# Circadian incubation – the next generation of modular, single stage technology

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Modern poultry production requires birds that grow uniformly and efficiently, which means that most of the bird's nutrition is directed to production. Efficient birds are resistant to stressful conditions and use only small amounts of nutrients for the maintenance of basic physiological systems.

Geneticists have introduced the concept of robustness to describe these efficient, modern birds in more biological terms, so that today, robustness also defines an important trait for selection, related to animal health and welfare.

Robustness is a health criterion that originates in embryonic life and correlates with growth and the resistance of the individual chicks under different farm conditions.

We define a robust day old chick as a first class chick that delivers predictable growth and production under different farm designs and fluctuating conditions, such as high and low temperatures. Batches of robust day old chicks show low mortality, need less medication and have the potential for optimum growth even under adverse farm conditions.

## Circadian incubation

To support the development of robust day old chicks, Pas Reform has introduced circadian incubation as a natural and progressive development of single stage incubation. Circadian incubation is based on observations that embryonic 'training' – or the imprinting of body functions – stimulates robustness on the farm.

This 'imprinting' is achieved by exposing the embryo to environmental triggers during critical periods of the maturation of physiological control systems – and has been shown to cause long lasting alterations in the perinatal epigenetic programming of body functions.

In poultry, the best understood physiological system is the maturation of thermoregulation and its dependence on incubator temperatures. Study shows that embryos exposed to short periods of heat or cold develop an improved capacity to control body temperature during periods of heat or cold in the farm.

Consequently, these birds retain most of their feed for growth – and use much less for the maintenance of body functions.

Circadian incubation is a single stage protocol that includes periodic stimulation, by increasing temperature during certain sensitive periods of embryonic development. The term 'circadian' literally means 'about a day' as it derives from the latin ('circa'= about and 'dies'=day).

Circadian thus refers to daily biological rhythms observed in most organisms, such as the day-night rhythm in body temperature. The biological rhythms, also called the circadian or biological clock, are essential for regulating the daily metabolic rhythm and other physiological functions. In contrast to nature, embryos hatched in a conventional incubator are not exposed to a daily rhythm. This changes when the circadian incubation technique is implemented.

## Embryo development

To understand day old chick robustness, we need first to understand the development and maturation of physiological systems in the embryo.

Embryonic development comprises a number of complex physiological interactions between cells and groups of cells, best understood by simply observing the development that takes place in different phases.

- The first phase of development is called the Differentiation Phase. This is when the different embryonic structures and premature organ fields are determined and differentiated.
- The second phase – the Growth Phase – is so called because this is when the different organs and tissue grow to their final structure and size. Not only do the organs develop their final form, they also acquire the capability to function physiologically, although at this point they are not yet integrated into a physiological control system.
- The third and final phase of embryonic development is known as the Maturation Phase, characterised by the maturation of physiological functions and the development of integrated physiological and endocrinal controlling systems.

Embryonic development is a continuous process. Each embryonic phase overlaps, while the embryo moves gradually from an embryonic state to that of a hatchling.

Normal post-natal performance is only possible when functional maturation of the organs and fine-tuning of the integrated physiological circuits have taken place, during the final days of incubation.

A good example of an 'integrated physiological circuit' is the thermoregulatory system, which controls body temperature in the late stage embryo and the chicken.

Organs involved in thermoregulation – such as the hypothalamus, thyroid and pituitary gland – develop and grow during the mid-period, or Growth Phase, of incubation.

Final maturation of the thermoregulatory systems, however, occurs during the last days of the maturation phase in the embryo and the first days post-hatch.

## Differential gene expression

To further understand the route to achieving a robust day old chick that can cope with varying farm conditions, we need to look at a lower level of embryonic development: that of cell-to-cell interaction and differential gene expression.

Each phase of embryonic development described above is recognised by specific cellular interactions and the expression of genes.

As the embryo develops after fertilisation, the number of cells increase – and these cells become differentiated as each adopts the characteristics of its ultimate restricted fate.

Some cells grow to form muscle tissue, while others become part of the skeleton.

The differentiation of cells is the result of differential gene expression: muscle cells express genes for contractive proteins, while bone cells produce proteins that can bind calcium, for example.

Thus differential gene expression is the fundament of the three phases of embryonic development – and

*Continued on page 15*

***Table 1. Temperature stimulation: Eggs (337) exposed for two hours/day at 38.5°C during the last four days of incubation (days 18-21) (Tschentke B. and Halle I. (2009). Influence of temperature stimulation during the last four days of incubation on secondary sex ratio and later performance in male and female broiler chicks (Br. Poultry Sci). 50(5):634-640).***

| | Overall (females + males) hatchability of fertile eggs (%) | Male: weight gain g/broiler/ day 1-35 | Male: final body weight (35d) | Males: feed conversion rate (1-35d) |
|---|---|---|---|---|
| Control incubator | 94.6 | 62.2 ± 2.9 | 2270 ± 203 | 1.50 ± 0.04 |
| Temperature stimulation | 97.0* | 64.6* ± 2.0 | 2336* ± 191 | 1.47* ± 0.02 |

*(P< 0.05)

*Continued from page 13*
the differential activation and expression of genes has formed a key focus for research and publication in the field of developmental biology.

It is now generally understood that minute variations in the environment of embryonic cells will induce variations in the expression of genes. Embryos derived from the same parents, having inherited basically the same genetic potential, develop to different phenotypes when exposed to different environmental inducing agents: the agents that prepare and adapt the embryo to cope with varying conditions after birth.

A term often used to explain embryo-environment interaction is epigenetic adaptation: the study of how changes in gene expression patterns mediated by the environment can cause variations in phenotypes.

Today's discussions on the embryonic origin of human health and heart failure in later life, for example, refers to epigenetic affects during the embryonic and foetal development of the baby.

## Epigenetic adaptation

In poultry research, the idea that the embryo can be acclimatised to a certain trigger for better performance later in life is becoming more widely accepted.

Currently, the most studied trigger for epigenetic adaptation is the exposure of the embryo to brief periods of high or low temperature.

Critical periods, when the embryo is prone to thermal adaptation, have been found during the early phase of development, when the differentiation of specific structures is being induced – and again in the later phase of development, when the organs and physiological systems mature.

A four-day thermal-manipulation during the differentiation phase has been shown to influence the proliferation of muscle-type cells in turkey embryos, to subsequently and positively affect post-hatch muscle development.

In the chicken, short periods of increased temperature from embryonic days 4-7 encouraged embryonic movement and activity, promoting leg and muscle growth in the embryo.

Broiler embryos can be thermally conditioned during their final days in the setter, such that they achieve tolerance to heat challenge at an early age in the farm, thus altering post-natal growth.

Short periods of cold exposure (60 minutes at 15°C) at days 18 and 19 of embryonic development shows an improved performance at 38 days of age.

Long lasting adaptation occurs when periodic thermal manipulation is applied during the last part of the Maturation Phase, when the integrated circuits for the thermoregulatory system are well developed – and therefore most responsive to 'training'.

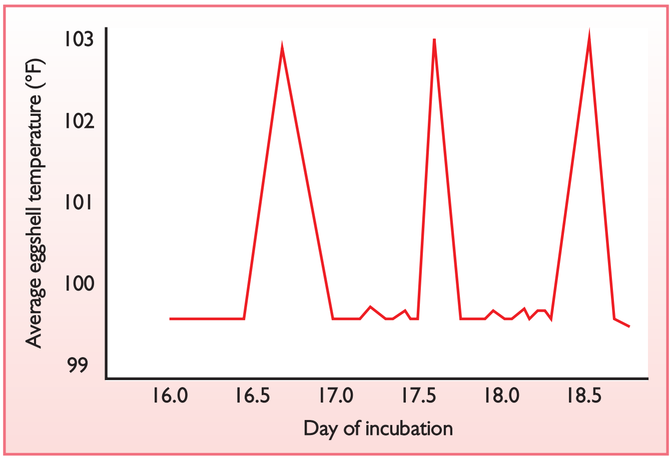


***Fig. 1. Temperature stimulation in a modular, single stage incubator, adapted to enable the circadian incubation principle. A thermal conditioning period of three hours was applied by increasing temperature set points from 98°F to 100.6°F for three hours on day 16.5, 17.5 and 18.5. Eggshell temperatures were measured automatically by means of contact thermistors.***

Thermal manipulation during this late phase in the setter and hatcher shows an improvement of 1.5% on hatchability, a 2.9% improvement in male growth and improved feed conversion: all indications of enhanced robustness in the day old chick.

More research will decipher specific, sensitive embryonic phases and conditions, to engender the further use of thermal stimulation in commercial incubators, to induce enhanced robustness in day old chicks from different flock ages and commercial breeds.

In the meantime, promising scientific results already ratify the development and introduction of circadian incubation.

## Single stage to circadian

If the goal of the modern hatchery is to produce uniform, robust day old chicks, the multistage system does not deliver the degree of control required – and single stage incubation requires further development.

Single stage incubators can of course be adjusted and set such that climate conditions match the needs of modern embryos, to improve day old chick quality and uniformity.

Today, the basic assumption for the design of single stage incubation programs is that optimal embryonic development occurs under constant conditions, without fluctuation.

However, the idea that the embryo can be adapted to certain stress factors (high or low temperatures, for example) to improve robustness and deliver better performance later in life is gaining significant acceptance. In the poultry sector, where substantial growth is indicated over the next two to three decades,

Circadian incubation signals an important bridge to meeting next generation demands and opportunities in commercial hatcheries.

The majority of thermal conditioning investigations have been performed under controlled experimental conditions, in small incubators. In collaboration with a commercial broiler hatchery and Wageningen University Research Centre, Pas Reform has undertaken trials on a commercial scale with four flocks of 35, 42, 48 and 56 weeks respectively.

In each experiment, Ross 308 eggs from three different suppliers were incubated in a modular, single-stage incubator, adapted to enable the circadian principle with a capacity of 115,200 hen eggs. For each batch of eggs, a thermal conditioning period of three hours was applied by increasing temperature set points from 36.7°C (98°F) to 38.1°C (100.6°F) for three hours on days 16.5, 17.5 and 18.5 in the setter.

In all four experiments, the egg shell temperatures raised immediately after increasing the set point.

At the end of the thermal conditioning period, average egg shell temperature was measured at 39.8-40.1°C (103.6-104.2°F).

On each experimental day, egg shell temperatures returned to normal and were comparable with egg shell temperatures in the control incubator 1.5 hours after completing the period of thermo-conditioning, by returning set points to normal (36.7°C/98°F) temperature.

Each batch demonstrated positive influences on hatching results, as a result of thermal conditioning. A clear, positive trend on growth performance was observed, with 1-2 points improvement in feed conversion ratios.

Further studies will improve the protocols for thermal conditioning in practice for different commercial breeds and flock ages.

For this reason, Pas Reform has initiated a collaborative research project with Dr B. Tzschentke from Institute of Biology, Working Group Perinatal Adaptation at the Humbold University of Berlin and Dr I. Halle from Friedrich-Loeffler-Institut, Federal Research Institute for Animal Health, Institute of Animal Nutrition, Braunschweig.

We know, however, that thermal conditioning is only beneficial when applied in a clear, controlled manner, for specific time points and duration.

A circadian incubation program can only be applied in commercial practice, if the hatchery's single stage incubation system contains individually controlled sections for accurate climate control and (thereby) delivers homogeneous eggshell temperature.

The system must also be equipped with sufficient, cooling and heating devices – to deliver short, highly accurate cold or heat stimuli for the incubating embryos to result in uniformly robust day old chicks.

Used correctly, circadian incubation opens the door for the hatchery manager to produce uniform, highly robust day old chicks that will go on to deliver robust, improved performance at farm level.

## Conclusions

The ultimate goal of modern hatchery management is to produce uniform, robust day old chicks.

Robustness is a health criterion that originates in the embryonic life stage of the chicken and correlates directly with the performance and resistance of individual chicks under differing farm conditions.

Robustness requires a specific incubation trigger during so-called critical periods, for example stimulation by heat or cold, to physiologically imprint the embryo such that the chicken thrives in its farm environment.

Short term thermo-conditioning using circadian incubation improves hatching results and produces long lasting effects, with 1-2% increase in final body weight and 1-2 points better feed conversion rates.

Batches of uniform, robust day old chicks improve uniformity at slaughter age and thereby improve efficiency and performance throughout the entire production chain.

However, to support the use of circadian incubation, the incubator should provide accurate climate control, to promote tight temperature uniformity.

Each egg must receive a consistent flow of conditioned air for optimum thermal conditioning. ■

Full paper including references are available from the author on request