Thermal comfort in young broiler chickens (*Gallus gallus domesticus*) inferred from metabolic expenses.

Veronika Karczmarz

LiTH-IFM- Ex—14/2876--SE

Supervisor: Jordi Altimiras, IFM Biology, Linköpings universitet
Examinator: Anders Hargeby, IFM Biology, Linköpings universitet
Title/Title: Thermal comfort in young broiler chickens (\textit{Gallus gallus domesticus}) inferred from metabolic expenses

Författare/Author: Veronika Karczmarz

Sammanfattning/Abstract:
The thermoneutral zone (TNZ) curve lies between the ambient temperatures ($T_a$) where an endothermic animal uses the least amount of energy to maintain a balance between the heat production from the animal’s own metabolism and the heat lost to the environment. If the animal is exposed to $T_a$’s over the upper critical temperature (UCT), which is the highest temperature that is still in the TNZ, the animals have to use energy to cool down. If they are exposed to temperatures lower than the lower critical temperature (LCT), which is the lowest temperature that is still in the TNZ, the animal have to use energy to warm up. In the present study oxygen consumption was measured at different $T_a$’s to determine the TNZ in two and three week old broiler chickens (\textit{Gallus gallus domesticus}). Two different protocols were used and compared, a pseudorandom protocol in which chickens were exposed to seven temperatures in two hour periods for each run and a more typical progressive protocol in which $T_a$ decreased gradually, one or two degrees per hour. The TNZ in two weeks old chickens was between 30.7 °C- 36.4 °C and between 28.8 °C- 32.7 °C in the three weeks old chickens. In chickens the TNZ shifts remarkably during the first few weeks of life towards lower temperatures as the animals acquire thermoregulatory competence. The method with a pseudorandom protocol takes more factors, like activity, into consideration than a typical progressive protocol.

Nyckelord/Keyword: Oxygen consumption, Thermoneutral zone, Young broiler chickens, Random ambient temperatures, $\text{msVO}_2$. 
## Contents

1 Abstract .................................................................................................................. 3

2 Introduction ............................................................................................................. 3

3 Material & methods ............................................................................................... 5

3.1 Housing for the chickens .................................................................................... 5

3.2 Random temperature protocol: part I ................................................................. 5

3.2.1 Programming of the climatic chamber ............................................................. 6

3.2.2 The experiment and handling of the chickens ............................................... 6

3.2.3 Handling of chickens after climatic chamber .............................................. 7

3.3 Random temperature protocol: part II ............................................................... 7

3.4 Gradually decreasing temperature protocol ....................................................... 8

3.5 Data analysis ...................................................................................................... 8

4 Results ................................................................................................................... 8

4.1 Thermoneutral zone ........................................................................................... 9

5 Discussion .............................................................................................................. 11

5.1 Social and ethical aspects ................................................................................... 18

5.1.1 Social aspects ................................................................................................ 18

5.1.2 Ethical aspects ............................................................................................... 19

5.2 Conclusions ....................................................................................................... 19

6 Acknowledgement .................................................................................................. 19

7 References ............................................................................................................. 20
1 Abstract
The thermoneutral zone (TNZ) curve lies between the ambient temperatures (Tₐ) where an endothermic animal uses the least amount of energy to maintain a balance between the heat production from the animal’s own metabolism and the heat lost to the environment. If the animal is exposed to Tₐ’s over the upper critical temperature (UCT), which is the highest temperature that is still in the TNZ, the animals have to use energy to cool down. If they are exposed to temperatures lower than the lower critical temperature (LCT), which is the lowest temperature that is still in the TNZ, the animal have to use energy to warm up. In the present study oxygen consumption was measured at different Tₐ’s to determine the TNZ in two and three week old broiler chickens (Gallus gallus domesticus). Two different protocols were used and compared, a pseudorandom protocol in which chickens were exposed to seven temperatures in two hour periods for each run and a more typical progressive protocol in which Tₐ decreased gradually, one or two degrees per hour. The TNZ in two weeks old chickens was between 30.7 °C - 36.4 °C and between 28.8 °C- 32.7 °C in the three weeks old chickens. In chickens the TNZ shifts remarkably during the first few weeks of life towards lower temperatures as the animals acquire thermoregulatory competence. The method with a pseudorandom protocol takes more factors, like activity, into consideration than a typical progressive protocol.

2 Introduction
All endothermic animals have different temperature intervals where they use the least amount of energy, this is called the thermoneutral zone (TNZ). In the TNZ the heat production from the metabolism and the loss of heat to the environment are at an equilibrium and the animal do not need to use energy to maintain the balance. The balance is maintained through conductance. The animal use the skin and blood vessels to either let heat out to the ambient air or to absorb the heat from the surroundings. The veins and arteries then either vasoconstrict or vasodilate. Through this the blood is regulated. If it is warm the arteries and veins vasodilate to let the heat out into the ambient air through the skin. When it is cold the arteries and veins vasoconstricts to keep the blood away from the skin surface so that less heat is released into the ambient air. The blood can then warm the body. Due to this the skin can work both as a radiator and an insulator (Porter, Kearney 2009). When the ambient temperature (Tₐ) raises either above or drops below the TNZ the animal have to use more energy to cool themselves down or to warm themselves up (UK Ag 2014). When exposed to cold ambient temperatures which are below the lower critical
temperature (LCT) chickens start to shiver (contraction of muscles) to produce heat. Different mechanisms in the body also starts, for example combustion of fats in the body to produce heat and energy. When chickens are together they tend to lie beside each other to keep warm. If the chicken is exposed to ambient temperatures over the upper critical temperature (UCT) instead they start panting to cool themselves down (Barott, Pringle 1946). This contributes to higher oxygen consumption (Hillenius, Ruben 2004).

The body temperature ($T_b$) of a full grown chicken normally lies between 40.5 °C- 41.7 °C (Daghir 2008, Rajkumar et al. 2011, UK Ag 2014) while a newly hatched chicken has a body temperature of 39.7 °C (UK Ag 2014). A newly hatched chicken’s body temperature increases a bit each day until three weeks of age when the body temperature becomes stable. If the ambient temperature should decrease or rise above the thermoneutral zone the body temperature of the chicken would become higher than normal (UK Ag 2014).

The TNZ for a full grown chicken is at around 18 °C -24 °C (UK Ag 2014), but less is known about the TNZ for younger chickens. Chickens are more ectothermic when newly hatched (Seebacher et al. 2006) and have a harder time thermoregulating than adult chickens, which contributes to that a newly hatched chicken needs a higher ambient temperature around them. Different methods have been used to determine the TNZ in younger chickens as well as full grown. Meltzer (1983) exposed the chickens to ambient temperatures that either gradually decreased or increased. The chickens were exposed to the gradual change in temperatures for 0.5 - 1 hour depending on the age of the chickens. Others used the method of not changing the temperatures but instead keep the chickens for a longer period of time at the same temperature. Misson (1982) kept 10, one week old chickens for 2 hours at one ambient temperature. This procedure was executed on different ambient temperatures. Misson (1982) studied where starved chickens as opposed to fed chickens TNZ was located. Some other scientists who also exposed the chickens to one ambient temperature for a long time was Barott and Pringle (1946). They used chickens of different ages and exposed them to one temperature for 24 hours. All these methods have been executed during the day, which is not the case in the present study. In the present study a novel method is used to determine the TNZ.

The aim of present study is to find out where the thermoneutral zone is in both two and three weeks old broiler chickens (Gallus gallus domesticus), and to see if there is any difference in TNZ between the two ages. The second aim is to see if the novel method, with a random temperature
protocol, used in this study can be used to determine where the TNZ is located in two and three week old broiler chickens.

3 Material & methods
The present study was executed on 36, two week old (between 14-18 days) broiler chickens (*Gallus gallus domesticus*) and 20, three week old (between 21-25 days) broiler chickens. Both male and female chickens were used but gender was excluded as a factor because no significant differences were found. Newly hatched chickens were obtained from SweHatch AB, Väderstad. The chickens were sacrificed at the end of the study. The study was executed according to ethical permit Dnr. 9-13.

Two different protocols were used, one with temperatures shifting pseudo randomly between high, medium and low temperatures (executed on two and three week old broiler chickens). After a high or low temperature for two hours the ambient temperature always returned to a middle temperature so that there would not be a too big fluctuation between the temperatures. For instance, it could have been bad from both a welfare and a health perspective if the temperature would have dropped from 39 to 18 °C or vice versa. That is why returning to a middle temperature within the expected TNZ was important. The other protocol used consisted of a progressive decrease in ambient temperatures from a middle temperature towards lower temperatures (executed on two week old broiler chickens). The two protocols are explained in more detail below.

3.1 Housing for the chickens
All the chickens were kept in an enclosure (180cm x 85cm) with cardboard flooring and with wood shavings over the flooring. A heating lamp kept the temperature in the enclosure at slightly over 30 °C but the animals could choose lower temperatures by moving away from the heating lamp. They also had *ad libitum* access to food and water. The lights in the enclosure were programed to be on from 06.00-18.00 so the chickens would get a normal circadian rhythm.

3.2 Random temperature protocol: part I
The first part of the experiment was executed on 28, two weeks old chickens with a random chosen temperature protocol. The animals to be measured were chosen based on body mass so the largest chickens in each batch of 20 individuals were run first in order to decrease size variation that could directly increase the variation in oxygen consumption. Each day the test was executed on four chickens (n=4).
3.2.1 Programing of the climatic chamber

Before the experiments each day the climatic chamber (RUMED, Germany) was programed to switch between different temperatures from 17.00 until 07.00 the next morning, (during which time the experiment was executed) with an interval of two hours at each temperature. The climatic chamber switched between seven temperatures during one run. The different temperatures for each run/day were determined pseudo randomly. The climatic chamber was programed each day with the same structure of the temperatures (four middle temperatures and three high/low temperatures). The first and last temperature during a run was always a middle temperature of either 27 °C or 30 °C. Because of this every other temperature during the run was a middle temperature (27 °C or 30 °C) with a high (33 °C, 36 °C or 39 °C) or low temperature (18 °C, 21 °C or 24 °C) in between.

Not all the temperatures were included during one test run, but all temperatures were included each week. Each of the high and low temperatures were in two of the runs each week, while there was always two middle temperatures in each run (eight times a week). Because there were seven intervals and three of them were a high or low temperature, each run had either two low temperatures and one high temperature or two high temperatures and one low temperature. The random temperature protocol was used to take the activity during different times into consideration. Different chickens were then exposed to the same temperature but during a different time of the run. This is what made the protocol pseudo random.

3.2.2 The experiment and handling of the chickens

On the test days, Tuesday- Saturday, four chickens were firstly weighted and then put alone in a respiratory chamber. The chamber used for the experiment had a volume of three liters (18.5 cm in diameter x 17.5 cm in height) and the lid was equipped with an in and out port for the air. The four chambers with the chickens inside were closed tightly to prevent leaks. The chambers were then placed in the climatic chamber and the inlet and outlet ports connected. Two tubes, one to let the air flow in and the other to let the air and CO₂ out, were connected to the ports in the lid so that the air could go around in the chamber (an open circuit system). The chickens had no access to food or water in the chamber and they were kept in complete darkness. The experiments were performed during the night to reduce the activity of the chickens and to get a basal metabolic rate. During the night the chickens do not normally eat or drink which did not make the lack of
food and water a big stress factor. The chickens were asleep or resting most of the time during the experiment.

To measure how much oxygen consumption each chicken used at the different temperatures throughout the run the gas tubes were connected to a FoxBox- C, field gas analysis system (Sable systems, Las Vegas, USA, S/N FX2C-1201-21). A custom built computer program was used to analyze oxygen consumption from all chambers in a permanent loop. The measurement of each metabolic chamber (lasting 120 s) was alternated with a measurement of baseline air (lasting 90 s). This procedure was carried out until 07.00 the next morning. To regulate the air flow to the chambers, an electromagnetic piston pump (Denmark) provided an air flow which was subsequently regulated by a FB8 flow measurement system (Sable systems, Las Vegas, USA) to control that all the chambers got the same amount of air flow. The air flow was set to around 1000 ml min\(^{-1}\). To the lid a thermometer was also plugged in through a hole to measure the real ambient temperature in the chamber during the experiment.

Four new chickens were used each day. On Saturdays the chickens were only taken out from the climatic chamber and handled as described.

3.2.3 Handling of chickens after climatic chamber

After the chickens had been in the climatic chamber for 14 hours (17.00-07.00) they were taken out and returned to their holding enclosure. There they were weighted again to see how much weight they had lost during the 14 hours. The chicken’s feces were also weighted by first weighing the whole chamber (without the lid) with the feces and then subtracting the weight of the chamber after it had been cleaned. After the weighing, the chickens were put back in the enclosure. All the weights were taken to determine how much the chickens had lost in body mass and how much water was lost through breathing during the experiment.

3.3 Random temperature protocol: part II

The same protocol (random temperatures) as in the first weeks of the experiment was then executed on 20, three weeks old broiler chickens. Part II of the experiment was executed in its whole in the same way as part I except from some changed details. The temperatures used in part II of the experiment were different. The middle temperatures were 24 °C, 26 °C, 28 °C and 30 °C. The higher temperatures used were 32 °C, 34 °C and 36 °C, and the lower temperatures used were 22 °C, 20 °C, 18 °C and 16 °C. Because the chickens were twice as big as when they were two weeks old the chambers used in part II of the experiment had a volume of six liters.
(18.5 cm in diameter x 26.5 cm in height). The flow through the chamber was also changed to a higher flow (2000-3500 ml min\(^{-1}\)) due to the increase in chamber volume and VO\(_2\). During each run the flow was increased with 1000 ml min\(^{-1}\). The two last runs had a flow at around 3500 ml min\(^{-1}\) (the highest flow that was able to achieve through the electromagnetic piston pump used). Humidity was measured with an iButton (Maxim Integrated, U.S) in each chamber.

3.4 Gradually decreasing temperature protocol
During the fourth week a different protocol was used to change the temperatures in the climatic chamber with a gradual decrease of 1 °C or 2 °C every hour from 32 °C (which was a temperature that was determined to be in the TNZ from the results from previous protocol) to 18 °C (from 17.00-07.00) instead of the random temperature protocol used before. The gradual decrease protocol was used on 8, two weeks old broiler chickens. Except for the programing of the climatic chamber the procedure of the test was done exactly the same, just with another temperature protocol.

3.5 Data analysis
The data was analyzed through a one way ANOVA to see if there was any difference in msVO\(_2\) between the different T\(_a\) in both the two week old chickens and the three weeks old chickens. A Tukey post hoc comparison test was then made to see where the difference in msVO\(_2\) was. From the results a TNZ could be determined for the two week old and the three week old broiler chickens. The analysis was executed in Minitab17.

4 Results
During the experiment the chickens were weighted before and after each test (Table 1). The chickens decreased in body mass during the test. The two weeks old broiler chickens lost a mean value of 31.4 g ± 5.3 g from their initial body mass, where 15.9 g ± 4.5 g of the loss was feces and the water loss was 6 % from the total body weight. In the three weeks old broiler chickens the decrease in initial mean body mass was 43.9 g ± 7.5 g, where 20.8 g ± 4.9 g stood for the weight loss due to feces and 4.5 % stood for the loss in water (Table 1). The weight was taken into account when analyzing the VO\(_2\) and a mass specific VO\(_2\) (msVO\(_2\)) was calculated and used for the analysis of all the data.
Table 1. Mean weights ± standard deviations and mean loss in body mass and water loss in two and three week old broiler chickens.

<table>
<thead>
<tr>
<th>Age</th>
<th>2 weeks</th>
<th>3 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body mass before test (g)</td>
<td>275.7 ± 30.9</td>
<td>536.1 ± 70.2</td>
</tr>
<tr>
<td>Body mass after test (g)</td>
<td>244.2 ± 28.8</td>
<td>492.2 ± 66.6</td>
</tr>
<tr>
<td>Total body mass loss (%)</td>
<td>12.9</td>
<td>8.9</td>
</tr>
<tr>
<td>Water loss (%)</td>
<td>6</td>
<td>4.5</td>
</tr>
</tbody>
</table>

4.1 Thermoneutral zone
To determine the TNZ in both two and three weeks old broiler chickens each chicken had their mass specific oxygen consumption (msVO₂) tested during different pseudo randomly chosen ambient temperatures (Figure 1a & 1b).

Figure 1a&b. Mass specific metabolic rate for two week old broiler chickens (n=28) at different Tₐ (18 °C - 39 °C; Figure 1a) and for three week old broiler chickens (n=20) at different ambient temperatures (16 °C - 37 °C; Figure 1b).
There was a significant difference (P=0.000; F=60.47; Figure 2a) between the mean msVO$_2$ (ml h$^{-1}$ g$^{-1}$) that were achieved during the different $T_a$ in the two week old chickens. From the analysis the TNZ was determined to be between 30.7 °C - 36.4 °C in the two weeks old broiler chickens (Figure 2a). The LCT lies at ~30.7 °C and the UCT at ~36.4 °C. In the three weeks old broiler chickens there was a significant difference (P=0.000; F=53.91; Figure 2b) between the mean msVO$_2$ (ml h$^{-1}$ g$^{-1}$). This result indicates a statistical significance between 28.8 °C - 32.7 °C which indicates a TNZ with the LCT at ~28.8 °C and the UCT at ~32.7 °C (Figure 2b). Figure 2c has the mean mass specific metabolic rate curve from both the two weeks old and the three weeks old chickens and indicates a shift in the TNZ towards lower $T_a$ in the three weeks old chickens.

Figure 2a&b. Mean mass specific metabolic rate ± standard deviations for two week old broiler chickens (n=28). With a LCT at ~30.7 °C and an UCT at ~36.4 °C. The orange line indicates at which $T_a$'s the TNZ is located (Figure 2a).

Figure 2b. Mean mass specific metabolic rate ± standard deviations for three week old broiler chickens (n=20). With a LCT at ~28.8 °C and an UCT at ~32.7 °C. The blue line indicates between which $T_a$'s the TNZ is located.
Figure 2c. The mean mass specific metabolic rate for the two (14-18d) and the three weeks (21-25d) old chickens. There is a shift towards lower temperatures in the three weeks old chickens.

The results from the gradual decreasing temperature protocol show a LCT at 28.6 °C. They also show a significant difference (P=0.000; F=22.99; Figure 3) in msVO$_2$ (ml h$^{-1}$ g$^{-1}$) during the different $T_a$.

Figure 3. Mean mass specific metabolic rate ± standard deviations for two week old broiler chickens (n=8). LCT at ~28.6 °C. Results from the decreasing temperature protocol. The dotted line shows the results from the random $T_a$ protocol for comparison purposes (Figure 2a).

5 Discussion

During the night when chickens do not eat there is a natural decrease in body weight. The decrease in body weight is due to different factors, e.g. feces, evaporation of water while breathing/panting and food being processed. During the experiment the chickens decreased in body size due to these factors. When exposed to the higher ambient temperatures, which were not within the TNZ, the chickens had to start panting to cool themselves down. This may have led to a bigger water loss than if they would have been exposed to temperatures only within the TNZ. In the
present study, the mean water loss was 6% in the two weeks old chickens and 4.5% in the three weeks old chickens. The water loss stood for almost half of the decrease in body mass while the other half was due to feces. As seen in Table 1 in the results both the decrease in body weight and the decrease due to water is bigger in the two week old chickens. The two week old chickens were exposed to higher temperatures than the older chickens but they did also have a higher TNZ. The older chickens were exposed to more temperatures above their TNZ and should therefore have lost more water due to panting than the younger chickens. This is however not the case. The younger chickens are less competent thermoregulators and therefore lost more water and due to that more weight than the older chickens did.

Barott and Pringle (1946) showed that when the chickens get older they start to have a lower msVO₂ than the younger chickens at the same temperatures. This can also be seen in present study, where the three weeks old chickens have a lower msVO₂ than the chickens who are two weeks old. These results indicates that when the chickens grow older and bigger they do not take up as much oxygen per gram as when they were smaller. This can be due to better thermoregulation skills or just an allometric effect. Weight normally scales to VO₂ with a mass exponent of 3/4 or 2/3 and do not scale linear with a mass exponent of 1. To determine if the msVO₂ is lower in the three weeks old chickens due to an allometric effect the equation (1) was used.

\[
msVO₂ = A \times (\text{Body mass})^b
\]  

The b was either a mass exponent of 2/3 or 3/4 and the A was calculated through the msVO₂ from the two week old chickens. A theoretical msVO₂ was calculated for the three weeks old chickens (msVO₂ ≥ 2.2 ml h⁻¹ g⁻¹). This value is the msVO₂ it should have been if it had been due to an allometric effect. The value calculated was much higher than the real msVO₂ (1.2 ml h⁻¹ g⁻¹). This indicates that the msVO₂ is not lower in the three week old chickens due to an allometric effect but maybe due to better thermoregulation skills. Barott and Pringle (1946) also showed that during the first few days and until two weeks of age there was quit a big increase in msVO₂. The increase in msVO₂ got smaller when the chickens grew older when reaching the ambient temperatures which were not in the TNZ. This is evidence that older chickens are better at thermoregulation than younger chickens.

In the results from the present study the TNZ for two weeks old chickens is between 30.7 °C - 36.4 °C. Barott and Pringle (1946) found that the
chickens had the smallest msVO$_2$ at 95 °F (35 °C) in both the chickens who were only a couple of days old and up to two weeks of age. Barott and Pringles (1946) results fits in the TNZ found in present study for the two weeks old chickens but not as a LCT (Table 2). When the chickens got older the lowest msVO$_2$ also started to shift towards lower ambient temperatures (Barott, Pringle 1946). This can also be seen in the three weeks old chickens in present study. Their TNZ is between 28.8 °C- 32.7 °C which is lower than the TNZ for the two weeks old chickens. Newly hatched chickens should have their lowest msVO$_2$ at a higher temperature than a two week old chicken, as shown by Barott and Pringle (1946), because it is during the first three weeks the TNZ changes the most. Meltzer (1983) studied the TNZ during different ages in chickens. During day zero the chickens had a TNZ between 35 °C- 37 °C. During day seven they had a TNZ between 32 °C- 35 °C and at the age of 14 days the TNZ was between 29.5 °C-33 °C. These results indicate that during the first two weeks the TNZ shifts towards lower temperatures quite a bit. The chickens do not have the same msVO$_2$ at the same temperature when newly hatched until two weeks of age either.

In the adult tinamou (N. ornata) which is a marginally larger bird than the broiler chicken the TNZ is between 25 °C- 35 °C (which is a larger temperature interval than for the broiler chicken). The msVO$_2$ is also lower and lies between 0.5- 1.3 ml h$^{-1}$ g$^{-1}$ in the tinamou (Ekström 2012) while for the broiler chickens in the present study the msVO$_2$ was between 1.2- 2 ml h$^{-1}$ g$^{-1}$ for the three week old chickens and between 1.4- 2.5 ml h$^{-1}$ g$^{-1}$ for the two week old chickens. From this we can see that when the bird is bigger the msVO$_2$ is lower. This is also true according to Barott and Pringle (1946). Wiersma et al. (2007) looked at the msVO$_2$ in tropical birds and compared it with the msVO$_2$ from temperate birds. They concluded that the tropical birds have a lower msVO$_2$ than the temperate birds. This may also be why the msVO$_2$ is lower in the tinamou than in the broiler chickens (which are temperate birds). The tinamou does not exactly qualify as a tropical bird because they live at high altitudes in the Andes, but they are more tropical than the broiler chicken. The tinamou do also have a small heart in ratio to its body, this does also have an effect on their oxygen consumption. However the msVO$_2$ also changes with age in the broiler chicken which is both proven in the present study and Barott and Pringle (1946).

Meltzer (1983) developed some equations to be able to calculate the LCT dependent on which weight the chickens had or which age they were. The broiler chickens used by Meltzer (1983) were of the Anak local strain. He
also had equations depending if the chicken was male or female. The equation to calculate the LCT for 1-21 days old chickens is;

\[ y = 34.2 - 0.32 \times x \]  (2)

(x is the chicken’s age in days). If this would be applied for a two week old chicken they would have a LCT at 29.7 °C which is between the two LCT’s found in present study (28.6 °C from the gradually decreasing temperature protocol and 30.7 °C from the random temperature protocol). Meltzer (1983) also had another equation for 14-63 day old chickens;

\[ y = 49.4 \times x^{-0.194} \]  (3)

From this equation the LCT would be 29.6 °C for the two weeks old chickens, which is just 0.1 °C less than the results from previous equation. For the three weeks old chickens the LCT would be 27.5 °C in the first equation and 27.4 °C in the second equation. These results are both 1 °C under the LCT found in present study.

There is also an equation dependent on weight (100 g-3000 g);

\[ y = 62.15 \times W^{-0.135} \]  (4)

(W= weight in grams) (Meltzer 1983). Which for the two weeks old chickens in present study would mean a LCT at 29.1 °C and for the three weeks old chickens a LCT at 26.6 °C. The results from the equation regarding the three weeks old chickens are 2 °C lower than the LCT found in present study (28.6 °C). Although the equations from Meltzer (1983) are not far away from the LCT found in present study they are all lower. When the equations are used for older or chickens with a higher weight the results are even further away from the LCT. These equations can be used to calculate the LCT but are not so trustworthy that they are 100% correct. If these equations are used to determine which ambient temperature should be used in an enclosure for chickens it is better to add approximately 3 °C- 5 °C. Then the temperature should at least be in the TNZ.

Meltzer (1983) had also done tests were he had found out the TNZ for different ages of chickens. For chickens at the age of 14 days (W= females; 250 g, males; 290 g) the TNZ was between 29.5 °C – 33.0 °C. For the chickens at age 21 days (W= females; 430 g, males; 490 g) the TNZ was between 27.5 °C -31.5 °C. Both of these TNZ’s are lower than the ones found in present study. The chickens at the age of 21 days in Meltzer (1983) weighted less than the ones used in present study. This can contribute to the difference in results. Although the chickens that weight the most should have a lower TNZ, which is not the case in this situation.
Table 2. Comparison between the TNZ and LCT from the present study and from other studies which are referred in the discussion.

<table>
<thead>
<tr>
<th>Studies</th>
<th>TNZ 2 weeks (˚C)</th>
<th>TNZ 3 weeks (˚C)</th>
<th>LCT 2 weeks (˚C)</th>
<th>LCT 3 weeks (˚C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Present study, random protocol</td>
<td>30.7 – 36.4</td>
<td>28.8 – 32.7</td>
<td>30.7</td>
<td>28.8</td>
</tr>
<tr>
<td>Present study, decreasing protocol</td>
<td>-</td>
<td>-</td>
<td>28.6</td>
<td>-</td>
</tr>
<tr>
<td>Meltzer (1983)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- calc. (weight)</td>
<td>-</td>
<td>-</td>
<td>29.1</td>
<td>26.6</td>
</tr>
<tr>
<td>- calc. (1-21d)</td>
<td>-</td>
<td>-</td>
<td>29.7</td>
<td>27.5</td>
</tr>
<tr>
<td>- calc. (14-63d)</td>
<td>-</td>
<td>-</td>
<td>29.6</td>
<td>27.4</td>
</tr>
<tr>
<td>- results (age)</td>
<td>29.5 - 33</td>
<td>27.5 - 31.5</td>
<td>29.5</td>
<td>27.5</td>
</tr>
<tr>
<td>Barott and Pringle (1946)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

There are many factors that can affect at which temperature the TNZ is located. We have already seen that weight and thereby age plays a part. But other factors that also take part are humidity, how much food is available, activity and time of the day. Barott and Pringle (1946) measured the msVO₂ during 24 hours at the same ambient temperature and came up with that the msVO₂ was the smallest at 08.00 pm (20.00) and the highest at 08.00 am (08.00). These results can also be dependent on activity since the chickens sleep during night and are active during the day. The oxygen consumption is different in amount depending on if they are active or asleep. This was seen through pilot tests. During the pilot tests for the present study we executed the random protocol during the day. From the results we got VO₂ values that showed almost the same VO₂ values at all the ambient temperatures. When exposed to temperatures between 28 °C-40 °C the chickens did not change their oxygen consumption much even though the temperature were above the TNZ. There was only a small increase in VO₂ in some of the experiment but when reaching the temperatures above the TNZ there was not a big increase. In total the VO₂ only changed with 2 ml h⁻¹ throughout the run while the VO₂ changed with 4 ml h⁻¹ in the random protocol. The chickens were too active during the day so a resting metabolic rate could not be attained. This was taken into consideration when determine the present method used. We concluded that all the methods would be done during the night (17.00-07.00) and that way a resting metabolic rate could be achieved. When using the random temperature protocol the ambient temperatures were distributed pseudo randomly during two hours at different times during the run, with different
animals. For example they could be exposed to 30 °C during 01.00-03.00 during one run and two days later new chickens could be exposed to 30 °C at 17.00-19.00. When using a random protocol, factors as time/activity could be neutralized.

Humidity may also affect the results. When exposed to the higher temperatures the humidity in the chambers can rise due to panting. This should also be taken to consideration by increasing the flow in the chambers or also increasing the volume of the chamber. To be sure that humidity will not affect the experiments pilots should be done so that humidity will not be an issue. The humidity will not affect the TNZ it may only increase the oxygen consumption at the higher ambient temperatures.

The availability of food also affects the TNZ. Misson (1982) found that when chickens are starved they have a higher UCT than when being fed. The UCT in the one week old chickens that were starved was at 36.2 °C, while the UCT was at 34.2 °C for the chickens being fed. There were no difference in LCT between the chickens. The starved chickens did not produce as much heat and the body temperature was lower. This contributed to a lower evaporative heat loss which is concluded by Misson (1982) to be the contributing factor to the higher UCT in the starved chickens.

Two different methods were used in present study to compare the results for the LCT. The first method used was with a random temperature protocol (method I) and got quite similar results as the second method where the temperatures decreased gradually in the climatic chamber (method II). From the method I the LCT determined was 30.7 °C instead of 28.6 °C which was the LCT determined from method II. If looking at Figure 3 in the results we can see that the msVO2 is actually very alike at the different ambient temperature from both of the methods in the experiment. If the experiment would have been made on additional chickens the result may have been more alike, at least near the LCT region. As mentioned before the first method with the random temperature protocol takes more factors into consideration so the results are not that affected by activity as the second method might be. The results of a higher msVO2 during the lower temperatures might have been higher because of the time. The lowest temperatures that differs the most from the random protocol were at the end of the run (04.00-07.00) and as shown by Barott and Pringle (1946) the highest msVO2 was at 08.00 (the increase in msVO2 started at around 24.00-08.00).
In previous studies the methods, to get an msVO₂ for respective ambient temperature, have been different. Misson (1982) had ten chickens in a calorimeter chamber for 2 hours at the same ambient temperature each test. The chicken were then returned to an enclosure after the test were they were starved. The next day the same chickens were tested in the same way again at the same temperature. This procedure was done four times with the same animals at the same temperature. The weight of the chickens was measured both before and after the 2 hour run. Barott and Pringle (1946) put chickens of different ages in a respiration calorimeter at one ambient temperature for 24 hours. The same chickens were exposed to an ambient temperature at every age. The oxygen consumption was measured every 2 hours. The chickens had no access to water or food during that 24 hour period. Meltzer (1983) did not just measure the oxygen consumption at one temperature for each animal. Meltzer measured the oxygen consumption during different ambient temperature during the same run with either a gradual decrease in the temperatures or a gradual increase during 0.5 or 1 hour (depending on the age of the chickens). Four or six temperatures were normally tested on during one run. A refrigerated incubator was used with an open circuit system. All these tests from the different studies have been executed during the day (Barott and Pringle’s (1946) experiments were executed both during the day and night). Because of that the activity of the bird was only considered in the Barott and Pringle (1946) experiment. The time which the chickens were exposed to a temperature also matters. In the experiment executed by Meltzer (1983) the chicken were only exposed to a temperature for a little while before changing to a new temperature. This may not have been enough to actually get the right temperature in the chamber where the chicken was located. During the higher temperatures the chickens can be affected by oxygen debt which can give a wrong VO₂ when exposed to the lower temperatures. The oxygen debt provides a postponed response so that the animal will still need to have a high oxygen consumption, to recover, even though they should have a much lower VO₂ at that temperature. If the time exposed to the temperature the animal may not have time to react properly. This is why we chose to expose the chickens for 2 hours in present study. We wanted to be sure to get a right VO₂ value and expected that 2 hours would be sufficient amount of time for the VO₂ to become stable. According to the results 2 hours seem to have been sufficient to get a reliable VO₂. Because the temperatures were in a pseudo random order in the present study also and more temperatures were used in one run more VO₂ values could be achieved during one run. By making it in a random order we could also normalize so that the VO₂ values were not affected by previous temperatures. Individual differences could also be
excluded. The most important part of the present study was that the experiments were executed during the night to get a resting metabolic rate that was not influenced by activity. The random order of the different ambient temperatures also standardized the activity because the same temperature was used during different times of the run. All the studies used a somewhat similar calorimeter chamber (just with different names) to program which ambient temperature the chickens would be exposed to during which time.

5.1 Social and ethical aspects

5.1.1 Social aspects

The results from this study may be of importance in the meat production industry to increase the welfare of the chickens and also get healthier chickens that grow better without eating as much. They will not need to eat as much if they could save more energy by not needing to thermo regulate.

In the hen production for e.g. Svenskfågel (2011) the temperature in the chicken stable is 30 °C in the beginning when the chickens are hatched and then the temperature decreases gradually to 20 °C while the hens get older and larger. This is also the recommended temperatures for broiler chickens, with the temperature starting at 32 °C -34 °C (which is higher than at Svenskfågel) but then decreasing to 30 °C the first week. The temperatures should then be decreased to 26 °C week two, 22 °C week three and for week four the temperature should be 20 °C. Although to decide the right temperatures some factors should be taken into consideration, e.g. health, humidity, body weight, food intake etc. (Hulzebosch 2006).

The results from present study show that a two week old chicken has a TNZ between 30.7 °C - 36.4 °C which is higher than the recommended $T_a$ of 26 °C. The $T_a$ recommended for a three week old chicken is 22 °C while the TNZ indicated from present study is between 28.8 °C- 32.7 °C. This is also higher than the recommended $T_a$. From these results we can see that the recommendations for at least the two week old and the three week old broiler chickens lies 4 °C- 6 °C below the LCT, which indicates that the chickens may need to thermo regulate much of their time (at least during the night) in the meat production and loses energy. This contributes to the chickens eating more to have more energy than what they would have needed if the temperatures would have been in the thermoneutral zone. However during the day when the chickens are active they may produce heat which can result in warmer air at chicken level. When it comes to temperatures outside the TNZ it is more harmful for the chickens if they are
exposed to temperatures above the TNZ than if they are exposed to temperatures below. The slope for the temperatures above the TNZ is much steeper than for the temperatures below the TNZ. This can be seen in Figure 2a&b (in the results) where the msVO$_2$ rises to higher values immediately after the TNZ instead of more slowly as with the temperatures below the TNZ. If the chicken are exposed to temperatures above the TNZ they could die but they have to be exposed to much lower temperatures than the TNZ to be in any real danger of dying.

5.1.2 Ethical aspects

The present study was done according to ethical permit Dnr. 9-13. The chickens were exposed to low and high temperature which are not in their TNZ, so they had to thermo regulate. During the higher temperature the humidity was a bit too high so this should be taken into consideration to make improvements for further studies. In the present study the temperatures were chosen so that the chickens would not be in any harm, but they were still stressed by the circumstances. After the experiments the chickens were behaving as before the tests when they were back in the enclosure. They just started to eat immediately to get more energy and because they were hungry as they are normally in the morning.

5.2 Conclusions

The first conclusion of this study is that two weeks old chickens has a higher TNZ than three weeks old chickens and that the msVO$_2$ becomes lower with age. This is due to better thermoregulation skills.

The second conclusion is that the method with a random temperature protocol works better than just decreasing the temperatures gradually because it takes more factors into consideration like the time/activity of the chickens.

The third conclusion is that if they would start using temperatures in the thermoneutral zone in the hen production then they would not need to spend as much money on food as they do today and it would contribute to a better welfare for the chickens.

6 Acknowledgement

I would like to thank my supervisor Jordi Altimiras for all the help and the opportunity to do this study. I would also like to thank SweHatch who provided the chickens, without them there would not have been a study to report.
7 References


University of Kentucky (UK Ag); College of Agriculture, Food and Environment (2014) POULTRY PRODUCTION MANUAL, CHAPTER 7 - Ventilation principles. 
http://www2.ca.uky.edu/poultryprofitability/Production_manual/Chapter7_Ventilation_principles/Chapter7_air_temperature.html (accessed 30 March 2014)