Commercial application of heat treatment during egg storage

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n the process of incubating eggs on an industrial scale, egg storage is a key element that cannot be ignored or avoided. Poor storage conditions inevitably lead to a significant decline in hatchability. Until recently, even storing eggs at the correct conditions but for a longer period automatically involved major losses in hatch results. However, recent findings indicate that some of these losses can be restored.

Egg storage: a necessary evil?

In a commercial hatchery, egg storage is an integral part of the logistical flow of the eggs. First of all, storing eggs cannot be avoided because commercial incubators are so large in size: from 10,000 eggs up to more than 100,000 eggs per machine. A single day's production of hatching eggs from a breeder flock is not enough to fill an entire incubator.

Using smaller incubators is not an option because they cannot be justified economically. Secondly, market conditions fluctuate. On the one hand, the supply of incubation eggs is not constant as breeding flocks have to be replaced on a cyclical basis. On the other hand, the demand for day-old chicks can change from day to day as customers place orders that change in size and timing and as market conditions vary.

This means that there is a need for a buffer of hatching eggs in the hatchery. Therefore, eggs are kept in a storage room for several days. The goal of storage is to arrest embryonic development until the eggs are loaded into the incubator.

Egg storage in practice

In order to achieve the desired arrest in embryonic development, the storage temperature should be at or under the threshold or physiological zero for development.



In literature there is a broad range to be found as what is defined as physiological zero, from 19-20°C to 29°C. There is no agreement about the real value of this physiological zero temperature, but it was found on an empirical basis that the longer the storage time, the lower the storage temperature should be; that is why storage temperatures of 10-12°C are applied for storage times of 2-3 weeks.

Drawbacks of long egg storage

It is well known that storing eggs longer than seven days decreases the hatchability. In fact, the longer eggs are stored, the higher the losses in hatchability. Stored eggs have a higher rate of embryonic mortality between days two and three of incubation, and need more time to complete incubation. This causes some live chicks to be rejected at take-off because they hatch too late.

The causes of this hatchability loss are deterioration of the albumen, and necrotic cell death. The developmental stage of the embryo, the number of viable cells and the pH of its micro environment probably affect embryo viability most during storage and early incubation, and are hence important for the ultimate hatchability. With increased storage periods, there is a rise in the number of mitotic (blocked in metaphase and dying during storage) and necrotic indexes. As initiation of mitosis can probably occur in eggs stored at or below 20° C, eggs stored for a long period should be stored at a temperature below 20° C (between 10-15°C) in order to reduce this cellular activity.

Moreover, changes in albumen pH and albumen viscosity may be important factors in embryo viability. The embryo is in direct contact with the yolk on one side (yolk pH being 6) and close to the thick layer of albumen on the other side (pH being close to 9 after four days of storage).

This difference in pH between albumen and yolk is necessary for particular transport functions through the vitelline membrane, but prolonged exposure to high albumen pH levels, due to prolonged storage, can become detrimental to the embryo, since optimum pH for embryonic development during the first few days of incubation is between 7.9 and 8.4.

Restoring hatchability?

Several studies have investigated the possibility to limit the hatchability loss after long storage, with differing results. Fasenko et al. (2001) and Lourens (2002) reported a reduced loss in hatchability for broiler eggs that were heat treated.

In recent years, more and more successful attempts have been reported applying heat treatment during storage, even for large *Continued on page 9*

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scale trials. Nicholson (2012) and Aviagen (2014) have shown a consistent improvement of the hatchability of long stored eggs (Ross 308 and Ross 708 broiler eggs, as well as various GP and GGP lines) by applying one or more heat treatments in 34 small to large scale trials.

Fig. I shows how the potential for improvement in hatchability increases with storage time. Similar results have been reported for layers: Schulte-Druggelte (2014) reported an improvement in hatchability of 11.5% for eggs from a grandparent flock of commercial Lohmann White Leghorns (LSL) stored for 20 days, when the eggs were heated for six hours on day one of storage.

Biological background

The biology of the processes behind it needs to be examined in detail, in order to understand why heat treatment can partially restore hatchability losses caused by egg storage as well as the limitations to this method. It is known that the embryonic developmental stage at the moment of oviposition (the moment the egg is laid) is variable and may be different for different genetic lines as well as for parental age.

This may be a direct effect of the genetically determined speed of early cell division and development or it may be indirectly linked to variations in oviductal transit time and/or body temperature.

Indeed, development of the avian embryo begins immediately after fertilisation in the infundibulum and continues as albumen and shell are deposited over the next 24-26 hours. It has been reported that embryos at the pre-gastrula stage at oviposition are less able to withstand prolonged storage compared to embryos at the gastrula stage.

Therefore, pre-storage incubation may improve hatchability of long term stored embryos, since it can advance embryos to the developmental stage in which hypoblast formation was complete (stage XII according to Eyal-Giladi).

However, if development is already well advanced and embryos have started to form the primitive streak (stage XIII and beyond), pre-storage incubation may even be detrimental since it brings the embryo in a more advanced stage of primitive streak formation which is a period of active cellular migration and differentiation, and storage during such a period could impede critical embryonic processes.

So there is some sort of 'point of no return'. Once this is reached, the embryonic development cannot be stopped anymore.

Pre-storage incubation and periodic heat treatment or short-term incubation during prolonged egg storage must be clearly distinguished although both heat treatments may interact. Some partial, but not a global or proportionate development can take place at so-called subthreshold



Fig. 1. Improvement in hatchability after heat treatment during storage (Aviagen, 2014).

temperatures during storage. Different cells or tissues in these early embryos may have different threshold temperatures for development, resulting in uneven or disproportionate development.

If this disproportionate development progresses too far, it may interfere with embryonic viability and hence also hatchability. Periodic warming during prolonged storage allows the embryo to redress disproportionate development and ensures the required degree of embryonic development for all tissues in a proportional way.

However, this technique may interact with the stage of development at oviposition or at the start of egg storage; when this stage is too far (for example beyond stage XIII) it may even be detrimental, similar as a periodic warming (during storage) which is too long and takes the embryo beyond the optimal stage (advanced gastrula stage) for prolonged storage.

Commercial application

Realising the potential of this treatment has been the challenge for the industry and equipment manufacturers. There have been numerous attempts both in the commercial and academic environments to achieve consistent gains with this principle but both have reported variable results. It is clear that inaccurate treatment frequency will at best give limited gains or at worst take the embryo beyond the 'point of no return' and result in destructive consequences.

Equally critical are the treatment parameters in terms of absolute temperature and transition durations. An excessive treatment, for example when the eggs are heated up too fast or up to a too high temperature for too long, will bring the embryos beyond the point of no return.

This means that the embryos have advanced to beyond stage XIII and that the embryonic development cannot be stopped anymore. Putting these eggs back into the cold storage room will negatively affect their viability. The research work carried out by Aviagen has made major strides in identifying the optimum temperature, timings and operating temperature bandwidth for heat treatment during storage. It has become clear that, in order to achieve consistent gains in hatchability and post-hatch performance, the key process parameters in the incubator need to be controlled very accurately.

It is crucial to achieve the correct egg shell temperature. The eggs need to be heated up to an egg shell temperature of more than 32°C; yet keeping the eggs above 32°C for too long will negatively affect hatchability. Another important factor in the process is the warming-up and cooling-down phases.

Therefore, Petersime has developed an incubator dedicated to heat treatment during storage. Equipped with the patented OvoScan technology, it precisely monitors and controls the egg shell temperature during the entire heat treatment process. The machine guarantees an accurate, controlled and uniform warm-up and cooldown phase as this is vital for the consistency of the results. In practice, applying heat treatment during storage on a regular basis can become very time consuming for the hatchery staff. With the dedicated Petersime incubator, it is possible to automate several of the steps in this process. The desired temperature trajectory can be fully programmed in advance by the hatchery manager, and the OvoScan will control the process throughout the cycle. Petersime has run several large scale trials, using a dedicated incubator with a capacity of 57,600 eggs. In a first phase, trials were conducted with grandparent flock eggs with a successful outcome in terms of egg shell temperature control, temperature uniformity throughout the incubator, consistency of the warm-up and cool down phases and hatchability restoration. During the incubation process, the internal environment throughout the entire treatment cycle was controlled based on egg shell temperatures.

In a second phase, trials were conducted in a commercial broiler operation. During these trials, hatchability, chick quality and post-hatch performance data have been acquired and analysed. Initial investigatory heat treatment tests were done with variable results, but ultimately an optimum parameter configuration was derived.

Based on the identified parameters, a series of trials were started where eggs from the same source were given different treatments: half of the eggs were incubated after three days of storage, the other half after 12 days of storage with heat treatment. A total of four trials were completed with no loss in hatchability $(\pm 0.2\%)$ and post-hatch data showed no significant difference (Fig. 2).

A further series of identical trials were undertaken targeting 15 days of storage with similar consequences (Fig. 3). This confirms *Continued on page 11*



Fig. 2. Comparison of hatchability between three days storage versus 12 days of storage with heat treatment.

95.5 95.0 94.5 94.0 93.5 93.0 1 2 3 4 Avg Trial number

Fig. 3. Comparison of hatchability between three days storage versus 15 days of storage with heat treatment.

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the huge potential of the heat treatment methodology. Further optimisation trials are ongoing, investigating both extending the storage duration and the effect of earlier treatments on post-hatch performance.

Conclusions

Storing eggs is an inevitable practice when incubating eggs on a commercial scale. It is impossible to synchronise breeder egg production with final product production, which often generates high levels of losses. Heat treatment during egg storage allows a significant reduction in these logistical discrepancies. There is undoubtedly a huge potential in restoring the hatchability of stored eggs, and even improving post-hatch performance. The initial series of commercial trials demonstrated that the operational parameters required in order to achieve a degree of benefits was relatively broad; however achieving the optimal gains considerably narrowed the parameter limitations.

Add to this the logistical need to minimise space usage within the hatchery, it was clear there is a need for dedicated, accurate equipment that has a practical capacity. It is crucial to accurately and consistently control the key incubation parameters, as inadequate application of the technique will result in suboptimal results or might even lead to major losses.

The precise measurement and control of the egg shell temperature in the incubator, as well as a controlled and uniform warm-up and cool-down phases of the eggs, are key to achieve consistent, optimal gains.

References are available from the authors on request

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