Kannibalismus und Federpicken ohne Schnabelbehandlung nicht erreichen.

Eine Europäische Richtlinie zu Eingriffen gibt es bisher nur für das Schnabelstutzen von Legehennen. Einzelne Mitgliedsstaaten der EU können schärfere Regeln gegen Eingriffe erlassen und haben dies z.T. bereits getan. Die Praxis folgt im Allgemeinen den gesetzlichen Vorgaben.

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