Fertile eggs – a valuable product for vaccine production

M. Kock and G. Seemann, Cuxhaven, Germany

Since a long time, fertile eggs are being used as the preferred substrate for vaccine production. The total number of eggs used for this purpose is estimated at currently about 600 million eggs per annum. The market for fertile eggs, commonly called “vaccine eggs”, consists of different segments with specific requirements concerning production and quality.

Market segments for vaccine eggs

Generally speaking, there are two major categories of eggs for vaccine production: “Clean Eggs” and “SPF-Eggs”.

**Clean Eggs**: The largest volume of vaccine eggs is used for the production of human flu vaccines. These eggs are produced under conditions similar to parent flocks for the production of day-old commercial layer chicks. The same quality of eggs is the standard product to produce killed vaccines for veterinary use in Europe, whereas SPF-eggs are generally required for this purpose in the USA. The quality specifications for these so-called “Clean Eggs” are laid down by the customers in cooperation with the producers. Main criteria are the cleanliness and the fertility.

The main reasons for using Clean Eggs instead of SPF-Eggs for human flu vaccine production are the huge quantities of eggs needed seasonally and the higher cost of producing SPF eggs. One fertile egg is needed to produce a single vaccine dose against a specific flu virus. Since normally the “flu shot” consists of a combination of flu viruses, for one shot up to 3 eggs are needed for production. If SPF-eggs were required, this would make flu vaccination unaffordable for many people, especially in low-income countries. As an example from the production of poultry vaccines, one SPF-Egg is good for the production of 17,000 doses of IB vaccine.

To meet specific requirements of customers, Clean Eggs can be produced without antibodies for specific disease agents by adjusting the vaccination program for the source flocks. The market volume for vaccine eggs is estimated to be 530 million eggs per annum.

**SPF-Eggs**: Specified Pathogen Free Eggs (SPF-Eggs) are produced under strictly isolated conditions to guarantee the absence of all disease agents and antibodies against these agents which are laid down in the European Pharmacopoeia. SPF-Eggs are generally used for all live vaccines and also for killed vaccines for veterinary use in some areas of the world, as mentioned above. The use for human vaccines is currently limited to four products and requires a limited volume of SPF-Eggs. The total volume of SPF-Eggs used worldwide is estimated to be 60 – 65 million. Only three major producers are active in this field worldwide who have the necessary know-how and experience to meet the high quality requirements.

Marketing of Eggs for Vaccine Production

Eggs for vaccine production are marketed in one of two forms: cold or pre-incubated. **Clean Eggs** are predominantly marketed pre-incubated. Since these eggs are needed in large quantities within a short period of time, the vaccine producers are negotiating delivery contracts on a yearly basis according to the anticipated volume of vaccine production. Pre-incubated eggs have the advantage of being candled before delivery. Only eggs with a developed and vital embryo are entering the site of vaccine production. This reduces not only the volume of eggs and labor requirement, but also the risk of contamination for the vaccine producer.

The production of **SPF-Eggs** is a highly specialized business with production facilities concentrated in a few locations mainly in Europe and the USA. On the other hand SPF-eggs are a necessary source material for world-wide vaccine production. It is therefore of utmost importance to secure the delivery of SPF-eggs also in the event of transport restrictions due to national, notifiable disease outbreaks.
Major producers like VALO have spread out their production units to different countries and are able to supply from this network in case of import restrictions from one of these locations. In co-operation with customers, SPF-egg producers are trying to get regulations that will allow free transport of SPF-eggs also in case of restrictions for ordinary hatching eggs. This could help the poultry industry especially at times when SPF-eggs are desperately needed for vaccine production.

Production of SPF-Eggs

As for the quality of SPF-Eggs some conditions for producing SPF-eggs are laid down in the European Pharmacopoeia. However, the consistent quality of SPF eggs depends more on the long-term experience of the whole staff involved in the production and processing chain than on studying manuals and text books in case problems are noticed. VALO, for example, has more than 40 years of experience in producing SPF-Eggs and developed a technology which enabled VALO to be first in producing SPF-Eggs completely free of chicken anaemia virus (CAV). Over the years, VALO had an impressive record of consistent quality, with rare failures in individual flocks, in which case customers were informed immediately and eggs used for less sensitive purposes or the flocks have been culled.

To achieve and maintain the SPF-status, all production steps must be under complete control. Products entering the production units must be safe and in case of a potential risk must be treated effectively before being used. Since SPF-Eggs for commercial purposes are produced by large flocks, the amounts of feed, water and air that have to be supplied in a safe way are staggering. A typical flock of 5000 hens will need about 200 tons specially treated feed, 400,000 liter water and 4-17 million m³ of air during the life cycle. The caretaker will enter and leave the building 450 times, 42,000 egg flats must be delivered into the house and 1.25 million eggs, dead birds and 500 tons of manure must be removed from the house safely. How are these processes organized in the VALO organization?

Personnel

Generally speaking, access to the chicken houses is minimized. Ideally only the caretaker enters the house throughout the whole life cycle. To reduce the necessity of entering for repair and maintenance, all standard maintenance work is done during the service period when the house is empty. Important technical equipment is serviced routinely or will be replaced as a safety measure in the downtime of the house.

The caretaker enters the house through double barriers: the first barrier requires a change from street clothes and shoes into farm owned clothes and boots, the second barrier before entering the SPF-house requires a complete change to house specific clothes after a shower with hair wash. The house specific clothes remain within the house during the complete production cycle, which requires a washer and dryer for each house.

Feed consists of organic material and is therefore a potential carrier of all kinds of contaminants. To minimize the risk for all VALO flocks, the feed is produced in a company owned state of the art feed mill, using an effective heat treatment system. In this feed mill, finished feed is exclusively produced for the VALO SPF-flocks and elite breeding flocks and grandparents in the Cuxhaven area under the control of the Veterinary Laboratory of Lohmann Tierzucht. Only selected raw materials from proven sources are used. Currently the feed consists of corn, wheat, soybean meal, vegetable fat plus vitamin and mineral premixes. The decontamination treatment consists of heat (85°C for 6 minutes minimum) and the addition of an acid mix after cooling.

Fat and vitamins are added after the heat treatment and need to be decontaminated separately. Fat is kept in the storage tanks at a constant temperature of 70°C and is therefore clean when added to the feed. The vitamin premix is delivered in 25 kg bags which are irradiated with 15 kgy minimum dose prior to being transported to the mill. The bags are emptied into a container in the HEPA-filtered section of the feed mill through sterile stainless steel tubes. Fat and vitamins are added to the feed in the second mixer located in the overpressure ventilated area of the mill. This mixer serves as dryer and cooler as well as final mixer for the mash feed. The process air for drying and cooling and also the air to overpressure ventilate the cooling and finished feed storage bins is HEPA-filtered.
An important part of the feed supply concept is clean delivery. A dedicated tanker type vehicle is used to deliver the feed into a storage building close to the production sites on Monday or Tuesday just after the mill and the delivery vehicles have been thoroughly cleaned and disinfected during the weekend. Supply to the SPF-chicken houses is organized with farm own transport vehicles which never leave the farm area. All SPF houses are supplied with town water which is controlled very strictly by the state authorities. Regular tests are performed internally and by an independent laboratory.

Manure handling and removal

All VALO – SPF flocks are kept in family cages with manure belts. The manure is removed once or twice a week, using specially designed screw augers. These augers have a double shut-off system to ensure that there is no backflow of material into the house and that no rodents or other potential disease carriers can enter the house. This auger system can only handle wet manure which, from a management point of view, is sub-optimal. However, it is the safest system we know to handle the manure removal from the SPF houses.

Material transfer

All material brought into or taken out of a SPF-house is a potential risk for contamination. Our general policy is therefore to minimize the transfer of material. All material needed during a life cycle is brought into the house before final disinfection prior to the placement of day-old chicks. Other material, dead animals and eggs must pass a disinfection chamber with an effective fumigation procedure in place. All outgoing material is fumigated and the emptied chamber is fumigated again before the chamber can be opened again from the clean side.

Air

Dust can carry a substantial contamination load. All air delivered to a SPF-chicken house must therefore be filtered before entering the house. We currently use a system with three filter steps. The last step is a HEPA–filter which filters out 99.95 % of all particles in the air. To insure that all air passes through the filtration system, the technical equipment must be designed to avoid false air entering the house. This is possible by using a filtered air, positive pressure (FAPP) system. The FAPP system we are using also has active ventilation elements on the exhaust side to avoid wind pressure into the house and to keep off insects. All filters are exchanged before a new flock is placed and are checked for leaks with a particle counter.

Pest Control

Rats and mice are important carriers of diseases and contaminants. To keep them from entering a house, all walls are sealed properly and doors are protected by shields. An essential part of rodent control is to catch them outside the houses if possible. Therefore, all VALO – houses are equipped with rodent traps along the walls at a distance of only 3 m from each other. As soon as a house is depopulated, a very intensive pest control program must be carried out inside the house. Since no chicken feed is available for the rodents at this time, they can be caught more easily than while a house is populated with birds.

Grading, disinfection and final inspection

The final step before shipping SPF–Eggs to the customer is grading and disinfection. Although the eggs at VALO are fumigated during the transfer from the chicken house to the grading facility, the eggs may have been re-contaminated during handling and transport and are therefore disinfected again. The grading process consists of a visual inspection where soiled eggs and eggs with obvious shell defects are removed before the eggs pass through a crack detector unit and a weighing system. Eggs with shell defects, undersized and oversized grades are directed to separate packing lines and sold to an egg breaker. Saleable VALO SPF-eggs are sorted into three weight classes and packed.
into cardboard boxes. This process gives VALO customers a more uniform egg size and supports automatic processes in egg handling during the vaccine production. The first disinfection is by fumigation, the final disinfection before packing the eggs by spraying. For both processes, formaldehyde free products are used.

**Quality control to confirm the SPF status**

Besides the visible inspection of egg quality, the SPF status of each flock has to be guaranteed at all times during production to ensure the requirements of the European Pharmacopoeia. At present, the list of agents consists of 22 different diseases, of which absence of antibodies or antigens has to be demonstrated by validated, approved testing protocols. During the rearing period 5% of the birds have to be tested twice, during the production period 5% on a monthly basis (or 1.25% weekly). All this testing is done in the DIN ISO 17025:2005 certified Veterinary Laboratory of Lohmann Tierzucht.

All efforts in production, handling and marketing of VALO SPF–Eggs focus on the same goal: to supply the vaccine industry with a safe and reliable source material of high quality in line with the current and future product specifications of our customers.

**Zusammenfassung**

**Bruteier als hochwertiges Ausgangsmaterial für die Impfstoffproduktion**


In dieser Arbeit werden die Unterschiede zwischen diesen beiden Kategorien und die speziellen Produktionsbedingungen für SPF-Eier beschrieben.

**Address of authors:**

Marcus Kock and Dr. Gerhard Seemann  
Lohmann Tierzucht GmbH  
Am Seedeich 9-11  
27472 Cuxhaven  
Germany  
E-Mail: Kock@ltz.de