# Is control of single stage incubation using metabolic parameters appropriate?

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**The history of commercial incubation has been a progression from multi-stage to single stage (all-in all-out) incubators. Having advantages in biosecurity single stage incubators allow matching of incubation parameters to the eggs and have increased in popularity over the past 10–15 years. They rely on a programme that provides optimal conditions for temperature, humidity and aeration (*i.e.* supply of fresh air). Many programmes have developed through trial and error but developments in the technology of monitoring of the incubation environment, linked with our understanding of embryonic development, have allowed some programmes to be based on first principles. Much attention has focussed on the metabolism of the embryo and how this affects egg temperature. Control of incubation through monitoring of metabolic parameters, *e.g.* carbon dioxide levels and eggshell temperatures, has increasingly dominated incubator design and marketing. This has been coupled with practises that restrict air exchange during the first half of incubation and keeping the eggshell temperature constant. Many commercial developments lack good scientific explanation and the value of some is questionable. The level of $CO_2$ in an incubator is used to control aeration of the cabinet but levels of $CO_2$ in incubators are not toxic and typically there is no limitation on levels of oxygen being supplied. $CO_2$ control systems often lead to over-aeration of the cabinet and destabilisation of the incubation environment. Maintaining an incubation environment to provide a constant eggshell temperature ignores the fact that development takes place some 3 degrees Celsius lower than the core body temperature of the adult bird. A limited rise in egg temperature during the latter stages of incubation may acclimate embryonic tissues for the body temperature of the free-living bird. Incubation temperatures may have been established because incubating adults are unable to maintain a temperature in nests comparable to their own body temperature. There is a continuing need for more research so we can better understand developmental patterns of avian embryos within commercial single stage incubators.**

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## Introduction

Single stage incubation is rapidly becoming the predominant method for incubation of poultry eggs worldwide. Over the past 10–15 years the advantages of this system, over that of multi-stage incubation, have been recognised by the industry and actively promoted by the manufacturers of incubation equipment. The potential to control the incubation environment more closely, which can increase both hatchability and chick quality, combined with greatly increased biosecurity between incubation batches, have been great selling points for the system. The popularity of this system has also spurred on manufacturers to develop increasingly sophisticated equipment for monitoring and control of the incubation environment. The ability of a hatchery manager to monitor embryonic development has greatly increased and this information can be used to control the incubation environment. This paper briefly assesses whether we fully understand this new approach and if such techniques have any real commercial application.

## Principles of single stage incubation

Multi-stage incubation depends on eggs of different ages being set within one incubator cabinet. They then experience a fixed temperature and one humidity setting and exchange of fresh air (aeration) is also at a fixed rate. The system works by relying on the exchange of metabolic heat produced by well-developed embryos with the cooler, fresher eggs in adjacent trays. Unless eggs of different ages are mixed evenly within the cabinet hot and cold spots can develop, which disrupt the thermal uniformity of the cabinet and results will be poor. The disadvantages of this system is that it is inflexible – all eggs get the same conditions, and the cabinet is rarely emptied making effective cleaning very difficult.

By contrast, single stage incubation involves setting all of the eggs together and no eggs are added during the incubation cycle until they are removed at transfer – hence the common term "all in, all out". The system requires a programme to allow changes in temperature and aeration, and possibly humidity, in order to produce the highest hatchability and chick quality. Rather than having one temperature setting a single stage incubator needs temperature set points to change as incubation proceeds. The settings are relatively high at the start of incubation but during the second half of development the air temperature is progressively dropped so as to increase cooling and remove the metabolic heat produced by the embryo. Unlike multi-stage incubators there are no cool fresh eggs to absorb this heat and without the decreasing programme (and an effective cooling system) the embryos readily overheat and die.

The system has the potential to be very flexible and incubation conditions can be matched to individual batches of eggs. For instance, whilst it is not common practise, monitoring of weight loss during incubation can allow for fine-tuning of the humidity programme to optimise weight loss for batches of eggs from different aged flocks. Aeration rates can be adjusted according to the fertility of the eggs within the cabinet. Egg turning can be stopped part way through incubation if required. All of these variables allow the hatchery manager to work with the machine to optimise its performance not only to maximise hatchability but also to get the best possible chick quality. A major advantage of the single stage system is the fact that the machine is emptied after each incubation cycle, which allows for full cleaning and disinfection of the equipment. Higher biosecurity is a real selling point for this system.

Effective single stage incubation does rely on a relatively good understanding of the incubation process and the development of embryo. Some hatchery managers have this kind of experience and are well equipped to develop effective programmes for their type and breed of poultry. Unfortunately, many others used to the multi-stage system find the change to single stage incubation challenging. As a result, incubator manufacturers have invested heavily in computerised control systems that simplify single stage incubation; for instance changes in the programme take place automatically. There have been other innovations that have been designed to open up single stage incubation to those people less versed in incubation science or practise. This can simply involve providing programmes for hatcheries to operate or the provision of systems that monitor various aspects of the incubation environment and so can be used to control the conditions experienced by the eggs. The most prominent of these are systems that monitor carbon dioxide ($CO_2$) and use its levels to determine the rate of aeration of the cabinet, and systems that automatically monitor eggshell temperature and so control the heaters and coolers. Whether these systems for monitoring the metabolism of embryos are really useful in controlling the incubation environment is discussed below.

## Role of carbon dioxide in incubation

Carbon dioxide is an important gas in embryonic development and incubation. Produced in respiration it is released into the incubator cabinet through eggshell pores although its levels within the egg can be very high. Burton *et al*. (1989) reported partial pressure of carbon dioxide ($pCO_2$) of 9% in the air space of fowl eggs immediately before external pipping. By contrast, levels of 0.3–0.5% $CO_2$ are common in multi-stage setters but levels vary in single stage machines from 0.1–1.5% depending on the rate of air exchange. $pCO_2$ greater than 0.5% is often considered to be deleterious despite

evidence to the contrary (described below). Few studies have specifically examined the effects of $pCO_2$ on hatchability and embryonic mortality. However, those studies that have been carried out all involved artificially increasing $pCO_2$ within the incubator air rather than reducing rates of air exchange so as to allow natural levels of $CO_2$ to rise above normal levels.

Romanoff (1930) showed that embryo growth to 3 days was severely restricted by levels of $CO_2$ higher than 6% and sustained embryonic growth was only possible for lower levels of $CO_2$. Even so, at 6%/19.6% $CO_2/O_2$, embryos were only able to survive until day 12. As $pCO_2$ increased then the highest rate of embryonic mortality occurred earlier during incubation. Exposure up to 48 hours to a 10% $CO_2$ and 18.8% $O_2$ gas mixture resulted in a depression in embryonic growth rates particularly during the first half of incubation and for the longer exposure period. There was, however, no effect on embryonic mortality. Sadler *et al*. (1954) found no significant effect on hatchability despite levels of 5–6% $CO_2$ in the machines during the later stages of development although they did comment that chick quality seemed higher in $CO_2$-treated eggs.

Taylor and his colleagues carried out the most detailed study of the effects of carbon dioxide on hatchability to date (Taylor *et al*., 1956, 1971; Taylor and Kreutziger, 1965, 1966, 1969). In a series of experiments, the sensitivity of fowl embryos to varying amounts of $CO_2$ was tested at different stages of single stage incubation (1–4, 5–8, 9–12, 13–16 and 17–21 days of incubation). Exposure during 1–4 days of incubation showed that early embryos were very sensitive to $CO_2$ with a maximum of only 1% before there is a significant loss of hatchability and complete mortality occurring at 7.5% $CO_2$ (Taylor *et al*., 1956). The experiments revealed a higher tolerance to elevated $CO_2$ with increasing developmental age and the level at which there was complete embryonic mortality also increased (Figure 1). There was an adverse synergistic effect on hatchability when high levels of $CO_2$ were used in combination with low levels of $O_2$ and this could be partially reversed by raising $pO_2$ levels to normal levels. The changes in tolerance were attributed to increasing complexity of the embryo that allowed for more buffering against the acidosis caused by high levels of dissolved $CO_2$ (Taylor and Kreutziger, 1966). Unfortunately, none of these studies examined patterns of embryonic mortality so we do not know whether the age of embryonic mortality also changed.

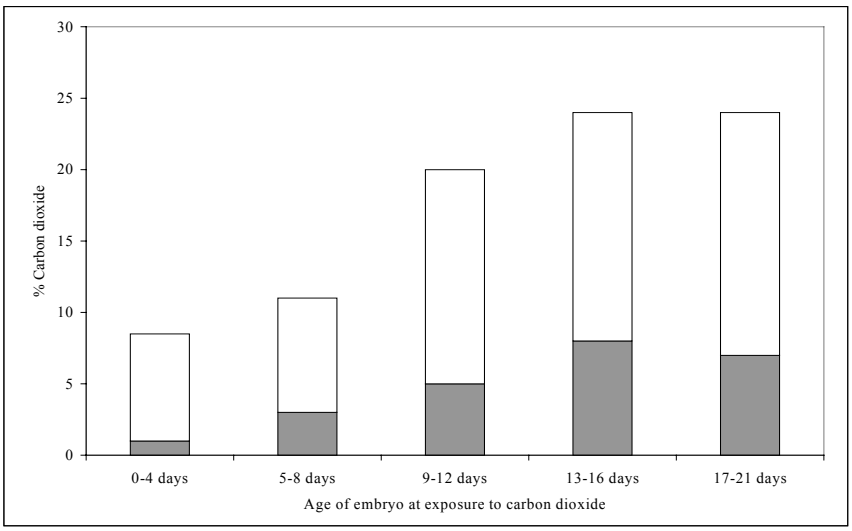


**Figure 1. Effect of age of the fowl embryo on the sensitivity to different levels of carbon dioxide. The top of the grey columns indicate the maximum level of $CO_2$ at which there is no effect on hatchability and the top of the columns indicate the level of $CO_2$ at which there is complete embryonic mortality (data from Taylor *et al.* [1956, 1971] and Taylor and Kreutziger [1965, 1966, 1969]).**

Working in a commercial hatchery Gildersleeve and Boeschen (1983) examined the effects of increasing $pCO_2$ (up to 0.3 or 0.5%) during the first half of single stage incubation of turkey eggs. Their results are variable with some instances increasing hatchability and others exhibiting no effect whilst other treatments decreased hatchability. Other than commenting on the pattern of mortality Gildersleeve and Boeschen (1983) had nothing to say about the reasons for changes in hatchability of these turkey eggs. It would have been interesting to see their thoughts on why most of the significant

effects on embryonic mortality were during the last 8 days of incubation yet their treatments were during the first half of incubation.

In past few years it has become easier and cost effective to automatically measure levels of $CO_2$ in the air and so it has become possible to control cabinet aeration on the basis of the actual $CO_2$ in relation to a pre-set level. The concept of this process is that the metabolism of the embryos, in generating $CO_2$, can be used to control the flow of fresh air (and presumably oxygen) into the cabinet. A level of $CO_2$, usually as a % or parts per million, is set at any stage of the incubation programme and if this set point is exceeded the machine opens the damper flaps to allow in more air. Presumably this is seen as a direct response to the respiration of the embryos and so that more oxygen can be supplied and the $CO_2$ can be removed from the cabinet.

Unfortunately, this logic does not make sense. A single stage setter holding 57,600 fowl eggs exhibiting a fertility of 90% has 51,840 fertile eggs. Peak oxygen consumption ($Vo_2$) occurs at 18 days and is around 30 $mlO_2 \cdot hr^{-1}$ (Burton and Tullett, 1983). This equates to a maximum $Vo_2$ of 1.555 $m^3 \cdot hr^{-1}$ and a $Vco_2$ of 1.089 $m^3 \cdot hr^{-1}$. If the setter's damper is half open and allows ~200 $m^3 \cdot hr^{-1}$ of fresh air into the cabinet, then it supplies 42 $m^3 \cdot hr^{-1}$ of oxygen. If respiration is constant then the $\%CO_2$ in the cabinet will be 0.54%. If $CO_2$ set point is 0.50% then machine will open damper by a minimum of 5% to 230 $m^3 \cdot hr^{-1}$ and the $CO_2$ level will drop to 0.47%. Other than lowering the $pCO_2$ what does opening the damper achieve?

Presumably the greater rate of aeration is required to supply more oxygen to match the needs of the embryos? However, as is shown above at 50% open the machine is being supplied with ~27 times the oxygen the embryos require before the damper opens. How can $CO_2$ level, which is supposed to indicate the metabolism of the embryos, be used to increase (or decrease) air supply to provide oxygen if the system is already being supplied with excess $O_2$ in the first instance? So what is point of $CO_2$ control of aeration? Lourens *et al.* (2006a) also doubt into the use of $pCO_2$ for controlling incubation following their work on the metabolic responses of embryos to short term changes in temperature (± 1º).

In practical terms this system of controlling aeration of the cabinet can have problems. Firstly, it cannot be always assumed that the $CO_2$ sensor is accurate and this questions the sense of relying on the system to control aeration of the cabinet. Many single stage programmes allow the damper to open in response to $CO_2$ levels at or above the set point and provide excessive amounts of fresh air that destabilise the thermal and hydric environment. This leads to reduced incubation efficiency and increased running costs. Excessive cold air reduces the accuracy of temperature sensing and control of the eggs even if the display exhibits no problem.

Owen (1991) showed that introduction of fresh air into an incubator has a greater role in controlling egg temperature and was important in controlling humidity within the cabinet. Aeration has many roles and so it is a much better approach to understand what the air requirements of the eggs are and to set the damper position to provide these conditions. The maximum ventilation rate suggested by Owen (1991) is 3.4 $m^3 \cdot hr^{-1}$ per 1,000 fowl eggs. With an understanding of how embryos grow it is possible to provide an aeration programme that matches the growth pattern of the embryos, which is the same as their $Vo_2$ (see Janke *et al.*, 2004 for data on strains of broiler eggs). In this way the damper position can be automatically opened as incubation proceeds to provide sufficient oxygen and fresh air for cooling.

## Constant eggshell temperatures

Egg temperature has long been recognised as being crucial in maximising hatchability and chick quality. Direct monitoring of the temperature of a developing egg has long been difficult to achieve outside of a laboratory environment. Whilst thermisters attached to the eggshell surface and linked to data loggers hare been available for many years they were expensive and not in wide use. They were primarily used to simply monitor egg temperature in situations when incubator operation was considered to be at fault. They revealed that fertile, living eggs had different temperature profiles to infertile eggs. Inserting a temperature probe into living egg to monitor its temperature is not possible because the embryo is killed and the egg reverts to a temperature profile comparable to an infertile egg.

Development of technology that could measure surface temperatures based on infrared radiation allowed eggshell surface temperature to be measured using thermometers originally designed for determining human ear temperatures. Such methods began to allow incubationists working in hatcheries to monitor shell temperatures and to adjust the single stage programmes to improve the thermal environment of the eggs. Based on the work of Meijerhof and van Beek (1993) a temperature of 100ºF (37.8ºC) has been adopted in the broiler industry as being a level at which eggs should be kept throughout incubation (Meijerhof, 2003). These two innovations have recently been combined with the development of a system of continuously monitoring eggshell temperature using IR thermometers during incubation and using this data to control eggs temperature during single stage incubation. It is now possible to allow the eggs to control the heating and cooling within the cabinet to maintain a set point temperature for the eggs rather than the air.

This development certainly assists in the development of single stage programmes that are better matched to the requirements of the egg. This begs the question of what are the requirements of the egg? It is known that the temperature of the egg and the air around it does not coincide. During the first half of incubation evaporation of water within the egg and its subsequent loss across the shell can lower egg temperature, albeit slightly. During the second half of development metabolic heat production can raise egg temperature well above that of the air. French (1997) showed that the temperature of turkey eggs in single stage incubation increased from the middle of incubation to a plateau level some 0.5ºC higher than in infertile eggs. Chick quality was not affected by this situation. Similar effects are seen in broiler chicken eggs, where strain mat also be important (Boerjan, personal communication), and Pekin duck eggs (personal observations). Therefore, egg temperature can exhibit a limited increase without any obvious detrimental effect but if the rise temperature is too great there will be deleterious effects on the embryos.

This is at odds with the concept of a uniform egg temperature during incubation and the production of good quality chicks (Meijerhof, 2003; Lourens *et al*., 2006b; Joseph *et al*., 2006). The assumption made by such a concept is that any rise in egg temperature during the second half of incubation is detrimental to embryonic development. However, whilst embryonic development occurs between 37–38ºC the body temperature of the adult birds is around 40.5ºC (McNab, 1966; Prinzinger *et al*., 1991; Rahn, 1991). Any attempt to incubate eggs at such temperatures are doomed to fail (see Romanoff and Romanoff, 1972) but certainly neonatal birds have to develop not only an ability to thermoregulate but at a much higher temperature than they have been previously been used to. This itself is an unusual circumstance – why is developmental temperature so low?

The answer may lie in part in the dynamics of contact incubation in a nest. Eggs receive heat energy from a brood patch that, whilst lower than core body temperature (Deeming, 2006), is still warmer than developmental temperature. However, this heat is lost to the nest and its surroundings and so the embryonic temperature that can be maintained by a sitting bird appears to be around the levels that are required in artificial incubation. Evidence from this perspective comes from a new type of incubator that uses a plastic bubble filled with warm air resting on the top of the eggs to warm the eggs. Deeming and Riches (2004) showed that for red-legged partridge (*Alectoris rufa*) eggs normal hatchability and incubation periods comparable to a force-draught set at 37.5ºC could only be achieved if the bubble temperature was at 40.5ºC. Lower bubble temperatures were unable to maintain normal development.

It is understood that incubation at high temperatures (*e.g.* 38.5ºC) can have detrimental effects on hatchability and chick quality (French, 1994, 2000; Lourens *et al*., 2005) but there is no evidence to suggest that a *limited* rise in egg temperature is deleterious. The possibility exists that the rise in egg temperature actually helps to pre-adapt the near-term embryo to a body temperature closer to one that it will adopt as a neonate. Lourens *et al*. (2005) report the rectal temperatures of day-old broiler chicks to be between 38.8–40.2ºC and by 7 days of age rectal temperatures are between 40.0–40.3ºC. Whilst maintaining a single egg temperature during incubation may yield high numbers of good quality chicks the possibility exists that an increased temperature during the latter stages of development may be advantageous. Moreover, domestic fowl and turkeys are less well developed than waterfowl at hatching and this may be important in the thermal requirements of these species towards the end of incubation. Technological developments that allow monitoring and control of eggshell temperature provide an ideal opportunity to investigate whether a uniform egg temperature is actually as important as is being suggested.

## Conclusion

Single stage incubation certainly has many advantages but it relies on a good understanding of the process of development within the egg and the incubator. Technological developments over the past 10 years has allowed us to improve our understanding of development of modern strains of poultry but we have a way to go yet. The value of adjusting the incubation environment based on carbon dioxide levels is questionable because it is likely that typical levels of $CO_2$ in a incubator are not deleterious, and increasing aeration of a cabinet in response to high $CO_2$ does not match the levels of oxygen already being supplied. Monitoring of eggshell temperature during incubation has potential for improving results but we first need to better understand the thermal requirements of embryos. Incubation and embryonic development continues to throw up new ideas and further research is a priority to help us understand the best way to operate single stage incubators. Such ideas will then need to be applied in practise through training of hatchery staff and perhaps development of new techniques and technologies.

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