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A study of haemoglobin and performance: egg laying, hatching, growth and summit metabolism

Haemoglobin and its functions in various organisms is well known; it increases the ability to obtain precious oxygen and it is crucial in aerobic performance. However, if high values of haemoglobin are always beneficial why is there a large natural variation? This study investigated the effects of varying concentrations of haemoglobin on several stages in the lifespan of Red Junglefowl: egg production and hatching, chicken growth, haemoglobin and summit metabolism. Red Junglefowl were tested for fertility in both eggs laid and eggs hatched. The offspring were individually measured for whole blood haemoglobin concentration and tested for growth and summit metabolism. The results show that there is a difference in haemoglobin after two weeks of age and that growth differs at the same time. High Hb animals do not lay smaller nor fewer eggs than low line birds but their offspring are smaller at the same times as there is a difference in haemoglobin levels. There was also a difference in the summit metabolism between the lines, where the high line animals performed better. Importantly the increase in haemoglobin did effect the growth of the animals negatively, and this would imply that higher levels of haemoglobin are detrimental to growth.

Haemoglobin, fertility, growth, summit metabolism, sliding cold exposure
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1 Abstract

Haemoglobin and its functions in various organisms is well known; it increases the ability to obtain precious oxygen and it is crucial in aerobic performance. However, if high values of haemoglobin are always beneficial, why is there a large natural variation? This study investigated the effects of varying concentrations of haemoglobin on several stages in the lifespan of Red Junglefowl: egg production and hatching, chicken growth, haemoglobin and summit metabolism. Red Junglefowl were tested for fertility in both eggs laid and eggs hatched. The offspring were individually measured for whole blood haemoglobin concentration and tested for growth and summit metabolism. The results show that there is a difference in haemoglobin after two weeks of age and that growth differs at the same time. High Hb animals do not lay smaller nor fewer eggs than low line birds but their offspring are smaller at the same time as there is a difference in haemoglobin levels. There was also a difference in the summit metabolism between the lines, where the high line animals performed better. Importantly the increase in haemoglobin did effect the growth of the animals negatively, and this would imply that higher levels of haemoglobin is not detrimental to growth.

2 Introduction

The aerobic capacity of birds is involved in many processes, one of them being regulation of body heat in response to increased or lowered temperature. Birds thermoregulate by several mechanisms such as increasing their plumage for better insulation, and by shivering (Schwab & Schafer, 1972). Shivering mainly uses the pectoral muscles and like all other muscle activity it is dependent on the oxygen delivered by haemoglobin (Vézina et al., 2006). Aerobic capacity is measured as oxygen consumption. The basal metabolic rate is the rate of which the animal consumes oxygen at rest and the maximal oxygen consumption (VO2 max) is the maximal amount of oxygen an organism can consume. For this report, the focus was on Red Junglefowl, and previous studies have shown a correlation between the basal metabolic rate (BMR) and both sex and body mass in Red Junglefowl (Hammond et al., 2000). Male Junglefowl have a lower BMR than females, but a higher VO2 max (Hammond et al., 2000). VO2 max is furthermore correlated to haematocrit values in females, but not in males (Hammond et al., 2000).

Summit VO2 is the maximum oxygen consumption value induced by cold temperatures in an atmosphere with helium and oxygen (helox) gas mix
(Swanson, Drymalski, & Brown, 1996). Summit VO$_2$ demonstrates the ability for the (endothermic) animal to thermoregulate, or produce heat and it is positively correlated to an animal’s cold resistance (Marsh & Dawson, 1989, as cited by Swanson, Drymalski, & Brown, 1996). There are two commonly performed summit measurements, the sliding cold exposure and the static cold exposure. The sliding cold exposure allows for smaller sample sizes and individual measurements. The animal is exposed to decreasing temperatures until the oxygen consumption plateaus (Swanson et al., 1996). Static cold measurements uses a variety of different temperatures, each being static. Several animals are being exposed to one of the different temperatures, each sample in different chambers, and the number of animals showing hypothermia is noted as a percentage of the total animals for each temperature (Swanson et al., 1996).

Haemoglobin is a protein responsible for oxygen transportation and oxygen delivery in mammals and avians alike. The amount of oxygen delivered to tissues is calculated by the haematocrit value: the percentage of erythrocytes (red blood cells) in full blood volume, and the amount of haemoglobin proteins per surface area of these cells (Kostelecka-Myrcha, 2002). The haematocrit levels in birds vary in response to different ambient temperatures, increased flight demand and even during egg laying (Limosa et al., 2016, Gayathri & Hegde, 2004). An increase in haemoglobin concentration leads to an increase in aerobic capacity (Minias, 2015). Because of this, the total blood concentration of haemoglobin is the best way of assessing a bird’s ability to satisfy its needs for oxygen (Minias, 2015).

Non-laying females have been shown to have higher haematocrit values than females laying eggs, incubating or even nest building. The difference is thought to be caused by the increased stress in laying, the thermogenesis during incubation and the diversion of nutrition from the mother to the egg yolk, called haemodilution (Gayathri & Hegde, 2006). The impact of haemoglobin on the fecundity, the actual reproductive performance of the birds, can be measured by the total amount of eggs laid and the amount of chicks reared. There is a negative correlation between high whole blood concentrations of haemoglobin, egg size (high concentrations of haemoglobin leads to smaller eggs) and amounts of eggs laid (Altimiras, unpublished), and because there is a correlation between the size of the eggs and the hatching success (Krist, 2011) the effect of haemoglobin could impact the fecundity overall.
Animals previously used in Haemoglobin studies at Linköpings University show a high variation in haemoglobin concentrations through many generations, despite them being bred for high and low lines of haemoglobin respectively. This would imply that, within this population, high haemoglobin is not necessarily a fully beneficial trait (Altimiras, personal communication). The assumption for this experiment was that there was a reason for the high variation, that there was a trade-off for the seemingly beneficial increased whole blood haemoglobin concentration.

A common concept in evolutionary biology is that of trade-offs, where a trait that is considered beneficial in one sense could have negative effects in other regards. Several factors have been examined to see if they could be a trade-off that causes the variations. Heart-size was measured, as an increased haemoglobin (and haematocrit) concentration was thought to cause a higher viscosity in the blood. The higher viscosity was thought to put a larger strain on the heart and demand larger cardiomyocytes. There was no difference between the high (high whole blood concentrations of haemoglobin) and the low (low whole blood haemoglobin) lines in this regard, although there was a sexual dimorphism in ventricular mass. Another factor that was measured was the amount of eggs laid and the size of these eggs, to see if haemoglobin could have an impact on the fertility of the birds. Here, the measurements showed that high line animals laid smaller and fewer eggs. Lastly measurements of VO$_2$ summit, oxygen consumption in correlation to temperature, were performed. Here there was also no difference between the lines.

As several studies would show, haematocrit is an important factor involving bird’s performance. Haemoglobin is a transporter protein for oxygen it has an important role in aerobic performance and although the haemoglobin protein’s function and mechanism is known, the effects in regard to whole blood concentration are less studied, although some studies have narrowed it down to the importance of haemoglobin. The aim of this study was to further see the physiological effects of whole blood haemoglobin concentration, a trait that has been observed to be heritable in avian species (Druyan, Shlosberg, & Cahaner, 2007) in Red Junglefowl. It also aimed at investigating the effects in several stages of the animals life cycle as this has not been done extensively in earlier studies. The study mainly focus on further researching the effects of haemoglobin on fertility, doing so by including hatchability but it will also study the effects of haemoglobin concentrations on growth and summit metabolism.
Considering the importance of the protein in oxygen deliverance, and the correlation between haemoglobin and aerobic performance it seems plausible that the high line birds will be able to thermoregulate more efficiently than the low line animals, and increase their summit oxygen consumption. Increased whole blood concentration of haemoglobin could also have a potential effect on the haemodilution of the laying animals. Haemodilution lowers the haematocrit value of the animals, and if there is more haemoglobin prior to egg laying, the effect of this dilution could be lowered, thus enabling a better performance. This correspond well with previous studies on Starlings (Fronstin, Christians, & Williams, 2015). Possibly, the amount of eggs laid could be a trade-off for increased haemoglobin concentrations and if this is true, the high line animals could decrease the amount of eggs laid in comparison to the low line animals, as previous studies on Junglefowl would imply (Altimiras, unpublished).

The summit metabolic rate is a measurement of an animal’s performance. The methods currently used in summit metabolism measurements are based on examining data projected on a screen to decide the length of the measurement. Neither the sliding nor the static method uses any means of core temperature measurements during the experiments and in the static cold exposure method, induced hypothermia is needed to calculate a metabolism value. To better understand the effect of the measurements on the subject animals, to be able to improve the method in manners of more controlled variables (such as end measurement core temperature) and to improve the method in manners of animal welfare it has been an important secondary aim of this study to improve upon summit metabolism methods. This has been done by continuously measuring the core temperature of the animals, not just the post-treatment temperature.
3 Material & methods

3.1 Animal management

14 birds of the species Red Junglefowl, *Gallus gallus*, were bred for either high or low whole blood haemoglobin concentrations. Of all birds, half had high levels of haemoglobin (six females and one male) and half had low levels of haemoglobin (six females and one male). The parentals used for these experiments were the third generation of animals bred for high and low haemoglobin values respectively.

The female animals were kept in two groups of six (in each group there was three high haemoglobin and three low haemoglobin line animals) and one group at a time was allowed to be in the pen together with the roosters while the other group was kept in individual cages for egg laying. Eggs from the animals in the individual cages were collected three times per week. After a two-week period, the hens in the pen and the hens in the egg laying cages were switched. This was repeated three times over a period of three months.

The collected eggs were labelled with the ID of the mother and the date of collection. They were measured for mass, length and width. After the measurements, the eggs were incubated at 37.8 degrees C with an air humidity of 45% in a Masalles 216 egg incubator (Model 25HS-SINF, Masalles, Barcelona, Spain). The day before planned hatch, the eggs were oved to an incubator with a temperature of 37.8 degrees Celsius and a humidity of 54%. Each egg was placed in individual compartments for pedigree hatching.

After hatch, the chickens were tagged and kept in 1x1 m pens, equipped with heat lamps and a wood shaving floor. The animals had a 12/12h day and night cycle and water and feed ad libitum. All animals were kept in the same enclosure, regardless of their line of heritage.

3.2 Growth and Haemoglobin measurements.

The chickens hatched from the collected eggs were weighed at hatch and then once per week. The birds were measured for haemoglobin two weeks after hatch and measured for summit oxygen consumption three weeks after hatch.
Haemoglobin measurements were carried out using a HemoCue haemoglobin reader (HemoCue®). The birds were pricked in the ulnar vein in the left wing and a blood sample was taken. The sample was measured twice in the HemoCue reader and from these measurements the animals with the highest and lowest values of haemoglobin could be used for the VO$_2$ summit measurements. The haemoglobin values were not compared to any control group.

### 3.3 VO$_2$ summit measurements

For the first two VO$_2$ summit measurements six three week old animals were used: three animals of the highest and lowest haemoglobin values respectively. For the remaining measurements 12 animals were used, with the 6 highest and 6 lowest individuals. Animals were subjected to the treatment at an age of 21-23 days of age.

VO$_2$ summit measurements were done using a combination between the sliding cold exposure method and the static method, with the modification that not only a helox gas mixture was used. The measurements were performed using a Gas Mixing Flow Meter (Cameron Instrument, Port Aransas) which was programmed to maintain a constant flow of two gases at a time. The following programs were used:

- 21 % O$_2$ & 79 % N$_2$ with a flow output of 1000 ml/min.

- 21 % O$_2$ & 79 % He with a flow output of 1000 ml/min.

The gas from the flow meter passed through a 3000 ml cylinder shaped container, via a plastic tube. For one batch of animals, a plastic container was used, for 2 measurements a metal container was used and for the last measurement a metal container with a glass bottom was used. The container was placed in a freezer kept at -25 degrees Celsius. All containers regardless of material was equipped with a tube for gas entry, a tube for gas exit and a temperature reader. The gas exit tube was connected to an Oxygen Analysis System (FoxBox sable system international), which analysed 300 ml/min, via an air dehumidifier.

Prior to the measurements, the gas mixer and analysis system was calibrated to a 20,95 % O$_2$ concentration. This was done using a N2 and O$_2$ gas mix in a closed loop system, keeping the flow at 300 ml/min. One animal at a time was measured for mass and body temperature before it was placed inside of the container.
The oxygen analysis system recorded the levels of oxygen, the flow of gas and the temperature inside of the container. The data was displayed by a LabChart7 program and the different variables were monitored during the experiment. The animals were measured with two different methods:

Method 1: 10 minutes of N2/O2 gas mix prior to switching to He/O2.

Method 2: Only He/O2

The first two batches were measured twice, once for each method on non-subsequent days. The remaining batches were only measured using the second method.

For the first batches (without a temperature reader), the period of time in which the animals were kept in the container depended on factors such as decrease in oxygen concentration and temperature in the cylinder. Both of these factors could be monitored with the LabChart programme. As long as the concentration of oxygen coming out from the container decreased and the temperature of the container increased, the measurement would continue. After the treatment the animals body temperature was measured once again and the oxygen measuring system recorded a second oxygen and flow baseline.

For the last measurement (on 12 animals) core temperature of the animal was measured using a Biomark tag. The tag was inserted into the crop via the oesophagus using two plastic tubes, the first tube was hollow and was inserted into the oesophagus. When the tube was placed properly the small tag was placed in the tube and the second tube was used to ensure that the tag went all the way down to the bottom of the first tube, and into the animal. During the measurements the core temperature was read every two minutes for all animals and the measurement was interrupted as soon as the core temperature was below 37.5 degrees Celsius (see appendix fig. 1). A temperature reading was done again after removing the animal from the container, both using the temperature reader and by a normal thermometer to obtain core and cloacal temperatures.

The summit values were calculated according the following equation:

$$mVO_2 = \frac{(O_2 \, diff. \times \frac{Flow}{100})}{(1 - 0,2 \times \frac{VO_2 \, min.}{100})} \times \frac{60}{\text{body mass}}$$
Where O2 difference is in percent and is the difference between the baseline oxygen concentration and the lowest value during the run. The VO2 minimum is the lowest value and is also expressed in percent. Flow is the flow entering the container per minute, meaning flow is in mL/minute.

3.4 Dissections of egg-laying hens

After laying the third and final batch of eggs, the parental animals were euthanized and the females were dissected to see if there were any differences in mass or number of mechanisms important for egg laying. The number of large yellow follicles (LYF) and post ovulatory follicles were counted. The mass of the oviduct, the stroma, the LYF and any egg in the egg gland was measured. LYF were considered if their diameter was larger than 10 mm.

3.5 Data analysis

All analysis was made using a level of significance (α) of 0.05.

To see if there was a difference between the normally distributed data from mass measurements, a 2 sample t-test was conducted. The 2 sample t-test was also used to see if there was a difference between the high and low egg layers came to total amount off eggs laid, eggs hatched and the mass and volume of said eggs. Dissection data regarding number of post ovulatory follicles (POF), mass of stroma, oviduct and the mass of the large yellow follicles were analysed using a 2 sample t-test.

The data from the haemoglobin measurements was not normally distributed and so a Mann-Whitney test was performed. From the dissection data, the number of large yellow follicles and the mass of the eggs in the egg gland was not normally distributed and these values were also analysed using a Mann-Whitney test.

For the summit oxygen consumption measurements, a general linear model ANOVA was performed with mass and haemoglobin levels at two weeks as covariates.

All data in the following results section is shown as mean (st.dev).
4 Results

Each group of hens (six animals, three high line and three low line animals) laid eggs for a combined length of six weeks. The high line animals laid an average of 21.5 eggs (3.02) during the entirety of the measurement, meaning 3.6 eggs per week (0.50).

For the low line animals, the average amount of eggs for six weeks was 20.16 (6.7) and 3.4 eggs per week (1.02). As such, there was no difference in the total amount of eggs laid during the full length of the egg-laying period ($t=0.44 \ p=0.674$, $N=250$, $df=6$).

There was also no difference in hatching success, i.e. the number of fertile eggs ($W=44.0$, significant at 0.47) where the high line animals hatched an average of 17.7 chickens (4.63) and the low line animals hatched 16.7 chickens (3.4).

![Figure 1: Individual value plots for mean of total amount of eggs laid ($N=257$, left) and total amount of eggs hatched (205, right). There was no difference between the eggs laid ($t=0.44 \ p=0.674$) nor the eggs hatched ($W=44.0$, significant at 0.47). Variation shows standard deviation.](image)

There was also no difference in the average mass ($t=0.08, \ p=0.942, \ df=8$) nor the average volume of the eggs ($t=0.28, \ p=0.801, \ df=9$). The high line animals laid eggs with an average mass of 35.01 g (2.79 g) and an average volume of 32.73 mL (2.47 mL), whereas the low line animals laid eggs with an average mass of 35.11 g (1.86 g) and an average volume of 33.07 mL (2.16 mL).
The egg-laying hens, six from high line and six from low line, were dissected and the data obtained shows that there was no difference in the amount of LYF (W=45.0) nor the mass of the LYF (t=0.27, p=0.794, df=6). There was no difference in the oviduct mass (t=0.05, p=0.960, df=7), the stroma mass (t=0.55, p=0.592, df=9) or the amount of post-ovulatory follicles (t=0.79 p=0.458, df=7). Out of all hens, 11 had eggs in the egg gland. However, there was no difference in the mass of these eggs (W=39.0).

Figure 2: Individual value plots over mean average volume (left) and mass (right) for all eggs laid. There was no difference between the lines for volume (t=0.28 p=0.801) nor mass (t=0.08, p=0.942). Variation shown as standard deviation

Figure 3: Dissection data with the mean number of large yellow follicles (LYF) and the number of post ovulatory follicles (POF). There was no difference between the high and the low line animals (N=12, 6 highs, 6 low) in number of LYF (W=45.0) nor POF (t=0.79, p=0.458). Variation shown as standard deviation
The chickens hatched were measured for weight at hatch and then once per week until 3 weeks of age. At hatch ($N_{\text{high}}=47$, $N_{\text{low}}=44$) there was no difference in mass between the lines ($t=0.54$, $p=0.613$), where high line animals had an average mass of 24.4 g (2.31 g) and the low line animals weighed on average 23.8 g (2.16 g). At one week of age ($N_{\text{high}}=46$, $N_{\text{low}}=44$) there was no difference between the lines ($t=2.37$, $p=0.2$), but at the two-week measurement ($N_{\text{high}}=46$, $N_{\text{low}}=44$) the low line animals were larger than the high line animals ($t=2.56$, $p=0.012$) with an average of 64.7 g for the high line birds (6.63 g) and 68.4 g for the low line birds (6.57 g). The difference was however lost at three weeks of age ($t=1.60$, $p=0.113$) where the high line animals averaged a 98.3 g of mass (13.93 g) and the low line animals had an average of 103 g (11.63 g). The overall growth of the animals is shown in the graph below.
At two weeks of age, the animals were tested for haemoglobin blood concentrations ($N_{\text{high}}=44, N_{\text{low}}=44$). The animals descending from high line mothers had higher haemoglobin values than the animals descending from low line mothers ($W=1697.5$, $p=0.0298$) with an average of 110.1 g/L (10.57 g/L) compared to the low lines average of 104.7 g/L (8.87 g/L).
Figure 6: Individual value plot of mean blood haemoglobin concentrations at two weeks of age. \( N_{\text{high}} = 44, N_{\text{low}} = 44 \). The high line animals had a larger whole blood haemoglobin concentration (\( W = 1697, p = 0.0298 \)). Variation shown as standard deviation.

The animals with the highest and lowest values of haemoglobin at two weeks were selected for summit metabolism measurements at three weeks of age. Analysis were made with 27 animals all treated with a single helox gas mixture and the metal container with glass bottom. There were significant differences for all investigated factors: line (\( p = 0.026 \)), mass at three weeks (\( p = 0.002 \)) and haemoglobin at two weeks (\( p = 0.004 \)) (see Table 1).

Table 1: Results from a general linear ANOVA with summit values as. This shows significant results for all investigated effects: mass haemoglobin and line (\( N = 27 \)).

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Figure 7: Individual value plot of mean summit measurement values (N=27). High line animals had a higher summit metabolism than the low line animals (p=0.026). Variation shown as standard deviation

5 Discussion

The aim of this project was to investigate the effects of high or low haemoglobin concentrations on fertility, growth and aerobic performance in Red Junglefowl. Earlier studies had shown the effects of high or low haematocrit values on such factors as aerobic capacity and fertility (Stanley, 1995, Fronstin et.al., 2016, Gayathri & Hegde, 2004).

The effects of haemoglobin on reproduction was examined in a study made by Fronstin et.al. in 2015. They studied European starlings and treated parental hens with phenyl hydrazine (PHZ), to lysate red blood cells. The animals with lower haemoglobin would, if treated during incubation, lay fewer eggs than the control group, treated only with saline. The low level animals would also initiate nesting later than the control (Fronstin et al., 2015). Another study on mallards showed that boron (B) and selenium (Se) affected whole blood concentrations of haemoglobin in parental mallards. These animals then laid smaller eggs and had a lower egg fertility. The eggs generated fewer chickens and the hatchlings were also smaller (Stanley et.al., 1995).
The possibility for haemoglobin to effect haemodilution during egg laying was also thought to be positive; if an individual has more haemoglobin to begin with, the effects of dilution could be less than for an animal with a lower concentration. This could mean that the birds that had higher levels of haemoglobin would be more productive. There was no difference in neither the total amount of eggs laid nor the amount of eggs hatched for both lines. Previous studies indicate that this is not the case in Starlings (Fronstin et al., 2015). Had the animals in this study been treated similarly to their study during egg laying, groups of high and low haemoglobin could have been compared. This study focused on increasing haemoglobin by selective breeding, while their study manipulated the concentrations of haemoglobin by haemolysis in the laying animals. The results also differ from previously made studies on Red Junglefowl (Altimiras, unpublished) which could be caused by a larger sample size for this project.

There was no difference in the volume nor the mass of the eggs. And furthermore there was no difference in any of the organs involved in egg laying. Physiological differences on organs, such as an increase in stroma mass to produce more follicles, could be plausible had there been a difference in egg productivity, but perhaps these changes would require more generations of breeding to be significant. All together this would imply that the laying of eggs and the fertility in this sense is not severely affected by increased concentrations of haemoglobin.

There was no difference in mass between the lines until two weeks of age. Previous studies (Altimiras, unpublished) shows that there is a significant difference in haemoglobin concentrations between high and low line hatchlings after 15 days. At this age, there was a difference in both mass and in haemoglobin between the line and perhaps the increase in haemoglobin is the reason for the difference in mass. This would require more study, but it could have importance for the ascites syndrome in rapidly growing broilers (Druyan et al., 2007). These animals grow too fast to supply sufficient amounts of oxygen to their tissue and the result is a pulmonary hypertension syndrome (Siegel, 2013). If the response to increased haemoglobin would be the same or similar to the response in Red Junglefowl it could result in being able to breed animals that can better sustain the oxygen demand of their rapidly growing bodies.

Here it would be important to note that all females were kept in a pen together with roosters from high and low line of haemoglobin. Therefore, there is no way of saying for certain that the high line animals do not
have a low line father. Had the animals been of fully high or low lines, the effect on growth could be examined with higher certainty. From these measurements, the only factor negatively affected by an increased haemoglobin concentration is growth, meaning that this would be the best estimate for the trade-off sought after. The method used by Fronstin et al. could be combined with selective breeding to possibly be able to quantify the effect of a gradient of haemoglobin concentrations.

Due to haematocrits impact on aerobic capacity the hypothesis was that an increased whole blood concentration of haemoglobin would enable an increased level of oxygen to the shivering tissue, and thereby more thermoregulation and higher values in Red Junglefowl when exposed to a summit metabolism measurement. This was thought to be true as haemoglobin is a component of haematocrit values.

The summit metabolism measurements would show that an increased haemoglobin concentration indeed improved upon the individual’s capacity to provide oxygen to the tissue needed for thermoregulation. However, the factor that affected the summit measurement the most was the body mass of the animal. This result is to be expected as the blood volume would be related to the size of the animal. The line would be shown to be the least important factor for the summit metabolism measurement. However, since the aim of this study was to examine the effects of haemoglobin concentration, the animal’s lineage is not as important as its two-week haemoglobin value.

Had the study been performed with animals of completely controlled lines, there could possibly be even higher values for the haemoglobin measurements, and as there was a correlation between the haemoglobin and the summit measurements the summit values could increase even further. Also, the sex of the animals was not controlled prior to the summit measurement. As males have previously been showed to perform better at summit measurements (Hammond et al., 2000), and because the largest difference in previous studies at Linköpings University were found in females (Altimiras, unpublished), noting the sex of all animals before the measurements could give important insight to the values.

Another aim in this study was to improve upon the summit metabolism method. This was achieved by continuously doing small alterations to the measurements, such as changing the container from metal to plastic and then glass, to avoid damage from cold on the feet of the animals. Using a plastic container was inferior to the metal container as it provided too
much insulation and thereby gave inconsistent and considerably lower results than the metal container.

Changing the bottom of the container to one made from glass showed no difference in measurement data, however it was believed to be a refinement to the method as it did not risk the animal’s welfare as much as having them stand on metal.

Using temperature readers meant opening the freezer to do temperature measurements and as such the temperature the animals were being exposed to was slightly higher than the temperatures without the reader. However, this showed no clear effect on the results, and it is a grave improvement to the method to be able to continuously measure the core temperature of the animals instead of only noting the temperature after the measurement.

5.1 **Ethical aspects**

All handling of animals had been approved by the ethical committee (Linköping), diary number 9-13. The project strived to work accordingly to the method “replace, reduce and refine”. This experiment could not fully replace animals, but could better reduce and refine the treatments. All treatments strived to be done in a controlled and humane way.

The study of haemoglobin and its effects on different stages of animal lives can give a better understanding of the correlation between genotype and phenotype. Apart from this the results could possibly be applied on the handling of commercial animals and be beneficial in the understanding of ascites syndrome.

6 **Conclusions**

The aim of this experiment was to investigate the effects of haemoglobin on several aspects regarding performance. The effects of haemoglobin on egg number and egg size was not significant between the animals, neither was the hatching success. Thereby the thought that fertility was the trade-off can be discarded.

There was a difference in the haemoglobin which would strengthen the belief that whole blood concentration of haemoglobin is hereditary. The growth of the animals differed at two weeks of age, at the same time as the haemoglobin levels started to differ. The mass was not different from
each other at three weeks of age, which could mean that the high line animals had a slower growth during the accumulation of haemoglobin.

There were significant differences in summit metabolism, and the high line animals showed greater success in these measurements. Further studies would be required to tell if these results are consistent and also if the difference is dependent on gender. There was some improvement done to the method, including using temperature tags, it is a great benefit to be able to monitor the core temperature of the animals throughout the measurement and an important step when trying to refine the method for ethical purposes.

Out of all factors looked at, the only factor that was negatively affected by an increase in haemoglobin was the growth. This would imply the trade-off sought after and as such it should be further studied to try to quantify these results.

7 Acknowledgement

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8 References


9 Appendix

Fig 1: Temperature measurements from the summit measurement using a metal container with glass bottom and temperature readers (N=11).