Effects of temperature and CO\textsubscript{2} during late incubation on broiler chicken development

It was previously found that a high EST of 38.9°C in the second or third week of incubation resulted in higher third week embryonic mortality, lower hatchability, and lower chick quality at hatch, expressed as a shorter chick length and lower yolk free body mass (YFBM) compared to a constant EST of 37.8°C throughout incubation. These negative effects of a high EST in the second part of incubation might be explained by a higher metabolic rate, which is the energy expenditure of the embryos, due to the high incubation temperature. It appears that at a high EST the balance between metabolic rate and O\textsubscript{2} availability is disturbed, which may result in higher embryonic mortality and impaired embryonic development. Lowering EST might restore the balance between metabolic rate and O\textsubscript{2} availability and may postpone or even prevent the embryos from experiencing O\textsubscript{2} shortage as incubation progresses.

What is the importance of temperature during incubation?

During incubation, temperature drives chicken embryonic metabolism, development, and growth. Incubation conditions need to be adjusted to meet embryonic requirements to obtain optimal chick quality and hatchability. To obtain optimal chick quality, embryo temperature, rather than incubator temperature, needs to be controlled. Eggshell temperature (EST) can be used as a non-invasive method to determine embryo temperature.
What is the importance of CO₂ during incubation?
Besides temperature, the CO₂ concentration during incubation seems to affect embryonic development. Several studies suggest potential effects of different CO₂ concentrations applied during week of incubation on embryonic development, hatching parameters, changes in acid-base balance, physiological responses of broiler and layer embryos, and post-hatch growth of broiler chicks. In the current thesis the effect of CO₂ and the interaction with EST was investigated during the hatching phase to investigate which of the two factors had the largest effect on embryonic development.

Aims of the thesis
Based on the potential effects of (lower) EST during the last week of incubation and of CO₂ during only the hatching phase, as described above, the following three aims were derived for this thesis:

1. To investigate effects of EST during the last phase of the incubation process, with special attention for EST below the general accepted optimal EST of 37.8°C.
2. To examine from which day of the incubation process onward EST should be changed from 37.8°C.
3. To investigate whether CO₂ concentrations are interacting with EST during the hatcher phase.

Materials and methods
In the first experiment, 600 Ross 308 eggs were individually monitored from embryonic day (E) 19 until hatch. Effects of an EST of 36.7, 37.8, and 38.9°C in combination with a CO₂ concentration of 2,000 or 10,000 ppm were investigated.

In the second experiment, 2,870 Ross 308 eggs were individually monitored from E15 until hatch. During that experiment, effects of an EST of 35.6, 36.7, 37.8, and 38.9°C starting from E15, E17, or E19 onward were investigated. In addition, 900 chicks were placed in a broiler grow-out facility to investigate effects on first week post-hatch development and growth performance. Both experiments were performed in climate respiration chambers with low air flow, where the climate could carefully be controlled. In both experiments, eggs were selected for an egg weight of 62 to 65 grams.

What is the effect of EST in the hatching phase on chick quality and physiology?
From the first experiment, it was concluded that time until hatch was longer at an EST of 36.7°C compared to 37.8 and 38.9°C, which might be caused by the lower metabolic rate of embryos that were exposed to an EST of 36.7°C. Although time until hatch was prolonged at an EST of 36.7°C, hatch of fertile (HOF) was not affected by EST. A similar result was found in experiment 2, where it was concluded that time until hatch was longer at an EST of 35.6°C, followed by 36.7, 37.8, and 38.9°C. The effect of EST on total incubation duration was mainly caused by the effect of EST on time until
IP, possibly because of the lower metabolic rate at an EST below 37.8°C which reduces O₂ requirement and subsequently ensures that the embryo can use fatty acids as an efficient energy source for a longer period than at a higher EST. Consequently, the energy produced from fatty acids can be used for growth and development, resulting in a higher YFBM at an EST of 36.7°C compared to 38.9°C in experiment 1, and a higher YFBM for an EST of 35.6 and 36.7°C compared to 37.8 and 38.9°C in experiment 2. The higher YFBM at a lower EST may suggest that protein from yolk and albumen is used for growth and development which was supported by the lower residual yolk weight at an EST of 35.6, 36.7, and 37.8°C compared to 38.9°C.

In the first experiment, a high EST of 38.9°C was found to affect chick metabolism, which was demonstrated by the lower sugar stores (glycogen) in the liver at hatch and lower lactate levels (which are a sign of sugar breakdown in the body) at 12h after hatch compared to an EST of 37.8°C. This was probably caused by the increased sugar utilization due to the higher metabolic rate. Sugar is vital to ensure a successful completion of the hatching process. If an increased metabolic rate during embryonic development depletes the glycogen stores, hatching success and subsequent chick quality may be poor.

How did EST affect heart weight?
In the first experiment, a high EST of 38.9°C resulted in a lower relative heart weight at internal pipping (IP), hatch, and 12h after hatch. This implies that a higher EST of 38.9°C compared to an EST of 36.7 or 37.8°C, even applied for a relative short treatment duration up to 38 h, starting from E19 onward, resulted in a lower relative heart weight. In the second experiment, it was found that start day of treatment affected relative heart weight. Differences in relative heart weight between EST treatments increased over time, resulting in an absolute difference of 0.39% at hatch between an EST of 35.6 and 38.9°C applied from E15 onward. The question remains whether this higher relative heart weight at low EST might contribute to an improved performance. Earlier research suggests a relation between a lower hearth weight at hatch found at an EST of 38.9°C and a higher mortality due to Ascites later in life.

From which moment onward should a lower EST be applied?
Although start day affected heart weight at hatch, YFBM and other organ weights were not influenced by start day. It is not yet clear how heart weight affects long term performance and health. In any case, chicks exposed to a lower EST had a later hatch moment, which lengthens the entire incubation process and may complicate the hatchery’s planning. Therefore, it is difficult to conclude which start date is optimal from the current data but it is definitely recommended to lower EST during the hatching phase (from E19 onward) to improve chick quality.
What were the post-hatch effects of a low EST?
An EST of 36.7°C resulted in a higher body weight (BW) and higher carcass weight at day 7 compared to all other EST, and a higher weight gain and gain to feed ratio between day 0 and day 7 compared to 35.6 and 38.9°C. At 7 days post hatch, relative heart weight remained larger at an EST of 35.6°C followed by 36.7, 37.8, and 38.9°C. This suggests that beneficial effects of a lower EST of 36.7°C applied during the last week of incubation found at hatch, might contribute to an enhanced development during the first week post-hatch. However, effects of EST on later performance until slaughter age remain to be studied.

What was the effect of CO\textsubscript{2} in the hatching phase on chick development and quality?
Effects of CO\textsubscript{2} on chick organ development and chick quality at hatch were marginal. Effects of CO\textsubscript{2} were mainly found before IP on chick weight, residual yolk weight, relative pipping muscle, stomach, intestines, and bursa weight, but effects mostly disappeared afterwards. At hatch, only an effect of CO\textsubscript{2} on relative stomach weight was found and at 12h after hatch on relative intestines and bursa weight. A high CO\textsubscript{2} level of 10,000 ppm resulted in lower blood pH and sugar stores in the liver at IP compared to a low CO\textsubscript{2} level of 2,000 ppm. Interactions between EST and CO\textsubscript{2} were found for several physiological variables at an EST of 36.7 and 37.8°C, but remained absent at an EST of 38.9°C. This suggests that chicks went into ‘survival mode’ at high temperatures and did not have the flexibility to respond to additionally high CO\textsubscript{2} anymore, while they were able to cope at a lower temperature.

Conclusions
Results of this thesis show that an EST below 37.8°C is beneficial for embryo development and growth performance during the first week post-hatch. Heart weight at hatch is highest when low incubation temperatures (<37.8°C) are applied from E15 onward, but it is not yet known whether an EST below 37.8°C leads to improved later life performance. The HatchTech research team will continue to investigate the optimal EST and starting day related to chick quality and long term effects on performance to further discover the optimal incubation conditions for chicken embryos.