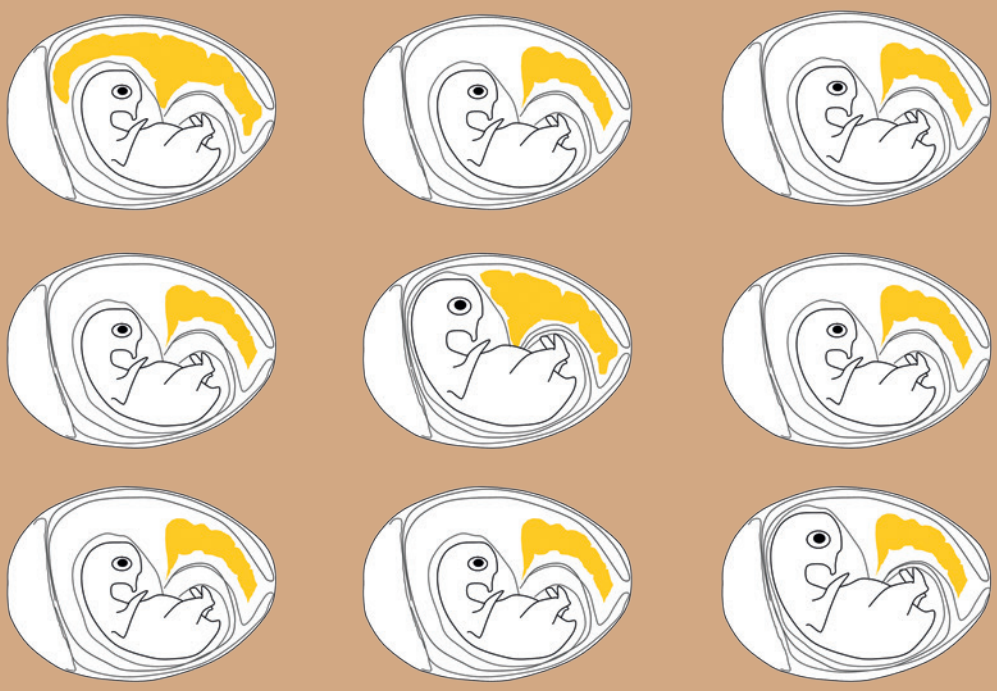
# Are all eggs equal?

## Embryonic development and nutrient metabolism in chicken eggs of different origins



Ampai Nangsuay

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**Thesis**

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# ABSTRACT

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Hatching eggs, supplied to hatcheries are originating from different origins varying in breed, strain, and breeder age. These hatching eggs can be different in size, composition and eggshell properties, which might influence nutrient and $O_2$ availability and consequently could affect embryonic development and nutrient metabolism. The aim of this thesis was therefore 1) to investigate effects of egg origin on nutrient and $O_2$ availability, 2) to investigate effects of egg origins on nutrient metabolism and embryonic development and 3) to investigate consequences of different egg origins on the incubation process and hatching characteristics. In five studies, effects of different egg origins on nutrient and $O_2$ availability, nutrient metabolism, embryo development and hatching characteristics were investigated. The first and second study focused on breeder age and egg size. The third study on breed; broilers and layers. The fourth study on broiler strain and the fifth study on breeder age, strain and eggshell temperature (EST). The overall findings in this thesis suggest that hatching eggs from different origins are not equal in availability of nutrients and $O_2$. Nutrient availability is altered through variation in yolk size, especially by the effects of breeder age and breed. $O_2$ availability is altered by differences in eggshell properties, which is influenced by especially breed and broiler strain. The availability of both nutrients and $O_2$ plays a role on nutrient metabolism measured as embryonic heat production (HP) and consequently on embryonic development. Between incubation day (E) E7 and E14, both nutrient and $O_2$ availability might affect nutrient metabolism as shown in the results of the broiler and layer comparison. Between E14 and hatching, the availability of $O_2$ becomes the most determinant factor for nutrient metabolism and consequently for embryonic development. An increase in EST from 37.8 to 38.9°C from E7 onward resulted in an acceleration of nutrient metabolism and embryonic development until E16, but thereafter a high EST resulted in reduced yolk free body mass development. Embryos with an accelerated metabolic speed at an early

stage of incubation, caused by an increased EST, might reach limited $O_2$ availability at a higher magnitude than the embryos at a normal EST. As a result, nutrient metabolism is restricted and embryonic development is depressed. It can be concluded that not only the HP, but also the availability of $O_2$ is crucial to be taken into account for developing incubator temperature. The principle is to obtain an optimal EST, which could maintain the balance between $O_2$ requirement (driven by nutrient metabolism) and $O_2$ availability for a continuing optimal nutrient metabolism to generate sufficient energy for embryonic development throughout incubation.

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# CHAPTER 1

# General introduction

# GENERAL INTRODUCTION

Hatching eggs supplied to hatcheries are usually originating from different breeder flocks, varying in selection traits and ages and they differ in egg size. These eggs vary in egg composition and shell properties, which may affect the amount of egg macro nutrients (protein, lipids and carbohydrates) and eggshell conductance. During incubation, chicken embryos take up $O_2$ via diffusion through the eggshell and use it for converting egg nutrients into energy. This derived energy is crucial for embryonic development and growth and for the maintenance of the embryos throughout the developmental process (Vleck, 1991). In the process of energy production, one of the by-products is heat and this needs to be removed from the system in order to maintain an optimal embryo temperature, as reflected in eggshell temperature (EST; Meijerhof and Van Beek, 1993). An optimal EST is important for hatching success, good chick quality and good broiler performance (Lourens et al., 2005; Hulet et al., 2007; Molenaar et al., 2011).

Although incubation in practice is continuously dealing with eggs from different origins, it is largely unclear how these eggs differ in availability of nutrients and $O_2$. In addition, it is not known whether embryos in these eggs differ in their nutrient metabolism and development and what could be the mechanisms that explain the possible differences.

### *Effects of Egg Origins on Egg Characteristics*

Newly laid eggs contain predetermined macro nutrients, which are stored in albumen and yolk. Egg yolk is rich in nutritional sources, containing approximately 48% water, 33% lipids, 17% protein, and 1% carbohydrates, whereas albumen is poor in nutritional sources, containing approximately 88 to 90% water, 10% protein, 1% carbohydrates, and no lipids (Romanoff and Romanoff, 1949). This implies that during the course of incubation, egg yolk serves as the major source of required lipids and protein for embryonic development and for energy production to sustain developmental processes. Egg composition, particularly albumen and yolk content, can be influenced by variations in egg origin (breeder age, breed, and strain), including variations in size of these eggs.

At similar age, broiler breeders produce eggs, which are approximately 10 g heavier than eggs from layer breeders (Everaert et al., 2008). The long term selection for growth in broilers resulted in an increase in egg weight (Christensen et al., 1995; Tharrington et al., 1999; Tona et al., 2004) and this led to a proportionately decrease in yolk size, whereas albumen was proportionately increased (Christensen et al., 1995; Tharrington et al., 1999; O'Dea et al., 2004; Joseph and Moran, 2005; Wolanski et al., 2007). However, if eggs are selected at the same egg size, Ho et al. (2011) reported that broiler eggs contained more yolk and less albumen than layer eggs. An influence of breeder age and egg size on egg composition has been shown in several studies (O'Sullivan et al., 1991; Vieira and Moran, 1998a; Peebles et al., 2000; Hamidu et al., 2007). These authors reported that as egg weight increases over a production cycle of breeder hens, yolk and albumen content are disproportionately changed. It appeared that percentage of yolk progressively increased, while proportion of albumen decreased. At the same hen's age, a larger egg size was mainly a result of an increase in albumen content (Vieira and Moran, 1998b).

Besides the effects on egg composition, differences in egg origins have an influence on eggshell properties, such as eggshell weight, eggshell thickness, eggshell pores, and water vapour conductance, which cause variations in diffusion rate for $O_2$ and $CO_2$ exchanges (Ar et al., 1974; Peebles and Brake, 1987). Eggshell properties change with breeder age and Peebles and Brake (1987) reported an increase in eggshell conductance with breeder age. At the same egg size, broiler eggs had lower shell weight than layer eggs (Ho et al., 2011), which suggested that the eggshell conductance of broiler eggs was higher than of layer eggs. Eggshell conductance was observed to be different between broiler strains. Wolanski et al. (2007) reported that a broiler strain, which was selected for growth rate and carcass yield had a higher relative eggshell conductance than a broiler strain which was selected for the whole bird market.

Collectively, the available information suggests that egg composition and eggshell conductance are altered by differences in egg origins. It can be hypothesized that alterations of egg compositions and eggshell conductance affect nutrient and $O_2$ availability, which can influence embryonic development and nutrient metabolism.

***Embryonic Development and Nutrient Metabolism***

**i.) Nutrients and $O_2$ availability:**

The available nutrients are transported from the yolk sac to the embryo by the function of the yolk sac vascular system, which starts after approximately 48 h of incubation (Romanoff, 1952). During the first week of incubation, when the chorioallantoic membrane (CAM) has not been developed yet, embryos have to rely on anaerobic glycolysis of carbohydrates as energy source. The CAM starts to develop at approximately incubation day 8 (E8) and its functions as respiratory organ permits embryos to have access to sufficient $O_2$ to complete lipid oxidation (Moran, 2007). Romanoff (1967) showed that during the second week of incubation the relative embryonic growth increases almost 50% on a daily basis and the embryo's absolute weight at E14 is already at approximately 30% of its final weight. The continuing process of development during the last seven days to achieve full term weight and to complete the hatching process is also an energy demanding process. The available protein is being used mainly from the second week of incubation onward for the purpose of embryonic development and growth (Romanoff, 1967), whereas yolk lipids are the main energy source providing approximately 90% of the total energy requirements (Noble and Cocchi, 1990). In the process of converting egg nutrients to energy, the by-products $CO_2$, water, and heat are produced and these will increase correspondingly with a high energy production and high growth rate. This leads to the hypothesis that variation in availability of nutrients and $O_2$ could influence embryonic development and nutrient metabolism and consequently the embryonic heat production (HP) will be altered. In addition, it can be hypothesized that if there is a difference in embryonic HP, the incubation temperature profiles to maintain optimal EST need to be adjusted accordingly.

**ii.) Incubation temperature and EST:**

During the developmental process, the higher the rate of embryonic growth, the greater the nutrients and $O_2$ demand for energy production are to sustain growth and maintenance. As a consequence, embryonic HP increases

correspondingly with high nutrient metabolism. Under normal incubation temperature, during the first 7 days of incubation, the embryonic HP is insignificant, but thereafter the HP rises steadily with the progress of incubation (Etches, 1996). An increased HP might lead to a higher incubation temperature and consequently a higher EST when the system is cooled insufficiently (Romijn and Lokhorst, 1956; Meijerhof and van Beek, 1993). Because the developing embryo before E18 is defined as poikilothermic (Tazawa et al., 1988), Zhang and Whittow (1992) and Mortola (2006) demonstrated that increasing incubation temperature during this period leads to an increase in $O_2$ consumption and embryonic growth. This means that nutrient metabolism and embryonic HP increase, which can result in a further increase in EST. Molenaar et al. (2010) demonstrated that increasing EST from 37.8 to 38.9◦C from E9 to hatch decreased body development at hatch, which was explained by a lower efficiency of converting egg protein to yolk free body (YFB). Since the main purpose of protein is building tissues for growth, a lower efficiency of protein suggests that there was allocation of protein for other purposes, such as production of energy as an alternative nutrient source under limited $O_2$, resulting in a decreased amount of protein available for development. The same authors also showed that a negative impact of a high EST at 38.9◦C can be partially restored when $O_2$ is increased to 25%. This information suggests a major influence of both incubation temperature and EST on nutrient metabolism and embryonic development. It is possible that embryos of eggs differing in composition and eggshell conductance as well as embryos at a certain incubation temperature or EST differ in developmental rate and embryonic HP. Consequently, eggs differing in availability of nutrients and $O_2$ need to be incubated at different incubation temperature.

### *Aim and Outline of the Thesis*

The aim of this thesis is to 1) investigate effects of egg origin on nutrient and $O_2$ availability and 2) to determine nutrient metabolism of eggs from different origins and examine its consequences on embryonic development during incubation.

Based on these aims, eggs of different origins were used and compared on their effects on embryonic development and metabolism. Chapter 2 and 3 is

focusing on effects of breeder age/egg size, Chapter 4 focuses on effects of breed, and Chapter 5 focuses on effects of broiler strain. These studies were performed by selecting eggs of different origins at similar egg weight ranges. The experimental eggs were incubated at a similar EST of 37.8 °C from the start of incubation until hatching. In Chapter 6 and 7, the consequences of differences in nutrient metabolism and embryonic development due to variations in egg origins were examined. Eggs were selected at a similar egg weight range from different breeder ages and broiler strains. The eggs were incubated at an EST of 37.8 or 38.9 ºC from E7 until hatching. In Chapter 8, the results obtained from the different studies described in Chapter 2 to 7 are discussed and hypotheses for underlying mechanism including practical implications are presented.

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# CHAPTER 2

# Yolk absorption and embryo development of small and large eggs originating from young and old breeder hens

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# ABSTRACT

To evaluate the effect of breeder age and egg size on yolk absorption and embryo development, a total of 4,800 Ross 308 hatching eggs were subjected to 4 treatments arranged in a 2 × 2 factorial randomized complete block design using 2 breeder ages (29 and 53 wk of age, or young and old) and 2 egg sizes (57–61 g and 66–70 g, or small and large). A significant interaction between breeder age and egg size was found for egg composition. Yolk weight increased with flock age, whereas a larger egg size resulted in higher albumen content. A significant interaction between breeder age and egg size was found for yolk-free body (YFB) weight only at d 7. Until the fourteenth day of incubation, eggs from the old flock yielded greater YFB weight than did eggs from the young flock. At hatch, chicks of both age groups had comparable wet YFB weight, chick weight, wet and dry residual yolk weight, and chick length. Dry YFB weight of chicks from the old flock was higher than that of chicks from the young flock. Compared with the small eggs, embryos and chicks of the large eggs had greater YFB weight from d 14 to hatching. At hatch, these chicks were also heavier, longer, and had higher wet and dry YFB and residual yolk weight. Yolk absorption at d 18 and at hatch of embryos and chicks of the old flock was higher than that of the young flock, both in absolute values and percentages. Rates of absolute and percentage yolk absorption through d 18 and percentage yolk absorption at hatch were higher in the small eggs than in the large eggs. It can be concluded that egg size influences chick length at hatch and embryo development when expressed in terms of total and YFB weight. Although yolk availability and rate of absorption may have influenced dry YFB weight, they did not influence hatching chick length or total and YFB weight.

**Key words**: breeder age, egg size, yolk absorption, embryo development

# INTRODUCTION



A newly laid egg contains all the essential nutrients an embryo needs (Vleck and Hoyt, 2004). These nutrients are utilized to build new tissue, maintain existing tissue and muscular activity, and sustain development through hatching (Vleck, 1991). The egg's nutrients are furnished in the albumen and yolk, which comprise 45% lipids, 48.5% protein, and 3.7% carbohydrates on a DM basis (Romanoff and Romanoff, 1949). Compared with the yolk, the albumen is a relatively poor source of nutrients, as it contains approximately 90% water (Freeman and Vince, 1974). The DM of albumen contains over 90% protein. The remaining part of the albumen is mainly carbohydrates (7.8%) and a small amount of fat (0.26%). The yolk solids are approximately 70.6% of the total egg solids, accounting for 99% of the lipids and 47% of the protein present in an egg (Romanoff and Romanoff, 1949). For this reason, the egg yolk functions as the main nutrient supply for the growth and energy production of embryos (Romanoff, 1967).

Flock age and egg size are major factors that determine the albumen and yolk content in eggs (O'Sullivan et al., 1991; Vieira and Moran, 1998a; Hamidu et al., 2007). Egg weight follows a curvilinear function in relation to flock age, and it reaches a plateau toward the end of the laying cycle (French and Tullett, 1991). Yolk and albumen weight increase with an increase in the egg's weight and a hen's age. A larger increment of yolk relative to albumen occurs as the hen gets older (O'Sullivan et al., 1991; Peebles et al., 2000), whereas an increase in egg size at the same hen age is mainly the result of an increase in albumen content (Vieira and Moran, 1998a). As egg nutrients are the only available nutritional source for developing embryos, variations in egg composition caused by breeder age and egg size can influence embryo development.

Embryos utilize egg materials for development from the early stages of embryo formation until hatching (Romanoff, 1967). Yolk nutrients are transported through the yolk sac membrane and the surrounding vascular system (Noble and Cocchi, 1990). This process accelerates after approximately 12 d of incubation, at which time a rapid transfer of lipids from the yolk to the embryo begins (Speake et al., 1998).The yolk sac is drawn into the body cavity on the nineteenth day of

incubation, and the entire yolk residue is located within the abdomen at the end of d 20. At hatch, approximately 30% of the original nutrients in the yolk are still present in the yolk residue, and these nutrients function as a major source of energy for the hatchling during the first few days of life (Etches, 1996; Noy and Sklan, 2001).

Several studies have demonstrated a strong relation between egg size and chick weight (Wiley, 1950; Wyatt et al., 1985; Vieira and Moran, 1998a, b). This relationship was observed in trials where an increase in egg weight was associated with an increase in flock age (Suarez et al., 1997; Vieira and Moran, 1998b) as well as an increase in egg size at the same hen age (Vieira and Moran, 1998a; Lourens et al., 2006). However, information regarding embryo development and the yolk absorption of chicks originating from different breeder hen ages and egg sizes is limited. A study of hatchling growth rates in Pekin hatchlings suggests that the faster resorption of a larger yolk from a larger egg results in a faster growth rate of Pekin ducklings from 75- versus 95-g eggs (Knizetova et al., 1988). Recently, Gous (2010) suggested that yolk sac utilization can be one of the limiting factors that restrict embryonic growth within small eggs. However, the study of Finkler et al. (1998) showed that the removal of 20% the yolk from eggs before setting did not affect the wet and dry embryo yolk-free body (**YFB**) weight at 20 d of incubation but simply decreased the amount of yolk sac, which means that it is unclear how yolk absorption and yolk availability interact with embryo development. To investigate the influence of these parameters, a trial was conducted to examine the effects of breeder age and egg size on yolk availability and embryonic growth.

# MATERIALS AND METHODS

### *Experimental Design*

The experiment was conducted with 2breeder flock ages (29 and 53 wk) and 2 egg size groups (small and large); which were arranged in a 2 × 2 factorial randomized complete block design. Eight replicates were used per treatment, resulting in 32 experimental units.

### *Hatching Egg*

A total of 4,800 Ross 308 hatching eggs from 2 different commercial broiler breeder flocks at 29 wk (young flock) and 53 wk (old flock) of age were obtained over a 3-d period. The breeder flock at 29 wk received a diet with 2,800 kcal of ME/kg and 16% CP. The breeder flock at 53 wk received a diet with 2,785 kcal of ME/kg and 15% CP. Both breeder flocks received the same management according to the recommendations of the breeding company. On the days of collection, the eggs of the young flock were selected from approximately 45,000 hatching eggs with an average egg weight of 56.85 g. The eggs of the old flock were selected from approximately 25,000 hatching eggs with an average egg weight of 67.15 g. For both age groups, 1,200 eggs were selected within the weight range of 57 to 61 g, and 1,200 were selected within the weight range of 66 to 70 g. Eggs between 57 and 61 g were classified as small (**S**), whereas eggs between 66 and 70 g were classified as large (**L**). This classification resulted in 4 groups of 1,200 eggs each: young small (29 S), young large (29 L), old small (53 S), and old large (53 L). One hundred fifty eggs from each of the 4 treatment groups were placed on each of 8 replicate incubator trays. The eggs were stored on the trays in an egg storage room for 3 to 5 d at a temperature of 18 to 20ºC and a relative humidity of 50 to 60%.

### *Incubation*

Preliminary embryo temperature measurements for each position in the setters were made. Specific positions in the machines had to be used to create a uniform embryo temperature over all experimental eggs in the same machine. To use these places, the experimental eggs were incubated in 2 single-stage HatchTech setters with a capacity of 57,600 eggs each (HatchTech B.V., Veenendaal, the Netherlands). Eight incubator trays with 150 eggs each from each of the 4 treatment groups were randomly assigned places in 2 setters containing 4 trays each that were determined to have the most uniform embryo temperature. The remaining places in the incubators were filled with hatching eggs that were not part of the experiment to ensure equal airflow. The temperature set point profiles for the 2 setters were applied to obtain an eggshell temperature (**EST**) of 37.8ºC (Lourens

et al., 2005). The EST, which was measured by an infrared ThermoScan IRT 4520 ExacTemp (Braun GmbH, Kronberg, Germany), was used as a reference for embryo temperature (Hulet et al., 2007). The infrared thermometers remained in the setters for the entire experiment and were moved only to take the necessary readings (Leksrisompong et al., 2007). On incubation d 6, 8, 10, 12, 14, and 17, the EST of 24 eggs (3 eggs/replication) was measured for each treatment. The mean EST values of each setter were calculated, and adjustments of the temperature set-point profiles were made to obtain the required EST. The variation of EST from the target was maintained at a maximum of 0.1 to 0.2ºC. The other incubator set points ($CO_2$, relative humidity, and opening of the setter valves) were set according to the normal procedures of the hatchery. On incubation d 18, the eggs were candled, and the fertile eggs were transferred to hatcher baskets and placed in 2 hatchers. The 2 hatchers were set at a temperature of 36.5ºC. On the day of hatch, the hatcher trays were pulled out according to the standard procedure of the hatchery for chick processing.

***Measurements***

From each experimental unit (setter tray), 5 eggs were randomly taken before setting to determine fresh egg composition. The egg weight was measured, and the eggs were opened to separate the shell, yolk, and albumen. The yolk and shell weight were measured, and the albumen weight was calculated as the difference between egg weight and the weight of the yolk and shell. The albumen and yolk were stored at -20°C for further analysis.

To determine embryonic development, 5 eggs containing live embryos per setter tray were randomly collected at incubation d 7, 14, and 18. The embryos were removed and separated from the remaining egg content for weighing. The yolk sac weight of the embryos at d 18 was measured. The length of the embryos at d 14 and 18 was measured by stretching them along a ruler and taking the length between the tip of the beak and the tip of the middle toe of the right foot (Hill, 2001; Molenaar et al., 2008). To maintain constant airflow over the trays, eggs from the non-experimental trays were used to replace the removed eggs. These eggs were marked and excluded from further measurements.

At pulling time, 5 chicks per experimental unit were randomly selected. The chicks were weighed, and their length was determined as described above. The chicks were then killed by cervical dislocation to determine the residual yolk (**RY**) and YFB weight. The yolk absorption at d 18 was calculated with the initial yolk weight (g, wet weight) minus the residual yolk weight at d 18 (g, wet weight). The calculation of yolk absorption at hatch was calculated as the initial yolk weight (g, wet weight) minus the RY weight at hatch (g, wet weight). The RY and YFB were stored at -20°C for further analysis.

***DM Analysis***

The albumen (fresh egg), yolk (fresh egg), RY and YFB (at hatch) of each experimental unit were pooled and homogenized. Thereafter, samples of each experimental unit were taken for DM analysis by the proximate method (AOAC, 1990). The yolk conversion rate (**YCR**) at hatch was calculated as yolk absorption at hatch (g, dry weight) divided by YFB weight at hatch (g, dry weight).

***Statistical Analyses***

The data were analyzed using the 2-way ANOVA of the R-program (R Development Core Team, 2009). The statistical model for the measurements of egg weight, yolk weight, albumen weight, yolk: albumen ratio, and solids content of albumen, yolk, and albumen + yolk was expressed as follows: $Y_{ijk} = \mu + A_i + B_j + AB_{ij} + e_{ijk}$, where $Y_{ijk}$ is the dependent variable, $\mu$ is the overall mean, $A_i$ is the effect of breeder hen age (i = 29 or 53 wk), $B_j$ is the effect of egg size (j = small or large), $AB_{ij}$ is the interaction between breeder hen age and egg size, and $e_{ijk}$ is the error term.

The 2 setters were included in the statistical model as blocks for the measurementsthroughout the incubation period. The model used was $Y_{ijkl} = \mu + E_i + A_j + B_k + AB_{jk} + e_{ijkl}$, where $Y_{ijkl}$ is the dependent variable, $\mu$ is the overall mean, $E_i$ is the effect of block (setters), $A_j$ is the effect of breeder hen age (j = 29 or 53 wk), $B_k$ is the effect of egg size (k = small or large), $AB_{jk}$ is the interaction between breeder hen age and egg size, and $e_{ijkl}$ is the error term. Data in the form of

ratios and percentages were transformed to an arcsine format before analysis, and the actual means are presented. The least significant difference for all statistically analyzed data was used to determine the differences among treatment means, and the differences were considered significant at $P < 0.05$.

# RESULTS

### *Egg Composition and Solids Content*

The yolk weight, albumen weight, and yolk: albumen ratio were influenced by the interaction between breeder age and egg size (Table 1). Both the small and large eggs of the old flock contained 2.92 and 4.55 g more yolk, respectively, than did those of the young flock ($P < 0.001$). Within the young flock, the difference in egg weight had no effect on yolk weight. In the older flock, the large eggs contained 1.9 g more yolk than did the small eggs. The small and large eggs of the old flock contained 3.02 and 4.33 g less albumen, respectively, than did those of the young flock ($P = 0.001$). The large eggs of both breeder age groups had a higher albumen weight than did the small eggs, but the difference between small and large eggs was greater in the young flock than in the old flock. The yolk: albumen ratio was higher in both the small and large eggs of the old flock, compared with the ratio in those of the young flock. The large eggs of both breeder age groups had a lower yolk: albumen ratio than did those of the small eggs ($P = 0.038$).

A significant interaction between breeder age and egg size was found for albumen, yolk, and yolk + albumen solids content (Table 1). Both the small and large eggs of the young flock had higher albumen solids content than those of the old flock ($P = 0.019$). The large eggs of both breeder age groups had higher albumen solids content than those of the small eggs, but the difference between small and large eggs was greater in the young flock than in the old flock. The small and large eggs of the old flock had higher yolk solids content than did those of the young flock ($P < 0.001$). The large eggs of the old flock had higher yolk solids content than did the small eggs, but there was no difference between the egg sizes of the young flock. Both the small-egg and large-egg groups of the old flock had -

**Table 1.** Effect of breeder ages and egg sizes on fresh egg composition and solid content of albumen, yolk and albumen + yolk.[1]

| Effect[2] | Egg(g) | Yolk (g) | Albumen (g) | Yolk: Albumen | Solid content (g, dry weight-basis) | | |
|---|---|---|---|---|---|---|---|
| | | | | | Albumen | Yolk | Albumen + Yolk |
| Breeder age × egg size | | | | | | | |
| 29 S | 58.31$^{c}$ | 15.91$^{c}$ | 35.88$^{c}$ | 0.44$^{c}$ | 4.73$^{b}$ | 8.23$^{c}$ | 12.97$^{c}$ |
| 29 L | 65.59$^{b}$ | 16.17$^{c}$ | 42.16$^{a}$ | 0.38$^{d}$ | 5.59$^{a}$ | 8.12$^{c}$ | 13.71$^{b}$ |
| 53 S | 58.39$^{c}$ | 18.83$^{b}$ | 32.86$^{d}$ | 0.57$^{a}$ | 3.95$^{d}$ | 9.50$^{b}$ | 13.45$^{b}$ |
| 53 L | 66.09$^{a}$ | 20.72$^{a}$ | 37.83$^{b}$ | 0.55$^{b}$ | 4.53$^{c}$ | 10.75$^{a}$ | 15.28$^{a}$ |
| Main effect mean | | | | | | | |
| Breeder age (wk) | | | | | | | |
| Young (29) | 61.95 | 16.04 | 39.02 | 0.41 | 5.16 | 8.18 | 13.34 |
| Old (53) | 62.24 | 19.77 | 35.35 | 0.56 | 4.24 | 10.13 | 14.37 |
| Egg size | | | | | | | |
| S | 58.35 | 17.37 | 34.37 | 0.51 | 4.34 | 8.87 | 13.21 |
| L | 65.84 | 18.44 | 39.99 | 0.47 | 5.06 | 9.44 | 14.49 |
| Source of variation | | | | *P*-value | | | |
| Breeder age × egg size | 0.008 | <0.001 | 0.001 | 0.038 | 0.019 | <0.001 | <0.001 |
| Breeder age | <0.001 | <0.001 | <0.001 | <0.001 | < 0.001 | <0.001 | <0.001 |
| Egg size | <0.001 | <0.001 | <0.001 | <0.001 | < 0.001 | <0.001 | <0.001 |
| SEM[3] | 0.035 | 0.079 | 0.089 | 0.003 | 0.027 | 0.056 | 0.052 |

[a–d] Means within a column with different superscript letters differ ($P < 0.05$). [1] For all variables measured, n = 5 eggs/experimental unit (setter tray). [2] Ages and sizes: 29 S = young small (29 wk of age); 29 L = young large; 53 S = old small (53 wk of age); 53 L = old large; small (S) = 57-61 g; large (L) = 66-70 g. [3] SEM is based on a pooled estimate of variance.

higher albumen + yolk solids content than did those of the young flock ($P < 0.001$). The large eggs of both age groups had higher albumen + yolk solids content than the small eggs, but the difference between the small and large eggs was greater in the old flock than in the young flock.

**Table 2.** Effect of breeder ages and egg sizes on yolk-free body (YFB) weight at incubation d 7, 14, 18, and at hatch (g, wet weight), chick weight (g, wet weight) and length of embryos and chicks at incubation days 14, 18, and at hatch (cm).[1]

| Effect[2] | YFB Weight (g) | | | | Chick Weight (g) | Length (cm) | | |
|---|---|---|---|---|---|---|---|---|
| | d 7 | d 14 | d 18 | Chick | | d 14 | d 18 | Chick |
| Breeder age × egg size | | | | | | | | |
| 29 S | 1.24$^{bc}$ | 15.22 | 31.44 | 37.40 | 40.28 | 12.59 | 17.88 | 19.89 |
| 29 L | 1.18$^{c}$ | 15.74 | 34.15 | 41.64 | 45.58 | 12.77 | 17.96 | 20.29 |
| 53 S | 1.29$^{ab}$ | 15.62 | 31.85 | 37.52 | 39.97 | 12.61 | 17.98 | 20.09 |
| 53 L | 1.35$^{a}$ | 16.44 | 34.25 | 41.77 | 45.53 | 12.82 | 18.45 | 20.49 |
| Main effect mean | | | | | | | | |
| Breeder age (wk) | | | | | | | | |
| Young (29) | 1.21 | 15.48$^{b}$ | 32.79 | 39.52 | 42.93 | 12.68 | 17.92 | 20.09 |
| Old (53) | 1.32 | 16.03$^{a}$ | 33.05 | 39.64 | 42.75 | 12.72 | 18.21 | 20.29 |
| Egg size | | | | | | | | |
| S | 1.27 | 15.42$^{b}$ | 31.64$^{b}$ | 37.46$^{b}$ | 40.12$^{b}$ | 12.60 | 17.93 | 19.99$^{b}$ |
| L | 1.27 | 16.09$^{a}$ | 34.20$^{a}$ | 41.70$^{a}$ | 45.55$^{a}$ | 12.79 | 18.20 | 20.39$^{a}$ |
| Source of variation | | | | *P*-value | | | | |
| Breeder age × egg size | 0.025 | 0.311 | 0.682 | 0.974 | 0.640 | 0.852 | 0.195 | 0.979 |
| Breeder age | <0.001 | 0.001 | 0.496 | 0.689 | 0.535 | 0.718 | 0.054 | 0.054 |
| Egg size | 0.978 | <0.001 | <0.001 | <0.001 | <0.001 | 0.066 | 0.072 | <0.001 |
| SEM[3] | 0.011 | 0.071 | 0.185 | 0.155 | 0.144 | 0.199 | 0.074 | 0.049 |

[a–c] Means within a column with different superscript letters differ ($P < 0.05$). [1] For all variables measured, n = 5 fertile eggs at d 7, 14, and 18 and 5 chicks at hatch/experimental unit (setter tray).
[2] Ages and sizes: 29 S = young small (29 wk of age); 29 L = young large; 53 S = old small (53 wk of age); 53 L = old large; small (S) = 57-61 g; large (L) = 66-70 g. [3] SEM is based on a pooled estimate of variance.

***Embryo Developmentand Yolk Absorption***

Table 2 shows that a significant interaction between breeder age and egg size was found for YFB weight at incubation d 7 ($P = 0.025$). On this day, the large egg group of the older flock yielded a higher YFB weight than both egg weight groups of the young flock, whereas the small egg group yielded a comparable YFB weight, regardless of breeder age. This interaction disappeared at the later stages of incubation. At d 14, the eggs of the old flock yielded a greater YFB than did those of the young flock ($P = 0.001$). At d 18 and at hatch, no effect of breeder age could be found on YFB weight, embryo and chick length, and chick weight. The embryos of the large eggs had consistently greater YFB than did those of the small eggs from d 14 to hatching (all $P < 0.001$). The chicks at hatch of the large eggs were also heavier ($P < 0.001$) and longer ($P < 0.001$) than those of the small eggs.

In Table 3, a significant interaction between breeder age and egg size was found for the absolute values of yolk absorption at hatch ($P = 0.024$). The yolk absorption was higher in both the small and large eggs of the old flocks than those of the young flock. This interaction disappeared when the yolk absorption at hatch was expressed by percentages of initial yolk content.

The yolk absorption at d 18 and at hatch was higher for the embryos and chicks of the old flock, compared with the young flock (Table 3). These results were observed for the absolute values and percentages of the initial yolk weight at d 18 (all $P < 0.001$). At hatch, the chicks of the old flock had a higher percentage of yolk absorption than did those of the young flock ($P < 0.001$). The yolk sac weight at 18 d of incubation was higher in the old flock compared with the young flock ($P = 0.005$). The RY weight at hatch was similar between breeder age groups. The embryos of the small eggs also had higher yolk absorption at d 18 than did those of the large eggs, both in absolute values ($P = 0.024$) and percentages ($P < 0.001$). The yolk sac weight at d 18 of the embryos of the small eggs was smaller than those of the large eggs ($P < 0.001$). At hatch, the chicks of the small eggs had higher percentages of yolk absorption than those of the large eggs ($P < 0.001$). However, the RY weight at hatch of the chicks of the large eggs was higher than that of the small eggs ($P < 0.001$). This result was also observed on a dry weight basis. In Table 4, the dry RY weight of the chicks of the large eggs was higher than that of the small eggs ($P < 0.001$). The dry YFB weight of the chicks of the old

flock was higher than that of the young flock ($P$ = 0.027). The chicks of the large eggs also had a higher dry YFB weight than did those of the small eggs ($P$ = 0.039). The chicks of the old flock had a higher YCR than did those of the young flock ($P$ < 0.001). The chicks of the small eggs also had a higher YCR than did those of the large eggs ($P$ = 0.029).

**Table 3.** Effect of breeder ages and egg sizes on yolk absorption at day 18, yolk absorption at hatch, and residual yolk (RY) weight at d 18 and at hatch (g) on wet-weight basis.[1]

| Effects[2] | Yolk absorption d18 | | RY weight at d 18 (g) | Yolk absorption at hatch | | RY weight at hatch (g) |
|---|---|---|---|---|---|---|
| | g[3] | %[4] | | g[3] | %[4] | |
| Breeder age × egg size | | | | | | |
| 29 S | 6.14 | 38.50 | 9.77 | 13.03[b] | 81.89 | 2.88 |
| 29 L | 5.06 | 31.28 | 11.11 | 12.23[b] | 75.63 | 3.94 |
| 53 S | 8.78 | 46.61 | 10.04 | 16.37[a] | 87.00 | 2.45 |
| 53 L | 8.38 | 40.43 | 12.34 | 16.96[a] | 81.82 | 3.76 |
| Main effect mean | | | | | | |
| Breeder age (wk) | | | | | | |
| Young (29) | 5.60[b] | 34.89[b] | 10.44[b] | 12.63 | 78.76[b] | 3.41 |
| Old (53) | 8.58[a] | 43.52[a] | 11.19[a] | 16.67 | 84.41[a] | 3.10 |
| Egg size | | | | | | |
| S | 7.46[a] | 42.56[a] | 9.90[b] | 14.69 | 84.45[a] | 2.67[b] |
| L | 6.72[b] | 35.85[b] | 11.72[a] | 14.59 | 78.73[b] | 3.85[a] |
| Source of variation | | | | *P*-value | | |
| Breeder age × egg size | 0.281 | 0.794 | 0.061 | 0.024 | 0.959 | 0.616 |
| Breeder age | <0.001 | <0.001 | 0.005 | <0.001 | <0.001 | 0.225 |
| Egg size | 0.024 | <0.001 | <0.001 | 0.721 | <0.001 | <0.001 |
| SEM[5] | 0.155 | 0.788 | 0.122 | 0.145 | 0.707 | 0.123 |

[a,b] Means within a column with different superscript letters differ ($P$ < 0.05). [1] For all variables measured, n = 5 fertile eggs at d 18 and 5 chicks at hatch/experimental unit (setter tray). [2] Ages and sizes: 29 S = young small (29 wk of age); 29 L = young large; 53 S = old small (53 wk of age); 53 L = old large; small (S) = 57-61 g; large (L) = 66-70 g. [3] Yolk absorption at d 18 and at hatch: fresh yolk weight (g, wet weight) – residual yolk (g, wet weight). [4] Yolk absorption (g, wet weight) / fresh yolk weight (g, wet weight) × 100. [5] SEM is based on a pooled estimate of variance.

**Table 4.** Effect of breeder ages and egg sizes on residual yolk (RY) weight, yolk-free body (YFB) weight and yolk conversion rate (YCR) at hatch on dry-weight basis.[1]

| Effects[2] | RY weight (g) | YFB weight (g) | YCR at hatch (g/g dry YFB)[3] |
|---|---|---|---|
| Breeder age × egg size | | | |
| 29 S | 1.31 | 8.21 | 0.85 |
| 29 L | 1.78 | 8.66 | 0.74 |
| 53 S | 1.19 | 8.70 | 0.96 |
| 53 L | 1.84 | 9.44 | 0.95 |
| Main effect mean | | | |
| Breeder age (wk) | | | |
| Young (29) | 1.55 | 8.43[b] | 0.79[b] |
| Old (53) | 1.52 | 9.07[a] | 0.95[a] |
| Egg size | | | |
| S | 1.25[b] | 8.46[b] | 0.90[a] |
| L | 1.81[a] | 9.05[a] | 0.84[b] |
| Source of variation | | *P*-value | |
| Breeder age × egg size | 0.372 | 0.593 | 0.081 |
| Breeder age | 0.777 | 0.027 | <0.001 |
| Egg size | <0.001 | 0.039 | 0.029 |
| SEM[4] | 0.051 | 0.120 | 0.013 |

[a,b] Means within a column with different superscript letters differ ($P < 0.05$). [1] For all variables measured, n = 5 chicks/experimental unit (setter tray). [2] Ages and sizes: 29 S = young small (29 wk of age); 29 L = young large; 53 S = old small (53 wk of age); 53 L = old large; small (S) = 57-61 g; large (L) = 66-70 g. [3] Yolk conversion rate [yolk absorption at hatch (g, dry weight)/YFB weight at hatch (g, dry weight)]. [4] SEM is based on a pooled estimate of variance.

# DISCUSSION

The observed egg compositions are in agreement with previous studies (O'Sullivan et al., 1991; Vieira and Moran, 1998a; Peebles et al., 2000; Hamidu et al., 2007). An increase in flock age within an egg weight group resulted in an increase in yolk content and a decrease in albumen content, whereas a larger egg size at a given age resulted in an increase in albumen content and a relative decrease in yolk content (see Table 1). This relationship led to an increase in the yolk: albumen ratio with increasing flock age and a decrease in the yolk: albumen ratio with increasing egg weight. The results indicate that the yolk size increases with flock age, and the amount of albumen increases with egg size. These resultsare in agreement with the results of Ahn et al. (1997), who demonstrated that 28-wk-old hens produced eggs with the lowest yolk: albumen ratio, whereas the highest ratio was observed in the eggs produced by 55- and 78-wk-old hens. Vieira and Moran (1998a) concluded that, at any given age, large eggs normally have greater albumen content and less yolk than do small eggs, which is in agreement with the present results.

The embryonic development during incubation was determined by length and the YFB weight of the embryos (Hill, 2001; Molenaar et al., 2008). At hatch, chick weight, YFB weight, and chick length were measured as indicators of chick quality (Willemsen et al., 2008). Several studies (Tufft and Jensen, 1991; Suarez et al., 1997: Vieira and Moran, 1998b) have shown an increase in chick weight with increasing flock age.The results of this study indicate that the YFB weight increased with flock age until 14 d of incubation. When the flock aged, the embryos from especially large eggs had a higher YFB weight at d 7 than did those from the eggs of the young flock. At d 14, theembryos of the eggs of the old flock had a higher YFB weight than did those of the young flock. However, at hatch, the YFB weight, chick weight, and chick length were similar for both age groups. The results of this study are similar to the studies of McNaughton et al. (1978) and Shanawany (1984), who demonstrated that flock age had no effect on chick weight from a particular egg size. In our results, the large eggs resulted in a greater YFB than the small eggs from d 14 to hatching. At hatch, the chicks of the large eggs also had longer chick length. This result is in agreement with the study of Mortola

and Al Awam (2010), who reported that the growth of embryos in small and large eggs followed different curves from d 11 onward. The present findings support the conclusion that embryo development, as expressed by YFB weight, chick weight, and chick length at hatch, is determined largely by egg size.



Byerly (1932) proposed that the growth rate of chick embryos parallels yolk sac weight. Gous (2010) suggested that yolk sac utilization could be one of the limiting factors for embryo development, especially within small eggs. These findings suggest an influence of yolk amount and yolk absorption on embryonic development and growth. The data obtained in our experiment indicate that this proposition might be limited until d 14. The eggs of the old flock, which had 3.73 g more yolk and 1.95 g more yolk solids content than those of the young flock, yielded embryos with a greater YFB at 14 d of incubation. However, the embryos and chicks of the eggs from the old breeder flock had higher yolk absorption, both in absolute values and percentages of initial yolk weight, than those of the eggs from the young breeder flock at d 18. At hatch, the absolute values of yolk absorption of the chicks from the small and large eggs of the old flock were higher than those of the young breeder flock. In addition, the chicks of the eggs from the old breeder flock had higher percentages of yolk absorption than those of the eggs from the young breeder flock. Noble and Cocchi (1990) suggested that the larger yolk sac membrane and vascular system of the large yolk compared with the small yolk may influence yolk nutrient utilization, which may result inhigher yolk absorption and a heavier YFB at d 14. However, these measurements were not conducted in the current experiment. In future studies, these measurements should be investigated in greater detail. The influence of breeder age on embryo development disappeared in the later stages of incubation. The present results lead to the conclusion that yolk availability and the rate of yolk absorption do not influence hatching chick length or total and YFB weight.

The effect of egg size on embryo development independent of yolk availability and yolk absorption was also observed from d 14 to hatching. At hatch, the large eggs, which had 1.07 g more yolk and 0.57 g higher yolk solids content than did the small eggs, produced chicks that yielded 4.24 g more YFB than did the chicks originating from the small eggs. These data complement the study of Wolanski et al. (2007), who concluded that egg size had a greater influence on chick weight than yolk size. Although the small eggs had smaller yolk sizes than

did the large eggs, the embryos of the small eggs had higher yolk absorption than did those of the large eggs. This result was observed at 18 d of incubation in absolute values and percentage of initial yolk weight. At hatch, the percentage of yolk absorption to the initial yolk weight was also higher in the chicks from the small eggs, compared with those of the large eggs. It is unclear why the embryos and chicks of the small eggs had a higher percentage of yolk absorption than did those of the large eggs. A possible explanation could be the contribution of albumen. Ovalbumin, which is 75% of the total albumen (Romanoff and Romanoff, 1949), is found in the amniotic fluid, serum, yolk, and organs, including the central nervous system of the chick embryo (Sugimoto et al., 1999). There are indications that the yolk protein intake per gram of YFB weight at hatch is higher in the small eggs than in the large eggs. (A. Nangsuay, unpublished data). It can be hypothesized that the larger amount of albumen and albumen solids content in large eggs compared with small eggs can result in less protein uptake from the yolk. In this case, less yolk would have been absorbed by the chicks of the large eggs compared with those of the small eggs. However, the nutrient uptake from the yolk sac of eggs differing in size should be further evaluated.

Several studies have demonstrated an increase of RY weight at hatch with the increase of flock age (Suarez et al., 1997; Vieira and Moran, 1998b; Sklan et al., 2003; Hamidu et al., 2007). However, in these trials, an increase in flock age was attributed to an increase in egg size. In our experiment, we observed that the chicks of different flock ages had comparable RY weight (wet and dry basis) when the egg weight was identical. The chicks of the large eggs had a larger RY weight than did chicks originating from the small eggs (wet and dry basis), regardless of flock age. This result indicates that breeder flock age had no direct effect on RY weight, but rather, the influence was through the egg weight.

Dry YFB weight was measured as an indicator of nutrient assimilation to the YFB. Furthermore, the YCR on a dry weight basis was calculated to evaluate the amount of yolk being utilized for building YFB. As a result of yolk absorption, the chicks from the old breeder flock had higher nutrient assimilation to YFB than chicks from the young breeder flock; this was observed both in dry YFB and YCR. This result is in agreement with the results obtained by O'Sullivan et al. (1991). These authors suggested that a higher availability of resources in the eggs, perhaps associated with changes in metabolic functions due to the advanced maturity of the

hens, can result in a higher nutrient assimilation to the embryos of old hens compared with those of young hens. The observed differences in YCR could have resulted in a difference in heat production. As the heat production was not measured in this experiment, this hypothesis cannot be evaluated. Another indication of a difference in heat production could be a difference in EST, as the air temperature was equal for all treatments. However, the results obtained did not show a difference in EST between the treatments. This result does not exclude the possibility that a difference in heat production existed, as the existing differences in EST may have been too small to be determined by the method used.

A better nutrient assimilation to YFB, even with an identical chick weight and RY weight of chicks originating from the old flock, may result in more available energy for growth (Tufft and Jensen, 1991) and it may result in a lower mortality rate after placement on the farm, compared with that for chicks originating from young breeder hens (McNaughton et al., 1978). It may be possible to interpret the higher yolk absorption and YCR of embryos and chicks coming from small eggs, compared with large eggs, in the same way as the differences in these parameters between young and old flocks. However, further study of yolk nutrient composition and assimilation to YFB as a function of breeder age and egg size is needed to evaluate the interaction between these parameters.

In conclusion, an increase in yolk size and yolk absorption by the function of breeder age affects early embryonic development. However, the limited space of the eggs may be a restriction factor for embryonic development during the later stages of incubation (Wiley, 1950). This effect can explain why the development measured by YFB weight, chick weight, and chick length of chicks from eggs of identical size was comparable regardless of breeder flock age. Egg size is a major factor determining YFB weight, chick weight, and chick length at hatch, irrespective of yolk availability and yolk absorption.

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# CHAPTER 3

# Energy utilization and heat production of embryos from eggs originating from young and old broiler breeder flocks

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# ABSTRACT

Two experiments were conducted to study the interaction between breeder age and egg size on the energy utilization (experiment 1) and heat production (experiment 2) of broiler embryos. In experiment 1, a total of 4,800 Ross-308 hatching eggs from 2 breeder ages (29 and 53 wk of age, or young and old) and, within each age, 2 egg sizes (57 to 61 g and 66 to 70 g, or small and large) were used. In experiment 2, a total of 240 Ross-308 hatching eggs from 2 breeder flocks at 29 (young) and 53 (old) wk of age, and which were selected from the same egg weight range (58 to 61 g), were tested in 2 replicate chambers. In experiment 1, it was shown that the amount of yolk relative to albumen was higher in the old flock eggs, and this effect was more pronounced in the large eggs. The old flock eggs, especially the larger egg size, contained more energy as a result of a greater yolk size. Energy utilization of the embryos was positively related to yolk size and the amount of energy transferred to yolk-free body (YFB) was largely determined by the available egg energy. The efficiency of converting egg energy into chick body energy ($E_{YFB}$) was equal for both egg sizes and both breeder age groups. Chick YFB weight of young and old flock eggs was equal. However, dry YFB weight of chicks from old flock eggs was higher than in chicks from young flock eggs, which was associated with more protein and fat content and thus more energy accumulated into YFB. As a consequence, embryos derived from old flock eggs produced more heat from d 16 of incubation onward than those of the young flock eggs. In conclusion, the higher energy deposition into chick YFB of old flock eggs, leading to higher embryonic heat production, is the result of a higher amount of available energy in the egg and is not due to changes in $E_{YFB}$.

**Key words:** breeder age, egg size, energy utilization, heat production

# INTRODUCTION



During incubation, embryos use yolk lipids as the main nutritional supply for energy production, accounting for 90% of the total requirements (Romanoff, 1967; Noble and Cocchi, 1990), whereas the other 10% is derived from proteins and carbohydrates (Fiske and Boyden, 1926). The biochemical processes to convert egg nutrients into body tissue require oxygen and produce carbon dioxide, metabolic water, and metabolic heat as by-products (Etches, 1996). It has been shown that changes in embryonic heat production (**HP**) influence the obtained eggshell temperature (**EST**; Meijerhof and Van Beek, 1993), which can affect hatchability and chick quality (French, 1997; Lourens et al., 2005). The HP of the embryos is determined by the total amount of energy used from the eggs and the efficiency of converting this energy into chick body tissue (**$E_{YFB}$**; Ar et al., 1987; Pearson et al., 1991). Recently, Lourens et al. (2011) demonstrated that only when eggs of the same size are incubated at different EST, HP is influenced by changes in $E_{YFB}$. However, differences in HP between small and large eggs incubated at the same EST are largely due to differences in the amount of energy used, rather than to differences in $E_{YFB}$. In an earlier study, Lourens et al. (2006) reported that large eggs contain more nutrients to be used for conversion to yolk-free body (**YFB**) than small eggs. This means that at a fixed EST, only variation in egg energy content can lead to differences in YFB energy deposition of the embryos and consequently in a different embryonic HP.

Several studies have shown an increase in yolk size with increasing flock age, whereas egg size is particularly determined by the amount of albumen (O'Sullivan et al., 1991; Vieira and Moran, 1998b; Peebles et al., 2000; Hamidu et al., 2007). As egg nutrients are stored in albumen and yolk, alterations of this ratio can affect energy content of the eggs and may lead to differences in embryonic HP, which may affect incubation results. However, the effects of flock age and egg size in most studies are strongly confounded, because older hens produce larger eggs. Information about the independent effects of breeder age and egg size on egg energy content, energy utilization, $E_{YFB}$, and the consequences for embryonic HP is limited or even absent. Therefore, the aim of this study was to examine effects of breeder age and egg size independent of each other on egg energy content and

energy utilization. Furthermore, effect of breeder age at a fixed egg size on embryonic HP was included in this study.

# MATERIALS AND METHODS

Two experiments were conducted. The first experiment aimed to examine the influence of breeder age and egg size on egg energy content and energy utilization during incubation. This study was conducted in a commercial hatchery in Thailand. The results of this experiment regarding embryonic development and yolk utilization are published by Nangsuay et al. (2011). The second experiment was performed to evaluate the effect of breeder age at a fixed egg weight and EST on heat production during incubation. This experiment was conducted at Wageningen University, Wageningen, the Netherlands.

### *Experiment 1*

#### *Experimental Design and Hatching Eggs*

A 2 × 2 factorial randomized complete block design was conducted using 2 breeder flock ages (29 and 53 wk; young and old) and 2 egg sizes (small and large). A total of 4,800 Ross-308 hatching eggs from 2 commercial broiler breeder flocks were obtained over a 3-d period. Breeder flocks of 29 and 53 wk received a diet with 2,800 kcal of ME/kg and 16% CP and 2,785 kcal of ME/kg and 15% CP, respectively. Both breeder flocks were managed following the recommendations of the breeding company.

At the days of collection, approximately 45,000 hatching eggs with an average egg weight of 56.85 g from the young flock, and approximately 25,000 hatching eggs with an average egg weight of 67.15 g from the old flock were available. From both age groups, 1,200 eggs were selected within the weight range of 57 to 61 g, and 1,200 eggs within the weight range of 66 to 70 g. Eggs between 57 and 61 g were classified as small, whereas eggs between 66 and 70 g were classified as large. This selection resulted in 4 groups of 1,200 eggs each: young small (29 small), young large (29 large), old small (53 small), and old large (53

large). Per treatment, 8 replicate incubator trays each containing 150 eggs were used. Eggs were stored on the trays in an egg storage room for 3 to 5 d at a temperature of 18 to 20ºC and a RH of 50 to 60%.

***Incubation***

Based on results of preliminary EST measurements, specific positions in 2 single-stage HatchTech setters (HatchTech B.V., Veenendaal, the Netherlands) with a capacity of 57,600 eggs each were used to obtain a uniform embryo temperature for experimental eggs. At the day of setting, the 32 incubator trays were randomly distributed over the assigned places at the specified positions, 16 trays per setter (4 trays of each treatment). The remaining trays in the incubators were filled with hatching eggs that were not part of the experiment to ensure uniform airflow. The temperature set point profiles for both setters were applied to obtain an EST of 37.8°C (Lourens et al., 2005). The EST, which was measured by an infrared Braun ThermoScan IRT 4520 ExacTemp (Braun GmbH, Kronberg, Germany), was used as a reference for embryo temperature (Hulet et al., 2007). The infrared thermometers remained in the setters for the entire experiment to avoid temperature fluctuation and inaccurate temperature readings (Leksrisompong et al., 2007). At incubation d 6, 8, 10, 12, 14, and 17, the EST of 24 fertile eggs (3 eggs/tray) was measured for each treatment. Mean EST values of each setter were calculated, and adjustments of the temperature set point profiles were made to obtain the required EST. The difference between average EST and target EST was maintained at a maximum of 0.2°C. The other incubator set points ($CO_2$, RH, and opening of the setter valves) were set according to the normal procedures of the hatchery. At incubation d 18, eggs were candled, and fertile eggs were transferred to hatcher baskets and placed in 2 hatchers. The temperature of both hatchers was set at 36.5°C. At the day of hatch, hatcher trays were pulled according to the standard procedure of the hatchery for chick processing.

***Measurements***

Prior to setting, 5 eggs from each replicate incubator tray (considered as experimental unit) were randomly taken to determine fresh egg composition. Eggs

were weighed and broken to separate the shell, yolk, and albumen. Yolk and shell weight were measured. Albumen weight was calculated by subtracting the yolk and shell weight from the egg weight. Albumen and yolk were stored separately at –20ºC for further analysis. At pulling time (516 to 518 h after start of incubation), 5 chicks from each replicate hatcher basket (considered as the experimental unit) were randomly selected. Chicks were weighed, and then killed by cervical dislocation. Chicks were opened to determine residual yolk (**RY**) and yolk-free body (**YFB**) weight. The YFB was calculated as chick weight – RY weight. The RY and YFB were stored at −20ºC for further analysis.

Samples of albumen and yolk (fresh eggs) and RY and YFB (chicks) of each experimental unit were pooled and homogenized. Thereafter, samples were analyzed for DM, ash, CP, and crude fat (AOAC, 1990). Carbohydrate (**CHO**) content was calculated with the equation: 100 − % moisture − % ash − % CP − % crude fat. Energy content of each sample was calculated using energy densities for protein, fat, and CHO of 16.8, 37.8, and 16.8 MJ/kg of DM, respectively (International System of Units, 1998). Energy utilization, energy lost, and efficiency of energy utilization during incubation were calculated as follows:

energy utilization = albumen (kJ) + yolk (kJ) – RY (kJ);

energy lost = [albumen (kJ) + yolk (kJ)] – [YFB (kJ) + RY (kJ)];

$$E_{YFB} = \frac{\text{YFB (kJ)}}{\text{Albumen (kJ) + yolk (kJ) - RY (kJ)}} \times 100\%$$

***Experiment 2***

Two trials (replicates) were conducted by utilizing a total of 240 first grade Ross-308 hatching eggs from 2 breeder flocks aged 29 wk (young flock) and 53 wk (old flock). Eggs within each flock age were selected within a range of 58 to 61 g. Per trial, 60 eggs from the young and old flock were separately incubated at a constant EST of 37.8°C and RH of 55% in 1 of 2 identical small open circuit climate respiration chambers (**CRC**; Lourens et al., 2006). To determine HP, oxygen and carbon dioxide concentrations were measured every 9 min in both chambers and in fresh air. Carbon dioxide concentration was measured with a nondispersive infrared $CO_2$ analyzer (type Uras 3G, Hartmann and Braun,

Frankfurt, Germany). Oxygen concentration was measured with a paramagnetic oxygen analyzer (type ADC7000, Analytical Development Co. Ltd., Hertfordshire, UK). The refreshed air volume was 2 L/min during the 18 d of incubation. Air volumes were measured with a Schlumberger G1.6 dry gas meter (Schlumberger, Dordrecht, the Netherlands). Eggs were candled at d 11 of incubation; infertile eggs were removed and opened to identify true fertility and age of dead embryos as described by Lourens et al. (2006). The HP was calculated as HP (kJ) = 16.18 × $O_2$ consumption (L) + 5.02 × $CO_2$ production (L) (Romijn and Lokhorst, 1961) and adjusted for fertility and embryonic mortality. Values were expressed in milliwatts per living embryo (fertile egg).



***Statistical Analyses***

In experiment 1, incubator tray was used as experimental unit for fresh egg variables and hatcher basket as experimental unit for variables at hatch. Variables were analyzed with the GLM procedure of the SAS 9.2 software package (SAS Institute, 2009). Variables obtained before incubation included egg weight, yolk weight, albumen weight, yolk: albumen ratio, DM, protein, fat, CHO, and energy content of albumen, yolk, and albumen + yolk were analyzed using model 1: $Y_{ijk} = \mu + A_i + B_j + AB_{ij} + e_{ijk}$, where $Y_{ijk}$ is the dependent variable, $\mu$ is the overall mean, $A_i$ is the effect of breeder hen age (i = 29 or 53 wk), $B_j$ is the effect of egg size (j = small or large), $AB_{ij}$ is the interaction between breeder hen age and egg size, and $e_{ijk}$ is the error term. Analyses of variables at hatch included RY and YFB weight, DM, protein, fat, CHO, and energy content in RY and YFB, energy utilization, energy lost, and $E_{YFB}$ were performed with model 1, added with setter as block (because of the use of 2 setters).

For heat production in experiment 2, the CRC was used as the experimental unit in the statistical analyses. The HP per day from d 0 to 18 d of incubation was analyzed using the MIXED procedure of SAS 9.2 software package (SAS Institute, 2009) for repeated measurements. The model used was $Y_{ijkl} = \mu + A_i + B_j + C_k + (A_i \times C_k) + e_{ijkl}$, where $Y_{ijkl}$ is the heat production, $\mu$ is the overall mean, $A_i$ is breeder hen age (i = 29 or 53 wk), $B_j$ is batch (j = 1 or 2), $C_k$ is incubation day (k = d 0 to d 18), $A_i \times C_k$ is the interaction between the breeder age and incubation day, and $e_{ijkl}$ is the error term. Distributions of the means and

residuals were examined to check model assumptions. Least squares means were compared using Bonferroni adjustments for multiple comparisons. Values are expressed as least squares means. In all cases, a difference was considered significant at $P \leq 0.05$. Pearson correlation analysis without correction for treatments was performed for the relationship between yolk weight and energy utilization.

# RESULTS

### *Egg Composition*

An interaction between breeder age and egg size was observed for egg weight ($P = 0.008$; Table 1). Large eggs of the old flock were 0.5 g heavier than large eggs of the young flock, whereas egg weight of small eggs from both flock ages was comparable. An interaction between breeder age and egg size was also found for yolk weight ($P < 0.001$), albumen weight ($P = 0.001$), and yolk: albumen ratio ($P = 0.038$). Eggs of the old flock contained more yolk and less albumen and had a higher yolk to albumen ratio than those of the young flock, and these effects were more pronounced in the large eggs.

### *Nutrient Density and Energy Content in the Albumen and Yolk of Fresh Eggs and YFB and RY of Chicks*

Albumen from eggs of the young flock contained more DM ($P < 0.001$) and protein ($P < 0.001$) and albumen + yolk contained more protein ($P = 0.002$) than those of the old flock (Table 2). Significant interactions between breeder age and egg size were found for albumen CHO content ($P = 0.040$), yolk DM ($P < 0.001$), fat content ($P = 0.001$), and albumen + yolk DM ($P < 0.001$) and fat content ($P < 0.001$). The large eggs of the young flock contained more albumen CHO than the other groups, which were not different from each other. In comparison with the same egg size of the young flock, yolk DM, and yolk fat content were lower in small eggs, but higher in large eggs of the old flock. In

albumen + yolk, eggs of the old flock contained more DM and fat than those of the young flock, and this difference was more pronounced in the large eggs.

**Table 1.** Effects of breeder ages at 29 (young) and 53 (old) wk and egg sizes of small (57 to 61 g) and large (66 to 70 g) on fresh egg composition.[1]

| Effect | Egg (g) | Yolk (g) | Albumen (g) | Yolk:Albumen |
|---|---|---|---|---|
| Breeder age × egg size | | | | |
| 29 small | 58.31[c] | 15.91[c] | 35.88[c] | 0.44[c] |
| 29 large | 65.59[b] | 16.17[c] | 42.16[a] | 0.38[d] |
| 53 small | 58.39[c] | 18.83[b] | 32.86[d] | 0.57[a] |
| 53 large | 66.09[a] | 20.72[a] | 37.83[b] | 0.55[b] |
| SEM | 0.07 | 0.16 | 0.18 | 0.01 |
| Main effect | | | | |
| Breeder age (wk) | | | | |
| Young (29) | 61.95 | 16.04 | 39.02 | 0.41 |
| Old (53) | 62.24 | 19.77 | 35.35 | 0.56 |
| SEM | 0.05 | 0.11 | 0.13 | 0.01 |
| Egg size | | | | |
| Small | 58.35 | 17.37 | 34.37 | 0.51 |
| Large | 65.84 | 18.44 | 39.99 | 0.47 |
| SEM | 0.05 | 0.11 | 0.13 | 0.01 |
| Source of variation | | | *P*-value | |
| Breeder age × egg size | 0.008 | <0.001 | 0.001 | 0.038 |
| Breeder age | <0.001 | <0.001 | <0.001 | <0.001 |
| Egg size | <0.001 | <0.001 | <0.001 | <0.001 |

[a-d] Means within a column and factor lacking a common superscript differ ($P \leq 0.05$).

[1] For all variables measured, n = 8 per treatment.

**Table 2.** Effects of breeder ages at 29 (young) and 53 (old) wk and egg sizes of small (57 to 61 g) and large (66 to 70 g) on DM, protein, fat, and carbohydrates (CHO) in albumen and yolk and albumen + yolk (g, dry basis/ g, wet weight).[1]

| Effect | Albumen[2] | | | | Yolk[2] | | | | Albumen + Yolk[2] | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | DM | Protein | Fat | CHO | DM | Protein | Fat | CHO | DM | Protein | Fat | CHO |
| Breeder age × egg size | | | | | | | | | | | | |
| 29 small | 0.132 | 0.115 | 0.001 | 0.009$^{b}$ | 0.518$^{a}$ | 0.159 | 0.313$^{a}$ | 0.029 | 0.250$^{b}$ | 0.129 | 0.097$^{b}$ | 0.015 |
| 29 large | 0.133 | 0.114 | 0.001 | 0.012$^{a}$ | 0.502$^{b}$ | 0.159 | 0.299$^{c}$ | 0.028 | 0.235$^{c}$ | 0.126 | 0.084$^{c}$ | 0.016 |
| 53 small | 0.120 | 0.106 | 0.001 | 0.008$^{b}$ | 0.505$^{b}$ | 0.158 | 0.303$^{bc}$ | 0.027 | 0.260$^{a}$ | 0.125 | 0.111$^{a}$ | 0.015 |
| 53 large | 0.119 | 0.106 | 0.001 | 0.008$^{b}$ | 0.519$^{a}$ | 0.159 | 0.310$^{ab}$ | 0.033 | 0.261$^{a}$ | 0.125 | 0.110$^{a}$ | 0.017 |
| SEM | 0.001 | 0.001 | 0.000 | 0.001 | 0.003 | 0.001 | 0.003 | 0.002 | 0.002 | 0.001 | 0.002 | 0.001 |
| Main effect | | | | | | | | | | | | |
| Breeder age (wk) | | | | | | | | | | | | |
| 29 | 0.132$^{a}$ | 0.114$^{a}$ | 0.001 | 0.011 | 0.509 | 0.159 | 0.305 | 0.028 | 0.243 | 0.127$^{a}$ | 0.090 | 0.016 |
| 53 | 0.120$^{b}$ | 0.106$^{b}$ | 0.001 | 0.008 | 0.512 | 0.159 | 0.306 | 0.029 | 0.261 | 0.125$^{b}$ | 0.111 | 0.016 |
| SEM | 0.001 | 0.001 | 0.000 | 0.001 | 0.002 | 0.001 | 0.002 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 |
| Egg size | | | | | | | | | | | | |
| Small | 0.126 | 0.110 | 0.001 | 0.008 | 0.511 | 0.159 | 0.308 | 0.028 | 0.255 | 0.127 | 0.104 | 0.015 |
| Large | 0.126 | 0.109 | 0.001 | 0.009 | 0.511 | 0.159 | 0.305 | 0.030 | 0.248 | 0.125 | 0.097 | 0.016 |
| SEM | 0.001 | 0.001 | 0.000 | 0.001 | 0.002 | 0.001 | 0.002 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 |
| Source of variation | *P*-value | | | | | | | | | | | |
| Breeder age × egg size | 0.628 | 0.515 | 0.507 | 0.040 | <0.001 | 0.602 | 0.001 | 0.114 | <0.001 | 0.144 | <0.001 | 0.622 |
| Breeder age | <0.001 | <0.001 | 0.189 | 0.002 | 0.585 | 0.488 | 0.686 | 0.519 | <0.001 | 0.002 | <0.001 | 0.943 |
| Egg size | 1.000 | 0.515 | 0.189 | 0.141 | 0.895 | 0.728 | 0.342 | 0.202 | 0.001 | 0.108 | <0.001 | 0.146 |

$^{a\text{-}c}$ Means within a column and factor lacking a common superscript differ ($P \leq 0.05$). [1] For all variables measured, n = 8 per treatment. [2] DM = g (dry basis) of albumen, yolk, and albumen + yolk/g of wet weight of albumen, yolk, and albumen + yolk. Protein, fat, and CHO = g (dry basis) of protein, fat, and CHO in the albumen, yolk, and albumen + yolk/g of wet weight of albumen, yolk, and albumen + yolk.

**Table 3.** Effects of breeder ages at 29 (young) and 53 (old) wk and egg sizes of small (57 to 61 g) and large (66 to 70 g) on RY and YFB weight (g, wet weight), DM, protein, fat, and carbohydrates (CHO) in residual yolk (RY) and yolk-free body (YFB; g, dry basis/g, wet weight).[1]

| Effect | RY[2] | | | | | YFB[2] | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Weigh | DM | Protein | Fat | CHO | Weight | DM | Protein | Fat | CHO |
| Breeder age × egg size | | | | | | | | | | |
| 29 small | 2.883 | 0.459 | 0.287 | 0.096 | 0.046 | 37.400 | 0.219 | 0.153 | 0.036 | 0.014 |
| 29 large | 4.069 | 0.450 | 0.304 | 0.076 | 0.045 | 41.376 | 0.201 | 0.140 | 0.031 | 0.014 |
| 53 small | 2.450 | 0.486 | 0.250 | 0.165 | 0.038 | 37.515 | 0.231 | 0.154 | 0.047 | 0.014 |
| 53 large | 3.758 | 0.493 | 0.269 | 0.156 | 0.043 | 41.771 | 0.226 | 0.149 | 0.046 | 0.013 |
| SEM | 0.246 | 0.008 | 0.012 | 0.018 | 0.002 | 0.290 | 0.004 | 0.002 | 0.003 | 0.001 |
| Main effect | | | | | | | | | | |
| Breeder age (wk) | | | | | | | | | | |
| 29 | 3.476 | 0.455[b] | 0.296[a] | 0.086[b] | 0.046 | 39.388 | 0.210[b] | 0.146[b] | 0.033[b] | 0.014 |
| 53 | 3.105 | 0.489[a] | 0.260[b] | 0.161[a] | 0.041 | 39.643 | 0.228[a] | 0.152[a] | 0.046[a] | 0.013 |
| SEM | 0.175 | 0.006 | 0.009 | 0.012 | 0.002 | 0.206 | 0.003 | 0.001 | 0.002 | 0.001 |
| Egg size | | | | | | | | | | |
| Small | 2.668[b] | 0.473 | 0.269 | 0.131 | 0.042 | 37.457[b] | 0.225[a] | 0.153[a] | 0.042 | 0.014 |
| Large | 3.914[a] | 0.472 | 0.287 | 0.116 | 0.044 | 41.573[a] | 0.213[b] | 0.145[b] | 0.038 | 0.013 |
| SEM | 0.174 | 0.006 | 0.009 | 0.012 | 0.002 | 0.206 | 0.003 | 0.001 | 0.002 | 0.001 |
| Source of variation | *P*-value | | | | | | | | | |
| Breeder age × egg size | 0.807 | 0.364 | 0.949 | 0.749 | 0.290 | 0.634 | 0.216 | 0.087 | 0.582 | 0.916 |
| Breeder age | 0.144 | <0.001 | 0.008 | <0.001 | 0.091 | 0.388 | 0.001 | 0.021 | 0.001 | 0.702 |
| Egg size | <0.00 | 0.937 | 0.161 | 0.420 | 0.435 | <0.001 | 0.019 | <0.001 | 0.338 | 0.756 |

[a,b] Means within a column and factor lacking a common superscript differ ($P \leq 0.05$). [1] For all variables measured, n = 8 per treatment. [2] DM = g (dry basis) of RY and YFB/g wet weight of RY and YFB. Protein, fat, and CHO = g (dry basis) of protein, fat, and CHO in the RY and YFB/g wet weight of RY and YFB.

In Table 3, effects of breeder age and egg size on DM, protein, fat, and CHO content of the RY and YFB are described. No interaction between breeder age and egg size was found for weight and nutrient content of RY and YFB. The RY weight of chicks from old and young flock eggs was comparable ($P = 0.144$). The RY of chicks from the old flock contained more DM and fat (both $P < 0.001$), but less protein ($P = 0.008$) than that of the young flock. The YFB weight of chicks from the young and old flock eggs was similar. The YFB of chicks from the old flock eggs contained more DM ($P = 0.001$), protein ($P = 0.021$), and fat ($P = 0.001$) than that of the young flock. Chicks of the large eggs had higher RY and YFB weights (both $P < 0.001$), but YFB contained less DM ($P = 0.019$) and protein ($P < 0.001$) than that of the small eggs.

In Table 4, effects of breeder age and egg size on energy content of the fresh eggs and chicks are described. In fresh eggs, an interaction between breeder age and egg size was found for the total amount of energy in albumen (P = 0.028), yolk ($P < 0.001$), and albumen + yolk ($P < 0.001$). Eggs of the old flock had less energy in the albumen, and more energy in the yolk and albumen + yolk than those of the young flock, and these effects were more pronounced in the large eggs. Chicks of old flock eggs had more energy in the YFB ($P = 0.001$) and YFB + RY ($P < 0.001$) than those of the young flock (Table 4). The large eggs yielded chicks with more energy in the RY ($P < 0.001$) and YFB + RY ($P = 0.005$) than the small eggs, but had comparable energy content in the YFB.

### *Energy Utilization, Energy Lost, $E_{YFB}$, and HP*

An interaction between breeder age and egg size was found for the amount of energy used ($P < 0.001$; Table 5). Chicks of the old flock used more energy than those of the young flock, and this effect was more pronounced in the large eggs. Breeder age and egg size had no effect on $E_{YFB}$. Energy lost during the incubation process tended to be higher for the old flock than for the young flock ($P = 0.059$). Figure 1 shows that the amount of energy used was positively related to yolk size ($R^2 = 0.88$). An interaction between breeder age and incubation day was found for HP ($P < 0.001$; Figure 2). The HP of embryos from the old flock was higher than those of the young flock from d 16 of incubation onward.

**Table 4.** Effects of breeder ages at 29 (young) and 53 (old) wk and egg sizes of small (57 to 61 g) and large (66 to 70 g) on energy content in albumen, yolk, albumen + yolk of fresh egg, and yolk-free body (YFB), residual yolk (RY), YFB + RY of the chick (kJ).[1]

| Effect | Fresh egg (kJ) | | | Chick (kJ) | | |
|---|---|---|---|---|---|---|
| | Albumen | Yolk | Albumen+Yolk | YFB | RY | YFB+RY |
| Breeder age × egg | | | | | | |
| 29 small | 76.15[b] | 238.28[c] | 314.43[c] | 157.06 | 26.15 | 183.20 |
| 29 large | 89.75[a] | 233.18[c] | 322.94[c] | 157.62 | 35.21 | 192.83 |
| 53 small | 63.32[d] | 274.30[b] | 337.62[b] | 173.79 | 26.89 | 200.67 |
| 53 large | 72.82[c] | 310.24[a] | 383.06[a] | 187.49 | 41.44 | 228.93 |
| SEM | 0.88 | 3.57 | 3.29 | 6.42 | 1.97 | 6.22 |
| Main effect | | | | | | |
| Breeder age (wk) | | | | | | |
| 29 | 82.49 | 235.90 | 318.39 | 157.32[b] | 30.38 | 187.69[b] |
| 53 | 68.07 | 292.27 | 360.34 | 180.64[a] | 34.16 | 214.80[a] |
| SEM | 0.62 | 2.52 | 2.33 | 4.54 | 1.39 | 4.39 |
| Egg size | | | | | | |
| Small | 69.73 | 256.29 | 326.02 | 165.42 | 26.52[b] | 191.94[b] |
| Large | 80.72 | 274.28 | 355.00 | 173.55 | 38.53[a] | 212.08[a] |
| SEM | 0.62 | 2.52 | 2.33 | 4.54 | 1.39 | 4.39 |
| Source of variation | | | *P*-value | | | |
| Breeder age × egg | 0.028 | <0.001 | <0.001 | 0.310 | 0.173 | 0.143 |
| Breeder age | <0.001 | <0.001 | <0.001 | 0.001 | 0.087 | <0.001 |
| Egg size | <0.001 | <0.001 | <0.001 | 0.280 | <0.001 | 0.005 |

[a-d] Means within a column and factor lacking a common superscript differ ($P \leq 0.05$). [1] For all variables measured, n = 8 per treatment.

**Table 5.** Effects of breeder ages at 29 (young) and 53 (old) weeks and egg sizes of small (57 to 61 g) and large (66 to 70 g) on energy utilization (kJ), energy lost (kJ), and $E_{YFB}$ (%).[1]

| Effect | Energy utilization (kJ) | Energy lost (kJ) | $E_{YFB}$ (%) |
|---|---|---|---|
| Breeder age × egg size | | | |
| 29 small | 288.28[c] | 131.22 | 54.52 |
| 29 large | 287.72[c] | 130.11 | 54.75 |
| 53 small | 310.73[b] | 136.95 | 56.03 |
| 53 large | 341.62[a] | 154.13 | 54.97 |
| SEM | 3.65 | 7.46 | 2.19 |
| Main effect | | | |
| Breeder age (wk) | | | |
| 29 | 288.02 | 130.70 | 54.63 |
| 53 | 326.18 | 145.54 | 55.49 |
| SEM | 2.58 | 5.27 | 1.55 |
| Egg size | | | |
| Small | 299.51 | 134.09 | 55.28 |
| Large | 316.47 | 142.92 | 54.87 |
| SEM | 2.58 | 5.27 | 1.55 |
| Source of variation | | *P*-value | |
| Breeder age × egg size | <0.001 | 0.240 | 0.781 |
| Breeder age | <0.001 | 0.059 | 0.685 |
| Egg size | <0.001 | 0.280 | 0.837 |

[a-c] Means within a column and factor lacking a common superscript differ ($P \leq 0.05$).

[1] For all variables measured, n = 8 per treatment. $E_{YFB}$ = efficiency of energy utilization.

# DISCUSSION



### *Egg Composition and Energy Content*

Although eggs were selected for an equal average egg weight in both age groups, large eggs of the old flock were 0.5 g heavier than those of the young flock. However, this difference was relatively small, and we do not expect a significant effect on the results obtained. Egg composition showed a higher yolk weight relative to albumen weight for both egg size groups of old flock eggs compared with eggs of the young flock, and was more pronounced in large eggs. This observation is consistent with results from previous studies, which demonstrated that the progressive increase in egg weight with breeder age was largely due to an increase of yolk relative to albumen (O'Sullivan et al., 1991; Peebles et al., 2000). The larger egg size of both young and old flock eggs was associated with a higher albumen content (Vieira and Moran, 1998b), and this difference was more pronounced in young flock eggs. It has been suggested that an increase of egg size along with age is a consequence of a decrease in egg production, which is partly caused by a reduction in the number of follicles reaching the final phase of rapid growth (Johnson, 2000). Because fewer follicles receive a proportionately greater quantity of yolk, yolk size of old flock eggs increases at a higher rate compared with an increase of albumen, resulting in a decreased proportion of albumen content (Johnston and Gous, 2007).

The higher yolk energy content observed in both egg size groups of the old flock eggs can probably be related to yolk nutrient density and especially to the yolk size. O'Sullivan et al. (1991) reported that an increase of yolk size with flock age is associated with an increased accumulation of DM content. Compared with the same egg size of the young flock eggs, the current results showed that yolk DM and yolk fat content were lower in small eggs, but higher in large eggs of the old flock. Because older flock eggs are normally larger, this finding agrees with Vieira and Moran (1998a), who reported a higher DM content in egg yolk derived from an old breeder flock (62 wk) compared with a young breeder flock (27 wk). Yadgary et al. (2010) observed a higher proportion of fat in fresh yolk derived from old flock eggs (50 wk) than that of young flock eggs (30 wk). These results suggest

that older flock hens deposit more yolk and fat in their eggs, particularly in large eggs, than younger flock hens. This is associated with changes in yolk size and in energy content of the yolk.

The higher albumen DM and protein content found in young flock eggs compared with old flock eggs is consistent with the study of Ahn et al. (1997). The higher albumen energy in eggs from the young flock, especially for the larger egg size, was due to both higher albumen content and albumen density. Ar et al. (1987) demonstrated that albumen has a limited contribution to the total amount of egg energy, due to low DM content (12 to 13%) and an almost complete absence of fat as shown in present results. Egg yolk contains about 50 to 51% DM and 30 to 31% fat, providing approximately 72 to 81% of the total energy in the egg. This makes yolk function as the main energy source for embryonic development (Romanoff and Romanoff, 1949). Because in our study the ratio of yolk: albumen increased with flock age, eggs derived from the old flock, especially the larger eggs, contained more energy than the young flock eggs. It can be concluded that the amount of energy in the eggs is determined mainly by yolk energy content through changes of yolk nutrient density and particularly yolk size.

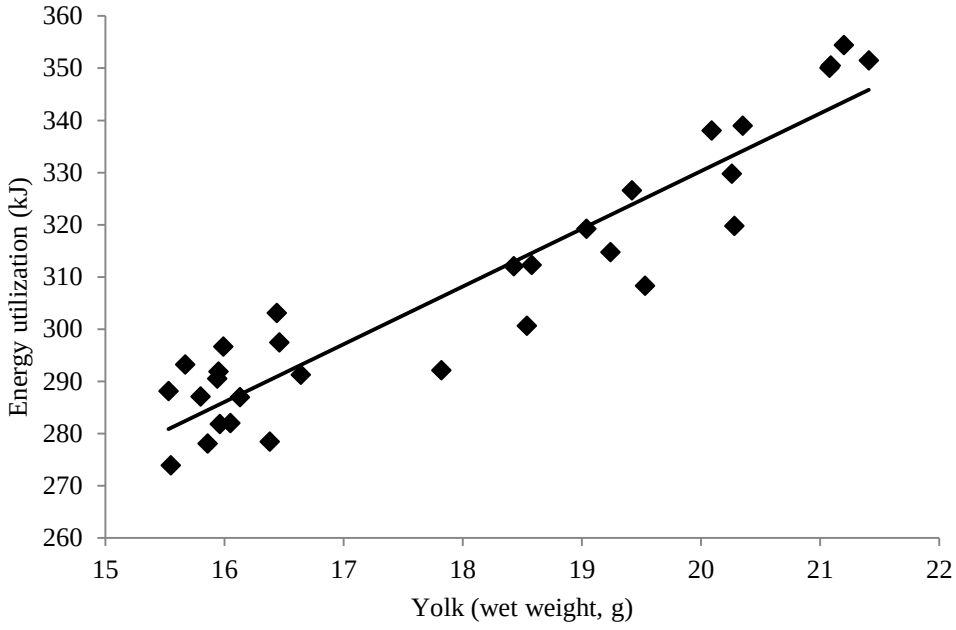


**Figure 1.** Amount of energy used by the embryos in relation with changes of yolk weight ($R^2$ = 0.88). Values are means of each experimental unit (setter tray or hatcher basket).

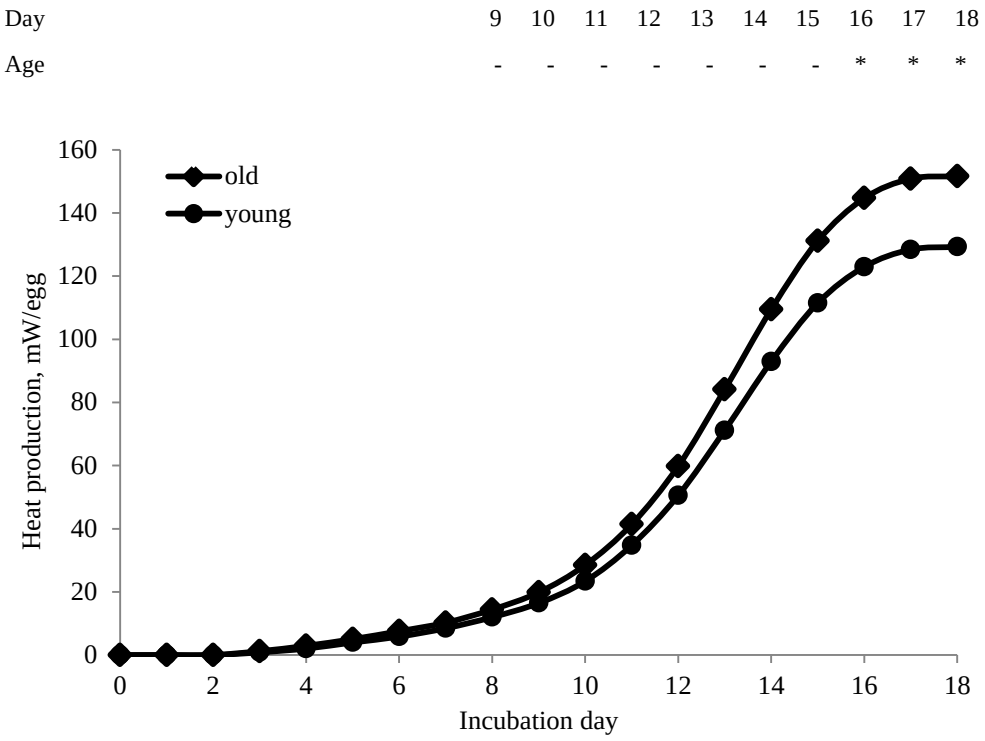


**Figure 2.** Effects of breeder ages at 29 (young) and 53 (old) wk on heat production (HP) in eggs weighing 58 to 61 g. The legend indicates the level of significance (* $P \leq 0.05$, pooled SEM = 4.48 mW).

### *Energy Utilization, $E_{YFB,}$ and Its Consequence on HP*

During incubation, oxidation of yolk fat contributes to approximately 90% of the total energy requirements of the embryos (Romanoff, 1967; Noble and Cocchi, 1990). This suggests an influence of available yolk energy on energy utilization of embryos, which is also demonstrated by the clear correlation between yolk size and energy utilization (Figure 1). Results of our experiment show that embryos of old flock eggs and then especially of the larger eggs used more energy than those of the young flock eggs. These results suggest that, at a fixed EST, an alteration of yolk energy content through variation in yolk size can lead to differences in energy utilization of the embryos.

Embryos of old flock eggs transferred more energy into chick YFB than those of young flock eggs, whereas embryos of small and large eggs transferred equal amounts of energy into YFB. These results suggest that the amount of energy incorporated into chick YFB is a reflection of energy availability in fresh eggs. Furthermore, at a fixed EST, this available energy is converted into chick YFB with equal efficiency ($E_{YFB}$). Breeder age and egg size had no effect on $E_{YFB.}$ Approximately 54 to 56% of energy used by the embryos is converted into chick YFB. Lourens et al. (2006) reported similar results when comparing small and large eggs incubated at an EST of 37.8°C, which resulted in an equal $E_{YFB}$. Recently, Lourens et al. (2011) demonstrated that $E_{YFB}$ is particularly influenced by EST. To minimize the effect of EST, the deviation of EST in the current study was maintained at not more than 0.2°C from the target at 37.8 °C during 18 d of incubation. The results obtained lead to the conclusion that embryos originating from different breeder age and different egg size deposit the available egg energy into chick YFB at an equal $E_{YFB}$. This suggests that a fixed EST, the factors that influence yolk size and consequently available energy in eggs may dictate the amount of energy deposition into chick YFB, whereas fluctuating EST may affect the results obtained.

Embryonic HP is produced as by-product of the biochemical processes to convert egg nutrients into chick body tissue (Etches, 1996). This suggests that a higher amount of energy deposited into YFB of chicks from old flock eggs would lead to an increase of embryonic HP. The results of experiment 2 showed that embryos of old flock eggs produced 22 mW/egg more heat than those of young flock eggs from d 16 to 18 of incubation. This finding is consistent with the data presented by Lourens et al. (2006), who showed that embryos of large eggs had a higher amount of energy transferred to chick YFB and consequently a higher embryonic HP than those of small eggs. Because embryos had an equal $E_{YFB}$, it can be suggested that differences in HP of embryos derived from young and old flock eggs as found in experiment 2 are caused by differences in the amount of available energy that is subsequently incorporated into chick YFB.

Because egg energy used during incubation is partitioned between tissue development, growth, and maintenance (Vleck, 1991), a higher amount of energy incorporated into YFB should be reflected in chick YFB weight. Results of the current study are contrary to our expectation. The YFB weight of chicks from large

eggs was higher than that of small eggs, but YFB energy content was equal. On the other hand, YFB energy content of chicks from the old flock eggs was higher than that of the young flock eggs, whereas YFB weight was similar. Two explanations can be attributed to these results. The first one is related with the amount of energy incorporated into chick YFB, which is largely influenced by available energy in the eggs. This would mean that the amount of energy deposited into chick YFB has no direct effect on YFB weight, but nutrient assimilation into YFB and then especially fat increases, resulting in higher YFB energy content. Second, present results can be explained by the influence of egg size on YFB weight. A limited space due to similar egg size of young and old flock eggs in this study may restrict embryonic growth (Wiley, 1950) to the same YFB size. However, the higher amount of protein and fat deposited into YFB of chicks from old flock eggs compared with those of young flock eggs may lead to a higher dry YFB weight. The ability of embryos from old flock eggs to deposit more energy with extra protein and particularly fat into YFB may be beneficial for the chicks. Weytjens et al. (1999) concluded that chicks from old flock eggs were more resistant to cold than chicks from young flock eggs, which might be an indicator of better thermoregulatory development. Noble et al. (1986) proposed that a lower yolk lipid transfer to the body of chicks from young flock eggs is a cause of higher chick embryo mortality compared with chicks from old flock eggs. However, these propositions should be further evaluated.

In conclusion, our results indicate that an egg of the same size should not be perceived as an identical egg, but is dependent on the age of the breeder flock. Older breeder hens produce eggs with more energy, especially through an increase of yolk size. Egg energy used during incubation was positively related to yolk size at equal $E_{YFB}$. Chick YFB weight was equal for both flock ages, but dry YFB weight of chick from old flock eggs was higher in association with more protein and fat content and thus more energy accumulated into YFB. As a consequence, embryos of the old flock eggs produced more heat from d 16 to 18 of incubation.

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# CHAPTER 4

# Differences in egg nutrient availability, development, and nutrient metabolism of broiler and layer embryos

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# ABSTRACT

Selection for production traits of broilers and layers leads to physiological differences, which may already be present during incubation. This study aimed to investigate the influence of strain (broiler vs layer) on egg nutrient availability, embryonic development and nutrient metabolism. A total of 480 eggs with an egg weight range of 62.0 to 64.0 g from Lohmann Brown Lite and Ross 308 breeder flocks of 41 or 42 weeks of age were selected in two batches of 120 eggs per batch per strain. For each batch, 30 eggs per strain were used to determine egg composition, including nutrient and energy content, and 90 eggs per strain were separately incubated in one of two climate respiration chambers at an eggshell temperature of 37.8ºC. The results showed that broiler eggs had a higher ratio of yolk: albumen with 2.41 g more yolk and 1.48 g less albumen than layers. The yolk energy content of broiler eggs was 46.32 kJ higher than that of layer eggs, whereas total energy content of broiler eggs was 47.85 kJ higher compared to layer eggs. Yolk-free body mass at incubation day 16 and chick weight and length at hatch were higher in broilers compared to layers. Respiration quotient of broiler embryos was higher than layer embryos during incubation day 8 to incubation day 10. A 0.24 g lower residual yolk at the hatch of broiler embryos than for the layer embryos indicated that broiler embryos used more yolk and had a higher energy utilization and energy deposition in yolk-free body mass. Heat production of broiler embryos was higher than that of layer embryos from incubation day 12 to incubation day 18, but efficiency of converting egg energy used by embryos to form yolk-free body mass was similar. In conclusion, broiler and layer embryos have different embryonic development patterns, which affect energy utilization and embryonic heat production. However, the embryos are equal in efficiency of converting the energy used to yolk-free body mass.

**Key words**: layers and broilers, nutrient availability, embryonic development, energy utilization, heat production

# INTRODUCTION

Broiler and layer strains are selected intensively for different production purposes, the former especially for growth and meat yield, and the latter mainly for egg production. This selection has led to physiological differences between both strains duringincubation as well as the post-hatch period (Jones et al., 1986; Muramatsu et al., 1990; Cooke et al., 2003; Ohta et al., 2004; Janke et al., 2004). Cooke et al. (2003) reported that broilers grew faster than layers at all ages during 4 to 20 weeks post-hatch. During incubation, it has been found that broiler and layer embryos show differences in yolk utilization (Sato et al., 2006; Everaert et al., 2008), yolk-free body mass (**YFBM**; Everaert et al., 2008), heat production (**HP**; Janke et al., 2004), and incubation duration (Janke et al., 2004; Everaert et al., 2008).



However, it has to be emphasized that in all the above studies, eggs of layers and broilers were incubated at the same incubator temperature. Lourens et al. (2005) demonstrated that temperature experienced by embryos or eggshell temperature (**EST)** rather than incubator temperature has a significant effect on embryonic development and nutrient utilization, and this was confirmed by Molenaar et al. (2010a). At the same incubator temperature, variation in embryonic HP significantly affects EST (Meijerhof and Van Beek, 1993; French, 1997). It was shown in studies with broiler eggs that embryonic HP during incubation was influenced by the size of the egg (Lourens et al., 2006) and age of the breeder flock (Nangsuay et al., 2013), which is probably the result of a difference in egg composition and, thus, egg energy content. This suggests that embryos of eggs from different origins, which have different egg composition and energy content, could have different EST and develop differently when kept at equal incubator temperatures.

At the same breeder age, Everaert et al. (2008) reported that broiler eggs were about 10 g heavier than layer eggs. Ho et al. (2011) showed that the eggs of broilers contained more yolk, and less albumen and eggshell compared to layer eggs. As yolk is the main nutritional source, it is possible that broiler and layer embryos differ in using nutrients for development and growth, which might be

reflected in the respiratory quotient (**RQ**; Romanoff, 1967), hepatic glycogen as well as blood plasma levels of metabolites (Molenaar et al., 2011).

Based on this information, we hypothesized that embryos of broilers and layers differ in energy utilization and, consequently, in embryonic HP, even when they are incubated at the same EST. The objective of this study was to investigate the influence of strain, "broilers versus layers," on egg nutrient availability, embryonic development, and nutrient metabolism.

# MATERIALS AND METHODS

In two subsequent batches, layer and broiler hatching eggs of the same egg weight range and breeder flock age were incubated at an EST of 37.8ºC. This EST was shown to give good embryonic development during incubation of broiler (Lourens et al., 2005) and layer eggs (Molenaar et al., 2010b). To minimize potential confounding factors, such as breeder age, egg size, and EST, eggs of the same breeder age, and egg weight range were incubated separately at the same EST of 37.8ºC. The experimental protocol was approved by the Animal Care and Use Committee of Wageningen University, the Netherlands.

### *Hatching Eggs and Incubation*

Good quality hatching eggs weighing 62.0 to 64.0 g from Lohmann Brown Lite and Ross 308 breeder flocks at 41 or 42 wk were obtained from two commercial hatcheries (Verbeek B.V., Lunteren, The Netherlands and Probroed & Sloot, Groenlo, The Netherlands). A total of 480 eggs were obtained in two batches, (120 eggs per batch per strain). For each batch, 30 eggs per strain were used to determine egg composition while the other eggs were stored at 18 to 20°C and a relative humidity of 50 to 60% for 4 or 5 days before incubation. Ninety eggs per strain per batch were incubated separately in one of two climate respiration chambers (**CRC**; Lourens et al., 2006). Five eggs per CRC were equipped with a temperature sensor attached to the equator of the egg with heat conducting paste and tape, as described by Lourens et al. (2006). The EST was measured every minute, and according to the median EST of the 5 eggs, the CRC temperature was

adjusted throughout the incubation period to maintain EST at 37.8ºC. Because of logistic reasons, egg candling and transfer were performed at incubation day (**E**) 13 and 16, respectively. At E16, the eggshell sensors were removed and the eggs were transferred to hatching baskets. Thereafter, the hatching baskets were returned to the same CRC and remained there throughout the hatching period. The temperature of the CRC was fixed at the last temperature before removal of eggshell sensors, and the EST was allowed to change during the remaining incubation period. Relative humidity was maintained at 50% in both CRCs and the concentration of oxygen ($O_2$) and carbon dioxide ($CO_2$) were at normal levels of approximately 20.9 and 0.4%. The $O_2$ and $CO_2$ concentrations during E0 to E18 were measured in 9-minute intervals in both chambers and in fresh air. Carbon dioxide concentration was measured with a non-dispersive infrared $CO_2$ analyzer (type Uras 3G, Hartmann and Braun, Frankfurt, Germany). Oxygen concentration was measured with a paramagnetic oxygen analyzer (type ADC7000, Analytical Development Co. Ltd., Hertfordshire, UK). The exact air volumes were measured with a Schlumberger G1.6 dry gas meter (Schlumberger, The Netherlands).



Eggs were candled at E13 of incubation, and infertile eggs and eggs containing dead embryos were removed. Infertile eggs and eggs containing dead embryos were opened and macroscopically inspected for fertility or moment of death (Lourens et al., 2006). Non-hatched eggs were opened as well and evaluated in the same way. The embryonic HP during E0 to E18 was calculated from oxygen consumption and carbon dioxide production according to Romijn and Lokhorst (1961) and adjusted for fertility and day of embryo mortality. The respiratory quotient (**RQ**) during E8 to E18 was calculated as the ratio of carbon dioxide production and oxygen consumption (litre per embryo per day).

***Hatching Egg and Hatchling Measurements***

A total of 30 fresh eggs per batch per strain were boiled, and albumen and yolk from each egg were separated and weighed. The eggshell was dried for 24 h at room temperature and weighed. Albumen and yolk were stored at –20ºC for further analysis.

All eggs were weighed at the start of incubation (E0). At E16 of incubation, eggs were reweighed to determine egg weight loss. Twenty eggs per

strain per batch were removed from the CRC at E16. Eggs were opened and the embryo and residual yolk **(RSY)** removed from the egg and weighed. Starting at E19 of incubation, eggs were checked every 3 h for hatching time. The incubation duration was defined as the time between setting of the egg in the incubator and the moment that chicks emerged from the shell. Percentage of hatch of fertile eggs was calculated by using the number of fertile eggs at E16 and the number of hatched chicks.

The hatchlings remained in the CRC for 6 h and were then removed. Chick weight and chick length from the top of the beak to the tip of the middle toe, excluding the nail (Hill, 2001), were determined. Thereafter, chicks were killed by decapitation, blood was collected, and the liver was removed, weighed, and immediately stored in liquid nitrogen. The RSY weight was determined and YFBM was calculated as chick weight minus RSY weight. The RSY and YFBM were stored at –20ºC for further analysis. Heart, stomach (gizzard plus proventriculus), intestine, and Bursa of Fabricius of hatchlings were weighed after defrosting the YFBM in a plastic bag in a water bath at 37ºC for 15 min.

***Chemical Analysis***

To obtain a sufficient number of samples for the chemical analyses, albumen and yolk from 2 eggs were pooled (n = 30 per strain). The YFBM and RSY of chicks at 6 h after hatch were selected equally distributed across the hatching period. The RSY of 2 chicks were pooled to obtain enough amount of samples (n = 30 per strain) and 30 YFBM samples per strain (n = 30) were used for the analyses. Proximate analyses were performed for dry matter (**DM**; ISO 6496, 1999), crude protein (**CP**; ISO 5983-2, 2005), and gross energy (**GE**; ISO 9831, 1998) in albumen and yolk from fresh eggs and RSY and YFBM of chicks at 6 h after hatch. Albumen, yolk, and RSY were dried in a freeze dryer before analyses of DM, CP, and GE. For YFBM analysis, one YFBM (without liver) was placed in150 mL of water and autoclaved for 2 h at 120ºC. Thereafter, YFBM in water was homogenized with an Ultra-Turrax disperser (IKA Werke GmbH & Co. KG, Staufen, Germany) for 10 min, and the suspension was used for DM and CP analyses. The remaining suspension was frozen at −20ºC and then dried in a freeze dryer to determine GE.

***Nutrient Utilization***

Energy content of protein in albumen and yolk of fresh eggs and RSY and YFBM of chicks at 6 h after hatch was calculated using an energy density for protein of 16.8 MJ/kg of DM (International System of Units, 1998).

Protein-free energy (kJ; albumen, yolk, RSY, YFBM) was calculated by;

= Energy content (kJ) – Protein energy (kJ)

Energy utilization (kJ; total, protein and protein-free) was calculated by;

= Albumen (kJ) + Yolk (kJ) – RSY (kJ)

Energy loss (kJ) was calculated by;

= [Albumen (kJ) + Yolk (kJ)] – [YFBM (kJ) + RSY (kJ)]

Efficiency of converting energy used to YFBM ($\mathbf{E_{YFB}}$,%; total, protein and protein-free) was calculated by;

$$E_{YFB} = \frac{\text{YFB (kJ)}}{\text{Albumen (kJ) + yolk (kJ) - RY (kJ)}} \times 100\%$$

***Plasma Metabolite and Liver Glycogen Determination***

After decapitation of the chicks at 6 h after hatch, blood was collected in a 4-mL blood tube containing 10 mg of sodium fluoride and 8 mg of potassium oxalate (BD Vacutainer, Franklin Lakes, NJ). An extra droplet of 10% heparin was added and mixed into the tube before sampling. Blood was centrifuged at 2,000 × g for 10 min at room temperature, and plasma was stored at −20ºC for further analyses. Plasma glucose, lactate, and uric acid concentrations were determined with commercially available enzymatic photometric kits (DiaSys Diagnostic Systems International, Holzheim, Germany).

After freezing in liquid nitrogen, livers were stored at −80°C until analysis of hepatic glycogen, as described by Molenaar et al. (2010b). All procedures for hepatic glycogen determination were carried out on ice.

### *Statistical Analyses*

For statistical analyses of DM, CP, and energy variables of albumen, yolk, albumen + yolk, and RSY, each experimental unit was a combination of samples from 2 eggs or 2 RSY of 2 hatchlings. An egg or a chicken was used as the experimental unit for other variables, except for HP and RQ, for which the CRC was used as the experimental unit. Distributions of the means and residuals were examined to verify model assumptions. The HP per day from E0 to E18 and RQ from E8 to E18 were analysed using the MIXED procedure of SAS 9.2 software package (SAS Institute, 2009) for repeated measurements. The model used was $Y_{ijkl} = \mu + A_i + B_j + C_k + (A_i \times C_k) + e_{ijkl,}$ where $Y_{ijkl}$ is the HP or RQ, $\mu$ is the overall mean, $A_i$ is the strain (i = broiler or layer), $B_j$ is the batch (j = 1 or 2), $C_k$ is the incubation day (k = E0 to E18 for HP and E8 to E18 for RQ), $A_i \times C_k$ is the interaction between the strain and incubation day and $e_{ijkl}$ is the error term. Hatching of fertile eggs was analyzed using the logistic regression analysis (PROC LOGISTIC) whereas all other variables were analyzed with the GLM procedure of SAS 9.2 software package (SAS Institute, 2009). Strain, batch, and their interaction were included as class variables. In none of the analyses was the interaction between strain and batch significant ($P > 0.05$), and thereafter this interaction was excluded from the model. Least square means were compared using Bonferroni adjustments for multiple comparisons. Values are expressed as LS means. In all cases, a difference was considered significant at $P \leq 0.05$.

## RESULTS

### *Egg Compositions*

Compositions of broiler and layer eggs are described in Table 1. By selecting eggs with the same egg weight range, broiler and layer eggs had similar average egg weight. Broiler eggs had a higher ratio of yolk: albumen ($P < 0.001$) with 2.41 g more yolk ($P < 0.001$) and 1.48 g less albumen ($P < 0.001$) than layer eggs. Eggshell weight of broiler eggs was 0.75 g less than that of layer eggs ($P < 0.001$).

**Table 1.** Egg compositions of broiler and layer hatching eggs.

| Variables[1] | Broiler | Layer | SEM | *P*-Value |
|---|---|---|---|---|
| Egg wt. (g) | 63.17 | 62.98 | 0.11 | 0.23 |
| Yolk wt. (g) | 19.52 | 17.11 | 0.15 | <0.001 |
| Albumen wt. (g) | 37.99 | 39.47 | 0.15 | <0.001 |
| Shell wt. (g) | 5.65 | 6.40 | 0.06 | <0.001 |
| Yolk: Albumen | 0.52 | 0.43 | 0.01 | <0.001 |

[1] n = 60 per strain.



**Table 2.** Dry matter (DM: %) and crude protein (CP: as % of DM) in albumen and yolk of fresh eggs and RSY and YFBM of chicks at 6 h after hatch.

| Variables[1] | Broiler | Layer | SEM | *P*-Value |
|---|---|---|---|---|
| Albumen | | | | |
| DM | 12.91 | 12.32 | 0.10 | <0.001 |
| CP | 87.64 | 87.99 | 0.16 | 0.111 |
| Yolk | | | | |
| DM | 48.17 | 47.16 | 0.11 | <0.001 |
| CP | 32.09 | 32.44 | 0.14 | 0.087 |
| RSY | | | | |
| DM | 49.77 | 47.41 | 0.27 | <0.001 |
| CP | 50.33 | 56.19 | 0.59 | <0.001 |
| YFBM[2] | | | | |
| DM | 19.56 | 17.44 | 0.15 | <0.001 |
| CP | 56.02 | 48.46 | 0.85 | <0.001 |

[1] n = 30 per strain. [2]YFBM without liver.

***Nutrient Content of Eggs, YFBM and RSY***

The percentage of DM was higher in the albumen (Δ = 0.59%; $P < 0.001$) and yolk (Δ = 1.01%; $P < 0.001$) of broiler eggs compared to layer eggs (Table 2). The percentage of CP in DM of the albumen and yolk of broiler and layer eggs was similar. The RSY of broiler chicks at 6 h after hatch had a higher percentage of DM (Δ = 2.36%; $P < 0.001$), but a lower percentage of CP in DM (Δ = 5.86%; $P < 0.001$) compared to layer chicks. The YFBM of broiler chicks at 6 h after hatch had a higher percentage of DM (Δ = 2.20%) and CP in DM (Δ = 7.56%) than that of layer chicks (both $P < 0.001$).

The amount of energy in the albumen and yolk of fresh eggs and RSY and YFBM of chicks at 6 h after hatch is shown in Table 3. The albumen of broiler and layer eggs was similar in the amount of energy from protein, but the amount of protein-free energy was higher in albumen of broilers than layers (Δ = 1.22 kJ; $P = 0.017$). Total amount of energy in albumen of broiler and layer eggs did not differ. The yolk of broiler eggs had a higher amount of energy from protein (Δ = 6.79 kJ) and protein-free energy (Δ = 39.54 kJ) than that of layer eggs (both $P < 0.001$). As a result, the total amount of energy in the yolk of broiler eggs was 46.32 kJ higher than that of layer eggs ($P < 0.001$). The albumen + yolk of broiler eggs contained more energy from protein (Δ = 7.09 kJ) and protein-free energy (Δ = 40.75 kJ) compared to layer eggs (all $P < 0.001$). Consequently, the total amount of energy in albumen + yolk of broiler eggs was 47.85 kJ higher than that of layer eggs ($P < 0.001$).

The RSY of broiler chicks had a lower amount of energy from protein (Δ = 2.65 kJ; $P = 0.010$) but a higher amount of protein-free energy (Δ = 7.02 kJ; $P = 0.001$) compared to the RSY of layer chicks. The total amount of energy in the RSY of broiler and layer chicks did not differ. The YFBM of broiler chicks contained more energy from protein (Δ = 11.23 kJ), protein-free energy (Δ = 12.68 kJ) and total energy content (Δ = 23.87 kJ) than the YFBM of layer chicks (all $P < 0.001$). The RSY + YFBM of broiler chicks contained more energy from protein (Δ = 8.85 kJ), protein-free energy (Δ = 18.76 kJ) and total energy (Δ = 27.76 kJ) than RSY + YFBM of layer chicks (all $P < 0.001$).

**Table 3.** Energy content (kJ) in hatching egg (albumen, yolk, albumen + yolk) and chicks at 6 h after hatch (RSY, YFBM and RSY + YFBM) of broilers and layers.

| Variables[1] | Broiler | Layer | SEM | *P*-value |
|---|---|---|---|---|
| Albumen | | | | |
| Protein | 68.94 | 68.64 | 0.61 | 0.727 |
| Protein-free[3] | 38.85 | 37.63 | 0.35 | 0.017 |
| Total | 107.79 | 106.27 | 0.89 | 0.232 |
| Yolk | | | | |
| Protein | 50.77 | 43.98 | 0.47 | <0.001 |
| Protein-free[3] | 263.15 | 223.61 | 1.98 | <0.001 |
| Total | 313.91 | 267.59 | 2.35 | <0.001 |
| Albumen + yolk | | | | |
| Protein | 119.71 | 112.62 | 0.63 | <0.001 |
| Protein-free[3] | 301.99 | 261.24 | 1.83 | <0.001 |
| Total | 421.71 | 373.86 | 2.11 | <0.001 |
| | | | | |
| RSY | | | | |
| Protein | 27.28 | 29.93 | 0.70 | 0.010 |
| Protein-free[3] | 59.69 | 52.67 | 1.45 | 0.001 |
| Total | 86.97 | 82.59 | 1.93 | 0.115 |
| YFBM[2] | | | | |
| Protein | 83.72 | 72.49 | 0.83 | <0.001 |
| Protein-free[3] | 97.65 | 84.97 | 1.65 | <0.001 |
| Total | 181.37 | 157.50 | 1.94 | <0.001 |
| RSY + YFBM[2] | | | | |
| Protein | 111.27 | 102.42 | 0.96 | <0.001 |
| Protein-free[3] | 156.57 | 137.81 | 2.06 | <0.001 |
| Total | 267.85 | 240.09 | 2.33 | <0.001 |

[1] n = 30 per strain. [2] YFBM without liver. [3] Protein-free = total – protein.

**Table 4.** Yolk-free body mass (YFBM, g) and residual yolk (RSY, g) at E16 and at 6 h after hatch, chick weight (g) and chick length (cm) at 6 h after hatch and incubation duration (h) of boiler and layer chick.

| Variables[1] | Broiler | Layer | SEM | *P*-Value |
|---|---|---|---|---|
| Weight loss E16 (%) | 8.15 | 7.67 | 0.21 | 0.113 |
| YFBM (g) | | | | |
| E16 | 20.88 | 16.86 | 0.27 | <0.001 |
| Hatch | 40.39 | 38.77 | 0.14 | <0.001 |
| RSY (g) | | | | |
| E16 | 13.22 | 12.41 | 0.28 | 0.046 |
| Hatch | 6.14 | 6.38 | 0.09 | 0.046 |
| Chick weight (g) | 46.48 | 45.10 | 0.12 | <0.001 |
| Chick length (cm) | 19.42 | 18.19 | 0.04 | <0.001 |
| Hatch of fertile egg (%) | 98.35 | 95.16 | na | 0.967 |
| Incubation duration (h) | 492.37 | 498.13 | 0.88 | <0.001 |

[1] at E16 n = 40 per strain; at hatch n = 119 and 118 for broiler and layer.

**Table 5.** Internal organ weight in absolute values (g) and relative to YFBM (%) of broiler and layer chick at 6 h after hatch.

| Variables[1] | Broiler | Layer | SEM | *P*-Value |
|---|---|---|---|---|
| Heart wt. (g) | 0.267 | 0.223 | 0.003 | <0.001 |
| Heart: YFBM (%) | 0.661 | 0.574 | 0.009 | <0.001 |
| Liver wt. (g) | 0.906 | 0.816 | 0.009 | <0.001 |
| Liver: YFBM (%) | 2.246 | 2.107 | 0.023 | <0.001 |
| Stomach wt. (g) | 2.289 | 2.202 | 0.018 | 0.001 |
| Stomach: YFBM (%) | 5.669 | 5.687 | 0.045 | 0.777 |
| Intestinal wt. (g) | 1.692 | 1.285 | 0.020 | <0.001 |
| Intestinal: YFBM (%) | 4.189 | 3.317 | 0.049 | <0.001 |
| Bursa (g) | 0.040 | 0.036 | 0.001 | 0.018 |
| Bursa: YFBM (%) | 0.100 | 0.093 | 0.003 | 0.152 |

[1] n = 119 and 118 for broiler and layer.

***Developmental and Physiological Status***

Egg weight loss at E16 and percentage of hatch of fertile eggs did not differ between broiler and layer chicks (Table 4). The YFBM of broiler embryos at E16 and chicks at 6 h after hatch were 4.02 and 1.62 g heavier, respectively, than those of layer embryos (both $P < 0.001$). At 6 h after hatch, broiler chicks were 1.4 g heavier and 1.2 cm longer than layer chicks (both $P < 0.001$). The RSY weight of broiler embryos at E16 was 0.81 g higher ($P = 0.046$) than that of layer embryos, but broiler chicks at 6 h after hatch had 0.24 g less RSY (P = 0.046) than layer chicks. Broiler chicks hatched 5.76 h earlier than layer chicks ($P < 0.001$).



Weights in absolute values and as percentage of YFBM for heart (Δ = 0.044g, 0.087%), liver (Δ = 0.090 g, 0.035%), and intestines (Δ = 0.407 g, 0.872%) were higher in broiler chicks than in layer chicks (Table 5; all $P < 0.001$). Broiler chicks had a heavier stomach (Δ = 0.087 g; $P = 0.001$) and bursa (Δ = 0.004 g; $P = 0.018$) than layer chicks, but these two values did not differ as a percentage of YFBM.

The hepatic glycogen at 6 h after hatch was higher in layer chicks than in broiler chicks (Δ = 12.75 mg/g; $P < 0.001$; Table 6). The level of lactate, uric acid and glucose did not differ between broiler and layer chicks.

***Nutrient Metabolism***

Broiler embryos used more energy from protein (Δ = 10.11 kJ) and protein-free energy (Δ = 32.15 kJ) compared to layer embryos (Table 7; both $P < 0.001$). As a result, total amount of energy used by broiler embryos was 42 kJ higher than that of layer embryos ($P < 0.001$). Energy loss during incubation was 19.02 kJ higher in broiler embryos than in layers ($P = 0.006$). The $E_{YFB}$ for protein energy and protein-free energy, and overall $E_{YFB}$ of broiler and layer embryos did not differ.

Respiration quotient (liter CO2 produced/liter O2 consumed, RQ) during E8 to E10 was higher in broiler embryos than in layer embryos (all $P < 0.05$; Figure 1). The RQ level at E8 was 0.75 for broiler and 0.74 for layer embryos and thereafter RQ of both strains decreased to an equal level of 0.71 at E12. From E12 to E18, RQ was approximately 0.71 for both strains. In both strains, HP increased

between E8 and E18 of incubation and reached a plateau stage at about E16 (Figure 2). Broiler embryos had higher HP than layer embryos from E12 to E18 (E12 to E18; $P < 0.05$).

**Table 6.** Hepatic glycogen (mg/g), lactate, uric acid, and glucose (mmol/L) of broiler and layer chick at 6 h after hatch.

| Variables[1] | Broiler | Layer | SEM | *P*-Value |
|---|---|---|---|---|
| Hepatic glycogen (mg/g) | 15.17 | 27.92 | 2.18 | <0.001 |
| Lactate (mmol/L) | 2.27 | 2.50 | 0.10 | 0.119 |
| Uric acid (mmol/L) | 0.23 | 0.27 | 0.02 | 0.167 |
| Glucose (mmol/L) | 10.94 | 11.25 | 0.13 | 0.090 |

[1] n = 30 per strain.

**Table 7.** Energy utilization (kJ), energy lost (kJ), and $E_{YFB}$ (%) of broiler and layer chicks at 6 h after hatch.

| Variables[1] | Broiler | Layer | SEM | *P*-Value |
|---|---|---|---|---|
| Energy utilization | | | | |
| Protein | 92.85 | 82.69 | 0.95 | <0.001 |
| Protein-free[2] | 241.04 | 208.89 | 2.32 | <0.001 |
| Total | 333.89 | 291.58 | 2.85 | <0.001 |
| Energy lost | 153.10 | 134.08 | 2.99 | <0.001 |
| $E_{YFB}$ | | | | |
| Protein[3] | 90.76 | 87.93 | 1.36 | 0.147 |
| Protein-free[4] | 40.18 | 40.70 | 0.81 | 0.651 |
| Total | 54.20 | 54.11 | 0.72 | 0.926 |

[1] n = 30 per strain. [2] Protein-free = total – protein. [3] EYFB protein = (protein energy in YFB/ protein energy used by embryos) × 100. [4] $E_{YFB}$ protein-free = (protein-free energy in YFB/ protein-free energy used by embryos) × 100.

# DISCUSSION

This study aimed to investigate the influence of chicken strain "broilers vs. layers," on egg nutrient availability, embryonic development, and nutrient metabolism during incubation. To eliminate possible confounding effects, hatching eggs were obtained from the same breeder age and egg weight range and incubated at the same EST.

### *Egg Compositions and Energy Content*



The results clearly demonstrate the effect of genetic strain on egg composition and amount of nutrients in the eggs. At similar egg weight, broiler eggs had a higher ratio of yolk: albumen and less eggshell weight than layer eggs. The higher energy content in broiler eggs compared to layer eggs was due to a combination of a higher DM of albumen and yolk and especially a larger yolk size. A major contribution of yolk size to amount of energy in the yolk and, consequently, in the egg is consistent with the study of Nangsuay et al. (2013). The current study indicates that despite an equal percentage of CP in yolk DM, a larger yolk size of 2.4 g led to an approximately 15% higher protein energy in the yolk of broiler eggs compared to layers. Furthermore, an equal amount of energy in the albumen clearly demonstrated that the higher energy content in broiler eggs was due to both protein and protein-free energy in the yolk. Since the yolk contained a negligible amount of energy from carbohydrates (Lourens et al., 2006; Nangsuay et al., 2013), the higher amount of protein-free energy in the yolk probably indicated a higher amount of energy derived from lipid sources. This means that at the start of incubation, broiler eggs had more energy from both protein and lipid sources, which could contribute to a higher growth rate.

### *Embryonic Development and Nutrient Metabolism*

Broiler and layer embryos had a different growth pattern during incubation. Broiler embryos developed and grew faster than layer embryos, as expressed by the embryo development at E16 and chick quality parameters at 6 h

after hatch. These results are in agreement with Ohta et al. (2004) who studied embryonic growth in broiler and layer eggs of similar egg weight and reported a higher growth rate of broiler embryos compared to layer embryos at E14 and E19. The current results show that a higher growth rate of broiler compared to layer embryos was accompanied by a higher weight of heart, liver, and intestines in absolute values and relative to YFBM. These findings clearly demonstrate that genetic background has an influence on embryonic development and growth pattern. Moreover, this result indicates that broilers, which have been selected for high growth rate post-hatch, have already developed heavier digestive and supply organs during the embryonic stages.

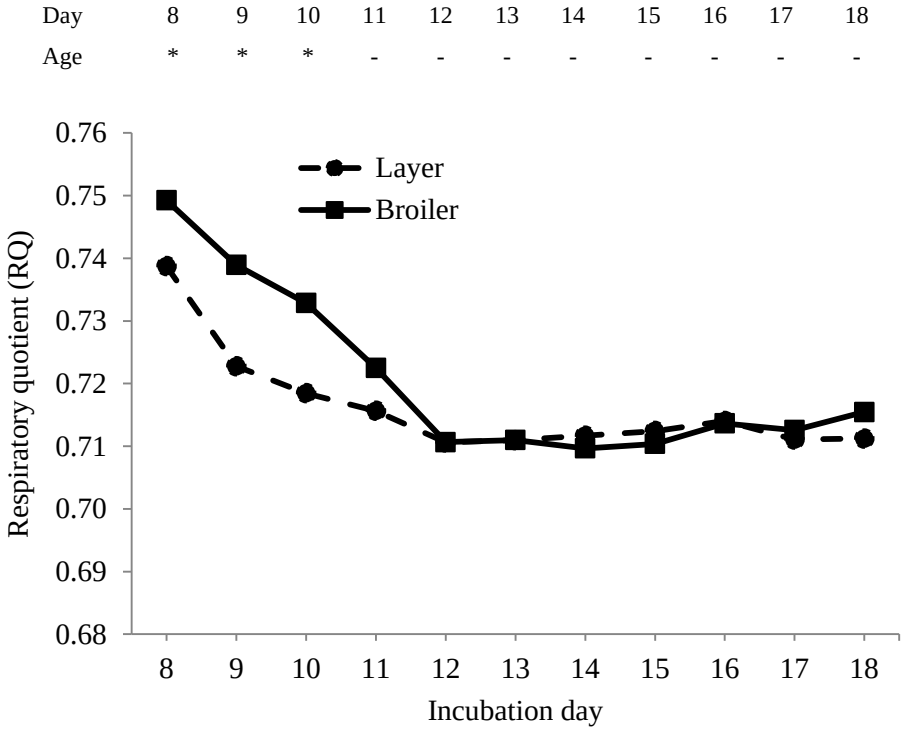


**Figure 1.** Respiratory quotient (RQ) of broiler and layer from E8 to E18 of incubation. The legend indicates the level of significance (*$P \leq 0.05$).

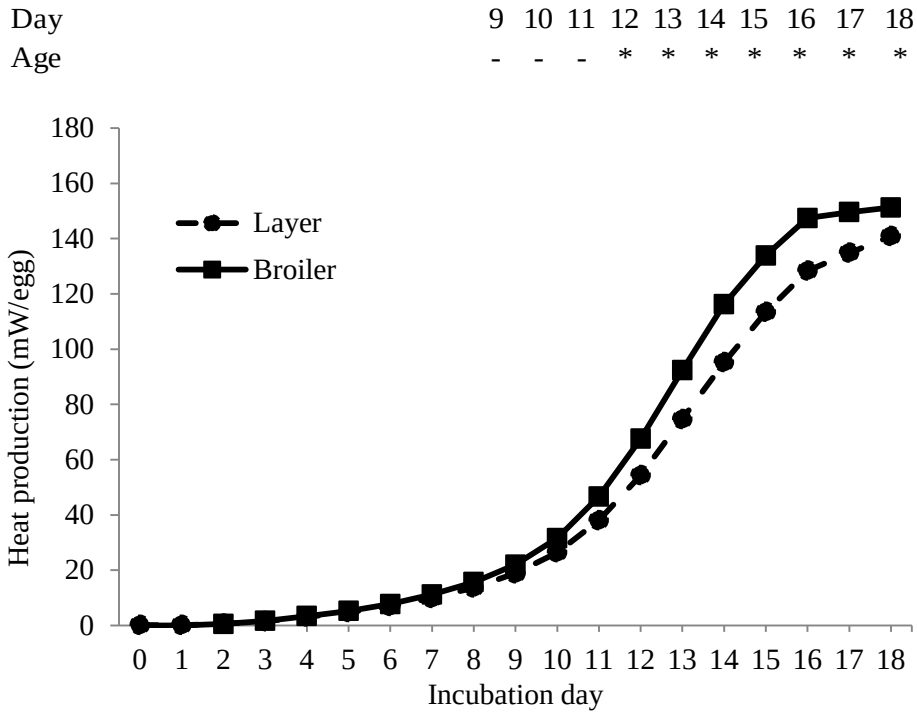


**Figure 2.** Heat production (HP; mW/egg) of broiler and layer embryo from E0 to E18. The legend indicates the level of significance (*$P \leq 0.05$).

A higher embryonic growth rate of broilers compared to layers can be related to two possible synergistic effects of strain-specific production traits. These traits are genetic growth potential and egg composition differences. It could be inferred from the current results that the genetic growth potential of the broilers has an influence on growth of developing embryos. To ensure sufficientenergy supply for growth, broiler embryos will require more nutrients and oxygen than layer embryos. The differences in nutrients and oxygen requirements might be fulfilled by the egg compositions, especially by the eggshell characteristics and yolk content. The lower shell weight of broiler eggs found in the current study might improve shell conductance (Wolanski et al., 2007) and lead to more oxygen availability. A combination of more oxygen and a higher nutrient availability due to especially yolk size suggests that broiler embryos have more energy stored and,

therefore, have more capacity to metabolize. Furthermore, it is possible that a larger yolk size of broiler eggs compared to layer eggs has an influence on yolk absorption capacity (Yadgary et al., 2013) and vitelline vascularization (Adair et al., 1990). This could mean that broiler embryos have a higher capacity to absorb and transport yolk nutrients to embryonic tissues. These synergetic effects of genetic growth potential and egg compositions might be mechanisms underlying a higher embryonic development and growth of broiler embryos compared to layer embryos.

To obtain energy, embryos consume oxygen and produce carbon dioxide and heat (Etches, 1996), and this can be measured. The RQ, which is the ratio of carbon dioxide production to oxygen consumption, can be an indicator of the nutritional sources used for metabolism. Romanoff (1967) related an RQ during early development of the embryos mainly to protein metabolism and to a lesser extent carbohydrate metabolism. Fiske and Boyden (1926) proposed that approximately 96% of the proteins absorbed during the first 13 days of incubation will be retained in embryonic tissues including membranes. It is possible that the higher RQ of broiler embryos compared to layer embryos during E8 to E10 is an indication of higher protein utilization. Although we cannot specify the usages during each embryonic stage, the current results show that broiler embryos use more energy from protein than layer embryos. These findings are in agreement with Ohta et al. (2004) who reported that growth and accumulation of protein in broiler embryos are higher than those in layer embryos.

A similar RQ at 0.71 of broiler and layer embryos from E12 onward indicates a shift to lipid metabolism as a main energy supply of both strains, which is known to occur in the second half of incubation (Romanoff 1967; Noble and Cocchi, 1990). A higher yolk utilization, which is exhibited in less RSY at hatch and higher utilization of protein-free energy in the yolk, may indicate that broiler embryos have used more lipid sources from the eggs than layer embryos. These findings are in agreement with Sato et al. (2006) who reported that broiler embryos absorb yolk as lipid source and use more lipids than layer embryos. As a result of both energy used from protein and protein-free energy, the total amount of energy used by broiler embryos was higher than that used by layer embryos. These findings indicate that broiler embryos had a higher metabolic rate than layer embryos. A higher energy loss results in more production of metabolic by products

by broiler embryos due to a higher metabolic rate, which is reflected in the higher HP from E12 to E18 of broiler embryos compared to layer embryos.

Although broiler and layer embryos differed in nutrient metabolism, they were equal in their efficiency to convert energy used from protein, protein-free energy, and total energy used to YFBM. A higher deposition of energy into YFBM of broiler chicks as a result of more energy used might be a reflection of the genetic difference, creating hatchlings with a higher growth rate in the grow-out period. Higher protein-free energy in the RSY of broiler chicks than in layer chicks suggests that broiler chicks have probably more lipids available post-hatch. However, protein available in the RSY was lower in broilers than layers as indicated in less protein energy. These findings may suggest different available resources for protein and lipids of broiler and layer chicks post-hatch.



The higher growth and metabolic rate of broiler embryos could be the reason for a shorter incubation duration and less glycogen in the liver of broiler chicks at hatch.A higher metabolic rate requires more $O_2$ consumption and results in a higher $CO_2$ production. This might drive broiler embryos to reach oxygen availability limitations or to have critically high $CO_2$ levels in the air cell earlier than layer embryos. Janke et al. (2004) and Everaert et al. (2008) reported that $O_2$ consumption in broiler embryos was higher than in layer embryos. Everaert et al. (2008) suggested that a higher $CO_2$ partial pressure (**$PCO_2$**) in the air cell caused an early internal pipping for broiler embryos compared to layer embryos. It is possible that a higher $PCO_2$ in theair cell triggers the hatching process of broiler embryos (Visschedijk, 1968) to start approximately 6 h earlier than layer embryos in the current study. Our finding for incubation duration is similar to that ofJanke et al. (2004) who reported that broiler chicks hatched about 24 h earlier than layer chicks. In contrast to our results, the study of Everaert et al. (2008) found no differences in incubation duration of broiler and layer embryos, although the moment of internal and external pipping of broilers was about 5 h earlier than layers. Variation of incubation duration found in these studies might be caused by differences in breeder age, egg weight, or EST of embryos. The results of the current study demonstrate that at the same breeder age, egg weight and EST, the broiler embryos have approximately 6 h shorter incubation duration than layer embryos, probably due to a higher growth rate.

Layer chicks hatched with more hepatic glycogen than broiler chicks, whereas the levels of glucose, lactate and uric acid did not differ. Glycogen synthesis and depletion can influence differences in hepatic glycogen of the chicks at hatch. Because hatching is a high energy demanding process, which occurs during the period that $O_2$ availability is limited, glycolysis of glucose becomes the main energy supply to meet the requirements of the embryo (Freeman, 1969). Glycogen depletion from the liver is an important source for glucose glycolysis. A difference in $O_2$ consumption of broiler and layer embryos might alter the magnitude of $O_2$ limitation and, as a consequence, the requirement for glucose during hatching process might be different. Everaert et al. (2008) demonstrated that layer embryos had relatively more $O_2$ availability than broiler embryos, as shown in a higher blood $PO_2$ at internal and external pipping. This means that during the hatching process, layer embryos require or have less glycogen depletion from the liver than broiler embryos. Moreover, as glycogenic amino acids and glycerol from metabolic processes are major precursors for glycogen synthesis (Sunny et al., 2011; Molenaar et al., 2013), it is possible that a lower nutrient metabolism of layer embryos enables more precursors available for glycogen synthesis.

In conclusion, the genetic background of “broilers vs. layers,” influences embryonic developmentand nutrient metabolism during incubation. Differences in especially the yolk size lead to a higher energy availability in the broiler eggs than the layer eggs. During incubation, the broiler embryos grew faster and used more energy than the layer embryos. However, broiler and layer embryos were equal in $E_{YFB.}$ As a result of more energy used, broiler embryos deposited more energy into YFBM and had a higher embryonic HP than layer embryos.

For practical implications, the results of the current study indicate that we should be aware of the possible influence of the selection traits on the physiological development of the embryos. Eggs of meat type chickens yield embryos with a higher growth and metabolic rate than the embryos derived from egg type chickens. As a result, these embryos produce different amounts of heat during incubation. This can affect the EST of the embryos and subsequent performance as presented in several studies. To obtain an optimal EST for the embryos, it is important to incorporate the differences of egg origins into the applied incubation temperature profile.

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# CHAPTER 5

# Development and nutrient metabolism of embryos from two modern broiler strains

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# ABSTRACT

A progressive selection for broiler live and processing performance traits has changed broiler growth patterns during the post hatch period. However, limited information is available to understand whether changes have also occurred during the embryonic stages. This study aims to examine influences of broiler strain on nutrient availability, embryonic development, and nutrient metabolism during incubation. Hatching eggs of Ross 308 and Cobb 500 fast feathering were selected from breeder flocks aged 43 to 46 weeks at an egg weight range of 60 to 63 g. Eggs were obtained in 2 batches, 120 eggs per strain per batch. For each batch, 20 eggs per strain were used to determine egg composition and nutrient availability. The remaining eggs were incubated separately in one of 2 climate respiration chambers at an eggshell temperature of 37.8ºC. The results showed that Ross 308 eggs had a higher yolk: albumen ratio with 0.9 g more yolk and 0.7 g less albumen than Cobb 500. Albumen + yolk of Ross 308 eggs had a higher dry matter ($\Delta$ = 0.24 g) and crude fat ($\Delta$ = 0.23 g) than that of Cobb 500 eggs, but a similar amount of crude protein. Albumen + yolk of Ross 308 eggs had a higher energy content ($\Delta$ = 8.9 kJ) compared to Cobb 500 eggs. At 3 h after hatch, Ross 308 chicks were 0.2 cm longer and had a 0.6 g heavier yolk free body mass (YFBM) than Cobb 500 chicks. During incubation, Ross 308 embryos used 13.9 kJ more energy than Cobb 500, and the efficiency of converting energy used to YFBM (EYFB) was approximately 7.6% lower compared to Cobb 500. Ross 308 chicks hatched approximately 4 h later and had less hepatic glycogen ($\Delta$ = 5 mg) than Cobb 500 chicks. It can be concluded that, Cobb 500 and Ross 308 differ in egg nutrient availability and have different trajectories for embryonic development and nutrient metabolism during incubation.

**Key words:** broiler strains, nutrient availability, embryonic development, energy utilization, heat production

# INTRODUCTION

Modern broiler strains are genetically selected for improved broiler live and processing performance traits (Zuidhof et al., 2014). Among the commercial available broiler strains, Cobb 500 and Ross 308 are most commonly used. Although the selection criteria for Cobb 500 and Ross 308 will be very similar, the offspring of those strains have shown some differences in the growth trajectory to achieve strain specific target performances (Marcato et al., 2008; Tona et al., 2010). To achieve certain growth trajectories, Tullet and Burton (1983) demonstrated that the growth rate of chickens during the post hatch period can be altered by changing growth patterns throughout their whole developmental period, including the embryonic stage. This suggests that genetic selection of current modern broiler strains Cobb 500 and Ross 308 might not only have changed broiler growth pattern during the post hatch period, but also during the incubation period.



During incubation, embryos rely solely on the nutritional supply from albumen and yolk in the egg. The nutrient reserves of the egg are metabolized with the exchange of oxygen and carbon dioxide through the eggshell and metabolic heat is produced. The combination of heat from the incubation environment and embryonic heat production (**HP**) can influence embryo temperature, reflected as eggshell temperature (**EST**; Meijerhof and Van Beek, 1993). The EST can subsequently affect embryonic development, nutrient utilization, chick quality, and broiler performance (French, 1997; Lourens et al., 2005; Molenaar et al., 2010a; Molenaar et al., 2011). Recently, Nangsuay et al. (2013) demonstrated that embryonic HP increased with an increase of energy utilization. The same authors proposed that the energy utilization of the embryos was influenced by yolk size and a result of a higher use of nutrients from the yolk. Although several studies have shown that albumen and yolk are altered by the genetic background of the hen (O'Dea et al., 2004; Joseph and Moran, 2005; Wolanski et al., 2007), there is no study investigating the differences of egg nutrient availability in the eggs of the major broiler strains of Cobb 500 and Ross 308.

Earlier studies have been conducted to investigate the influence of broiler strain on embryonic development and HP. Hamidu et al. (2007) reported that embryos of Cobb 500 had a higher HP than Ross 308 embryos at incubation day

(**E**) 19. The same authors reported a higher HP for Ross 308 embryos than for Cobb 500 embryos at E7, 16, 17, and 18. By using acoustic resonance as a measure for embryonic development, Tona et al. (2010) reported a faster development of Cobb embryos during E4 to E5, whereas Ross embryos developed faster in the second week of incubation. The same authors reported a similar HP of Cobb and Ross strains between 430 to 478 h of incubation. Because these studies were conducted by incubating eggs at the same machine temperature, it can be speculated that HP differences between strains have resulted in differences in EST which in turn affected relative growth rates. While differences in embryonic development between Cobb and Ross strains have been indicated by Tona et al. (2010), there is no study about the influence of broiler strain on nutrient metabolism in relation to embryonic development, which merits further investigation.

The objective of this study was to examine the influence of broiler strain (Cobb 500 vs. Ross 308) on the availability of nutrients, embryonic development, and nutrient metabolism during incubation. Because differences in embryonic development and nutrient metabolism can affect the metabolic status of the embryos, analyses of hepatic glycogen and some metabolic blood variables (glucose, lactate, and uric acid) are included in this study.

# MATERIAL AND METHODS

### *Experimental Design*

The experiment was performed in 2 successive batches using hatching eggs of the same egg weight range and breeder flock age of Cobb 500 and Ross 308. Eggs of each strains were incubated separately at an EST of 37.8ºC, which has been shown to give the optimal embryonic development for broiler embryos (Lourens et al., 2005). The experimental protocol was approved by the Animal Care and Use Committee of Wageningen University, the Netherlands.

***Hatching Egg, Incubation and Determination of Hatching Events***

Two batches of in total 480 Ross 308 and Cobb 500 fast feathering hatching eggs from parent stock aging 43 to 46 weeks were obtained from a commercial hatchery. Both broiler breeder strains received commercially available diets. For Cobb 500 the diets contained on average 14.2% CP, 4.5% crude fat, 2,830 kcal/kg ME, and 1.7% linoleic acid and for Ross 308, the diets contained on average 14.3% CP, 4.9% crude fat, 2,900 kcal/kg ME, and 1.8% linoleic acid. Storage duration of the selected eggs was 4 to 5 days. For each batch, a total of 240 eggs (120 eggs of each breeder strain) were selected within an egg weight range of 60 to 63 g. Per batch, 100 eggs of each breeder strain were incubated in 1 of 2 identical small open-circuit climate respiration chambers (**CRC**; Lourens et al., 2006) at a constant EST of 37.8ºC and relative humidity of 55%. The remaining 20 eggs per breeder strain were used to determine egg composition.



During incubation, 5 eggs per CRC were equipped with a temperature sensor attached to the equator of the egg, using heat conducting paste and tape, as described by Lourens et al. (2006). The EST was measured every minute, and according to the median EST of the 5 eggs, the CRC temperature was adjusted to maintain EST at 37.8ºC. Eggs were candled E11 and E18, and infertile eggs and eggs containing dead embryos were removed. At E18, the CRC temperature, which corresponded with the EST of 37.8ºC, was recorded before removal of the EST sensors from the eggs. Fertile eggs were reweighed to determine egg weight loss (**EWL**), transferred to hatching baskets, and placed back in the same CRC. Temperature of the CRC was fixed at the temperature measured before egg transfer at 37.1ºC for both strains. The EST after egg transfer until hatching time was allowed to change. Eggs were candled and checked every 3 h from 457 h of incubation onwards to determine the moment of internal pipping (**IP**), external pipping (**EP**), and hatching. The eggshell conductance was calculated as $EWL/\Delta P_{H2O}$ according to Meijerhof and Van Beek (1993); where EWL (mg/h) = EWL at E18/(18 ×24) and $\Delta P_{H2O}$ (kPa) = average vapour pressure deficit during E0 to E18.

### *Heat Production (HP)*

To determine HP, oxygen and carbon dioxide concentrations were measured every 9 min in both CRC and in fresh air. Oxygen concentration was measured with a paramagnetic oxygen analyser (type ADC7000, Analytical Development Co. Ltd., Hertfordshire, UK). Carbon dioxide concentration was measured with a non-dispersive infrared $CO_2$ analyser (type Uras 3G, Hartmann and Braun, Frankfurt, Germany). The refreshed air volume was 5 L/min throughout the incubation period. The exact air volumes were measured with a Schlumberger G1.6 dry gas meter (Schlumberger, The Netherlands). Clear eggs from candling at E11 and E18 and dead in shell at hatch were opened to determine true fertility and timing of embryonic mortality as described by Lourens et al. (2006). The HP was calculated from oxygen consumption and carbon dioxide production (Romijn and Lokhorst, 1961) and adjusted for fertility and day of embryo mortality.

### *Hatching Egg, Embryo and Chick*

#### *Measurements*

A total of 20 eggs per batch per breeder strain were boiled for 10 min and albumen and yolk for each egg were separated and weighed. Eggshell thickness (without membrane) of 3 regions of the egg, top (blunt end), middle, and bottom (pointed end) was measured, using a digital micrometer (Mitutoyo Corporation, Tokyo). The eggshell without the membrane was dried for 24 h at room temperature and weighed. Albumen weight was calculated as egg weight - yolk weight - shell weight.

Ten fertile eggs per breeder strain per batch were sampled during incubation at E11, E14, and E18. Eggs were opened and yolk free body mass (**YFBM**) was obtained at E11 and E14 and the YFBM and residual yolk (**RSY**) were obtained at E18. At 3 h after hatch (emergence from the egg shell), approximately 50% of the hatchlings were sampled and weighed. From the sampled chicks, chick length was determined by stretching chicks along a ruler and taking the length between the top of the beak and the tip of the middle toe of the right foot (Hill, 2001). Thereafter, chicks were decapitated, blood was collected

and the liver was removed, weighed, and immediately stored in liquid nitrogen. The RSY and heart were removed and weighed and the YFBM was calculated by subtracting RSY weight from chick weigh. The RSY and YFBM were stored at -20ºC for further analysis.

***Blood Metabolites and Hepatic Glycogen Determination***

After decapitation, blood was collected in a 4 mL blood tube containing 10 mg of sodium fluoride and 8 mg of potassium oxalate (BD Vacutainer, Franklin Lakes, NJ). An extra droplet (0.02 mL) of 10% heparin was added and mixed into the tube before sampling. Blood was centrifuged at 2,000 × g for 10 min at room temperature, and plasma was decanted and stored at -20ºC until further analysis. Plasma glucose, lactate, and uric acid concentrations were determined with a commercially available kit (DiaSys Diagnostic Systems International, Holzheim, Germany).



Determination of hepatic glycogen was carried out on ice as described by Molenaar et al. (2010b). A liver sample of 250 to 300 mg was taken and 1 $\mu$L of 7% HCLO4 was added per mg of wet tissue. Thereafter, the liver was homogenized and centrifuged (3,500 × *g*) at 4ºC for 15 min. The supernatant was decanted, cleaned with 1 mL of petroleum ether, and frozen at −80ºC until further analysis. Hepatic glycogen was determined by an iodine binding assay (Dreiling et al., 1987), and hepatic bovine glycogen (Type IX, Sigma-Aldrich Chemie GmbH, Steinheim, Germany) was used as a standard.

***Chemical Analysis and Nutrient Utilization***

To have a sufficient sample for chemical analyses, albumen and yolk samples were pooled from 2 fresh eggs and RSY were pooled from 2 chicks, resulting in 10 samples per strain per batch. Ten YFBM samples per strain per batch were used for analyses. Proximate analyses were performed for dry matter (**DM**; ISO 6496, 1999), crude protein (**CP**; ISO 5983–2, 2005), and crude fat (ISO 6492, ISO, 1999) in albumen and yolk from fresh eggs and RSY and YFBM of chicks at 3 h after hatch. Albumen, yolk, and RSY were freeze dried before analyses of DM, CP, and crude fat. For YFBM analysis, one YFB (without liver)

was placed in 150 mL of water and autoclaved for 3 h at 120ºC. Thereafter, the YFBM and water suspension was homogenized with an Ultra-Turrax disperser (IKA Werke GmbH & Co. KG, Staufen, Germany) for 10 min and the suspension was used for DM and CP analyses. The remaining suspension was frozen at -20ºC and later freeze dried to determine crude fat.

Energy content of protein and fat in albumen and yolk of fresh eggs and RSY and YFBM of chicks at 3 h after hatch was calculated using an energy density for protein and fat of 16.8 and 37.8 MJ/kg of DM (International System of Units, 1998), respectively. Due to a very low amount of carbohydrates in the albumen and yolk of fresh eggs and RSY and YFBM of chicks (Lourens et al., 2006; Molenaar et al., 2010a; Nangsuay et al., 2013) the energy content of carbohydrates was excluded from the calculations.

Energy utilization (kJ; for protein, fat, and protein plus fat) was calculated as;
= Albumen(kJ) + Yolk(kJ) −RSY(kJ)

Energy lost (kJ) was calculated as;
= [Albumen(kJ) + Yolk(kJ)] −[YFBM(kJ) + RSY(kJ)]

Efficiency of converting energy used to form YFBM ($E_{YFB}$,%; protein, fat, and protein plus fat) was calculated as;

$$E_{YFB} = \frac{\text{YFB (kJ)}}{\text{Albumen (kJ) + yolk (kJ) - RY (kJ)}} \times 100\%$$

***Statistical Analyses***

For statistical analyses of DM, CP, crude fat, and energy of albumen, yolk, albumen plus yolk, and RSY, the experimental unit was a combination of samples from 2 eggs or 2 RSY of 2 chicks. Egg or chicken was used as the experimental unit for other variables, except for the HP, where the CRC was used as the experimental unit. Distributions of the means and residuals were examined to verify model assumptions. HP per day from E1 to E18 was analyzed using the MIXED procedure of SAS 9.2 software package (SAS Institute, 2009) for repeated

measurements. The model used was $Y_{ijkl} = \mu + A_i + B_j + C_k + (A_i \times C_k) + e_{ijkl}$, where $Y_{ijkl}$ is the HP, $\mu$ is the overall mean, $A_i$ is the strain (i = Cobb 500 or Ross 308), $B_j$ is the batch (j = 1 or 2) and $C_k$ is the incubation day (k = E1 to E18), $A_i \times C_k$ is the interaction between the strain and incubation day and $e_{ijkl}$ is the error term. All other variables were analyzed with the GLM procedure of SAS 9.2 software package (SAS Institute, 2009). Strain, batch, and their interaction were included as class variables. The interaction between strain and batch was not statistically significant ($P > 0.05$) in any of the analyses and therefore this interaction was excluded from the model. Least square means were compared using Bonferroni adjustments for multiple comparisons. Values are expressed as LS means. In all cases, a difference was considered significant at $P \leq 0.05$.

# RESULTS



### *Egg Compositions*

At a similar average egg weight, Ross 308 eggs had a higher ratio of yolk: albumen ($P < 0.001$) with a 0.9 g more yolk ($P < 0.001$) and 0.7 g less albumen ($P = 0.020$) than Cobb 500 eggs (Table 1). Ross 308 eggs had 0.5 g lower shell weight and 0.05 mm thinner shells (both $P < 0.001$) than Cobb 500 eggs.

**Table 1.** Egg weight, egg composition, and shell thickness of Cobb 500 and Ross 308 eggs.[1]

| Variable | Cobb 500 | Ross 308 | SEM | *P*-value |
|---|---|---|---|---|
| Egg weight (g) | 62.58 | 62.17 | 0.20 | 0.152 |
| Yolk weight (g) | 19.60 | 20.51 | 0.16 | <0.001 |
| Albumen weight (g) | 37.30 | 36.56 | 0.22 | 0.020 |
| Yolk: Albumen | 0.53 | 0.56 | 0.01 | <0.001 |
| Shell weight (g)[2] | 5.68 | 5.10 | 0.07 | <0.001 |
| Shell thickness (mm)[2] | 0.38 | 0.33 | 0.01 | <0.001 |

[1] For all variables measured, n = 40 per strain. [2]Without membranes.

***Nutrient and Energy Content in the Eggs and Chicks***

Albumen of both strains had a similar amount of DM and CP, whereas the amount of fat in the albumen was higher in Ross 308 eggs ($\Delta = 0.005$ g; $P < 0.001$) than Cobb 500 eggs (Table 2). The yolk of Ross 308 eggs had a higher amount of DM ($\Delta = 0.30$ g; $P = 0.018$) and crude fat ($\Delta = 0.24$ g; $P = 0.009$) than that of Cobb 500 eggs, but the amount of CP did not differ between strains. Albumen plus yolk of Ross 308 had a higher DM ($\Delta = 0.24$ g; $P = 0.040$) and crude fat ($\Delta = 0.23$ g; $P = 0.008$) than that of Cobb 500, whereas the amount of CP was similar. The RSY of Ross 308 and Cobb 500 chicks at 3 h after hatch contained a similar amount of DM, CP, and crude fat. The YFBM of Ross 308 and Cobb 500 chicks at 3 h after hatch did not differ in the amount of DM and CP, but the amount of fat was higher ($\Delta = 0.34$ g; $P < 0.001$) in the YFBM of Cobb 500 chicks than of Ross 308 chicks. The RSY plus YFBM had a similar amount of DM and CP, but the amount of crude fat was higher in Cobb 500 than Ross 308 ($\Delta = 0.44$ g; $P < 0.001$).

Albumen from both strains had a similar amount of energy from CP, whereas energy from crude fat was higher in albumen of Ross 308 than that of Cobb 500 ($\Delta = 0.2$ kJ; $P < 0.001$; Table 3). Albumen of both strains had a similar amount of energy from CP plus crude fat. Yolk of both strains had a similar amount of energy from CP. The amount of energy from crude fat ($\Delta = 8.76$ kJ; $P = 0.009$), and CP plus crude fat ($\Delta = 9.98$ kJ; $P = 0.010$) was higher in the yolk of Ross 308 than that of Cobb 500. The albumen plus yolk of Ross 308 eggs had a higher amount of energy from crude fat ($\Delta = 8.96$ kJ; $P = 0.008$), and CP + crude fat ($\Delta = 8.88$ kJ; $P = 0.014$) than that of Cobb 500 eggs.

At 3 h after hatch, RSY of Cobb 500 chicks had a higher amount of energy from CP ($\Delta = 2.46$ kJ; $P = 0.029$) than that of Ross 308 chicks, whereas the amount of energy from crude fat and CP plus crude fat did not differ. YFBM of both strains had a similar amount of energy from CP. YFBM of Cobb 500 chicks had a higher amount of energy from crude fat ($\Delta = 12.13$ kJ; $P < 0.001$), and CP plus crude fat ($\Delta = 12.37$ kJ; $P = 0.004$) than that of Ross 308 chicks. A combination of RSY plus YFBM of Cobb 500 chicks at 3 h after hatch contained a higher amount of energy from CP ($\Delta = 2.7$ kJ; $P = 0.040$), crude fat ($\Delta = 14.62$ kJ; $P = 0.001$), and CP plus crude fat ($\Delta = 17.32$ kJ; $P = 0.001$) than that of Ross 308 chicks.

**Table 2.** Dry matter (DM), crude protein (CP), and crude fat in albumen, yolk, and albumen plus yolk of fresh eggs and RSY, YFBM, and RSY plus YFBM of Cobb 500 and Ross 308 chicks at 3 h after hatch (g).[1]

| Variable | Cobb 500 | Ross 308 | SEM | *P*-value |
|---|---|---|---|---|
| Albumen | | | | |
| DM | 4.44 | 4.38 | 0.04 | 0.307 |
| CP | 4.03 | 3.95 | 0.04 | 0.191 |
| Crude fat | 0.01 | 0.02 | 0.00 | <0.001 |
| Yolk | | | | |
| DM | 9.38 | 9.68 | 0.09 | 0.018 |
| CP | 2.99 | 3.06 | 0.03 | 0.102 |
| Crude fat | 5.79 | 6.03 | 0.06 | 0.009 |
| Albumen + yolk | | | | |
| DM | 13.82 | 14.06 | 0.08 | 0.040 |
| CP | 7.02 | 7.01 | 0.04 | 0.928 |
| Crude fat | 5.80 | 6.05 | 0.06 | 0.008 |
| RSY | | | | |
| DM | 3.29 | 3.10 | 0.07 | 0.068 |
| CP | 1.53 | 1.45 | 0.04 | 0.118 |
| Crude fat | 1.323 | 1.265 | 0.042 | 0.334 |
| YFBM[2] | | | | |
| DM | 7.15 | 7.18 | 0.08 | 0.762 |
| CP | 4.84 | 4.88 | 0.05 | 0.600 |
| Crude fat | 2.19 | 1.85 | 0.05 | <0.001 |
| RSY + YFBM | | | | |
| DM | 10.44 | 10.28 | 0.10 | 0.225 |
| CP | 6.37 | 6.31 | 0.05 | 0.400 |
| Crude fat | 3.51 | 3.07 | 0.07 | <0.001 |

[1] For variables of albumen, yolk, and albumen plus yolk, n = 20 per strain. For variables of RSY, YFBM, and RSY plus YFBM, n = 30 per strain. [2] YFBM without liver.

**Table 3.** Energy content (kJ) of Cobb 500 and Ross 308 hatching eggs (albumen, yolk, albumen plus yolk) and chicks at 3 h after hatch (RSY, YFBM, and RSY plus YFBM).[1]

| Variable | Cobb 500 | Ross 308 | SEM | *P*-value |
|---|---|---|---|---|
| Albumen | | | | |
| CP | 67.74 | 66.44 | 0.69 | 0.191 |
| Crude fat | 0.50 | 0.70 | 0.31 | <0.001 |
| CP + crude fat | 68.24 | 67.14 | 0.69 | 0.268 |
| Yolk | | | | |
| CP | 50.27 | 51.49 | 0.51 | 0.102 |
| Crude fat | 219.03 | 227.79 | 2.24 | 0.009 |
| CP +crude fat | 269.30 | 279.28 | 2.62 | 0.010 |
| Albumen + yolk | | | | |
| CP | 118.01 | 117.93 | 0.69 | 0.929 |
| Crude fat | 219.53 | 228.49 | 2.25 | 0.008 |
| CP + crude fat | 337.54 | 346.42 | 2.44 | 0.014 |
| RSY | | | | |
| CP | 26.37 | 23.91 | 0.77 | 0.029 |
| Crude fat | 51.31 | 48.82 | 1.97 | 0.379 |
| CP + crude fat | 77.68 | 72.73 | 2.49 | 0.168 |
| YFBM[2] | | | | |
| CP | 82.13 | 81.89 | 1.04 | 0.868 |
| Crude fat | 82.52 | 70.39 | 2.22 | <0.001 |
| CP + crude fat | 164.65 | 152.28 | 2.80 | 0.004 |
| RSY + YFBM[2] | | | | |
| CP | 108.50 | 105.80 | 0.90 | 0.040 |
| Crude fat | 133.83 | 119.21 | 2.96 | 0.001 |
| CP+ crude fat | 242.33 | 225.01 | 3.27 | 0.001 |

[1] For variables of albumen, yolk, and albumen plus yolk, n = 20 per strain. For variables of RSY, YFBM, and RSY plus YFBM, n = 30 per strain. [2] YFBM without liver.

**Table 4.** Yolk free body mass (YFBM) at incubation day (E)11, E14, E18, and at 3 h after hatch, chick weight, chick length, heart weight, heart/YFBM, liver weigh, and liver/YFBM at 3 h after hatch and residual yolk (RSY) weight at E18 and at 3 h after hatch of Cobb 500 and Ross 308.[1]

| Variable | Cobb 500 | Ross 308 | SEM | *P*-value |
|---|---|---|---|---|
| YFBM (g) | | | | |
| E11 | 4.67 | 4.58 | 0.10 | 0.490 |
| E14 | 13.76 | 13.43 | 0.29 | 0.434 |
| E18 | 30.02 | 30.64 | 0.26 | 0.099 |
| RSY (g) | | | | |
| E18 | 13.17 | 13.13 | 0.29 | 0.923 |
| 3 h after hatch | 6.63 | 6.26 | 0.14 | 0.060 |
| 3 h after hatch | | | | |
| YFBM (g) | 38.87 | 39.47 | 0.160 | 0.001 |
| Chick wt. (g) | 45.51 | 45.76 | 0.16 | 0.277 |
| Chick length (cm) | 19.29 | 19.49 | 0.04 | 0.003 |
| Heart wt. (g) | 0.34 | 0.36 | 0.006 | 0.073 |
| Heart/ YFBM (%) | 0.89 | 0.91 | 0.02 | 0.265 |
| Liver wt. (g) | 0.86 | 0.89 | 0.12 | 0.069 |
| Liver/ YFBM (%) | 2.22 | 2.27 | 0.03 | 0.281 |

[1] For all variables measured at E11, E14, and E18, n = 20 per strain. All variables measured at 3 h after hatch, n = 60 per strain.



### *Developmental and Physiological Status*

The weight of YFBM of embryos at E11, E14, and E18 and total chick weight at 3 h after hatch did not differ between strains (Table 4). At 3 h after hatch, Ross 308 chicks were 0.6 g heavier in YFBM ($P = 0.001$) and 0.2 cm longer ($P = 0.003$) than Cobb 500 chicks. RSY at E18 did not differ between strains, but RSY weight of chicks at 3 h after hatch tended to be higher in Cobb 500 chicks than in Ross 308 chicks ($\Delta = 0.37$g; $P = 0.06$).

Weight loss from onset of incubation to E18 ($P = 0.071$) and the eggshell conductance ($P = 0.057$) tended to be higher in Ross 308 than in Cobb 500,

whereas the moment of IP did not differ between strains (Table 5). External pipping occurred at approximately 4 h earlier ($P = 0.010$) in Cobb 500 than in Ross 308 chicks. Cobb 500 chicks hatched approximately 4 h earlier ($P < 0.001$) than Ross 308 chicks. At 3 h after hatch, heart, and liver weights in absolute values and relative to YFBM did not differ between strains.

At 3 h after hatch, hepatic glycogen concentration ($\Delta = 6.3$ mg/g, $P < 0.001$) and total hepatic glycogen ($\Delta = 4.9$ mmol/L, $P = 0.002$) were higher in Cobb 500 than in Ross 308 chicks (Table 6). The levels of glucose, lactate, and uric acid in blood plasma did not differ between strains.

**Table 5.** Weight loss and eggshell conductance at E18 and hatching events of Cobb 500 and Ross 308eggs.[1]

| Variable | Cobb 500 | Ross 308 | SEM | *P*-value |
|---|---|---|---|---|
| Weight loss E18 (%) | 9.78 | 10.08 | 0.12 | 0.071 |
| Eggshell conductance (mg/h/kPa) | 4.34 | 4.48 | 0.05 | 0.057 |
| IP (h) | 466.43 | 466.65 | 0.61 | 0.798 |
| EP (h) | 481.79 | 486.02 | 0.88 | 0.010 |
| Hatch (h) | 488.58 | 492.82 | 0.72 | <0.001 |

[1] For weight loss and eggshell conductance at E18, n = 141 for Cobb 500 and 144 for Ross 308. For variable of hatching events, n = 123 for Cobb 500 and 124 for Ross 308.

**Table 6.** Total hepatic glycogen (mg), hepatic glycogen concentration (mg/g), glucose, lactate, and uric acid in plasma (mmol/L) of Cobb 500 and Ross 308 chicks at 3 h after hatch.[1]

| Variable | Cobb 500 | Ross 308 | SEM | *P*-value |
|---|---|---|---|---|
| Total hepatic glycogen (mg) | 15.85 | 10.89 | 1.08 | 0.002 |
| Hepatic glycogen concentration (mg/g) | 18.11 | 11.81 | 1.12 | <0.001 |
| Glucose (mmol/L) | 9.90 | 10.14 | 0.15 | 0.259 |
| Lactate (mmol/L) | 3.12 | 3.14 | 0.13 | 0.918 |
| Uric acid (mmol/L) | 0.18 | 0.17 | 0.01 | 0.358 |

[1] For all variables measured, n = 60 per strain.

***Nutrient Metabolism***

The total amount of energy from CP plus crude fat used by Ross 308 embryos was higher than for Cobb 500 embryos (Table 7; Δ = 13.85 kJ; *P*= 0.013). The amount of energy lost was higher in Ross 308 than in Cobb 500 (Δ = 26.21 kJ; *P*<0.001). The $E_{YFB}$for energy from CP did not differ between strains. The $E_{YFB}$ for energy from crude fat (Δ = 9.57 kJ) and for CP plus crude fat (Δ = 7.59 kJ) was higher in Cobb 500 than in Ross 308 (both *P*<0.001). Heat production of Cobb 500 and Ross 308 embryos from E1 to E18 did not differ (Figure 1).

**Table 7.** Energy utilization (kJ), energy lost (kJ), and $E_{YFB}$ (%) of Cobb 500 and Ross 308 chicks at 3 h after hatch.[1]

| Variable | Cobb 500 | Ross 308 | SEM | *P*-value |
|---|---|---|---|---|
| Energy utilization | | | | |
| CP | 91.63 | 94.01 | 1.09 | 0.131 |
| Crude fat | 168.21 | 179.67 | 3.21 | 0.160 |
| CP + crude fat | 259.84 | 273.69 | 3.75 | 0.013 |
| Energy lost | 95.19 | 121.40 | 4.11 | <0.001 |
| $E_{YFB}$ | | | | |
| CP | 89.71 | 87.26 | 1.12 | 0.130 |
| Crude fat | 49.18 | 39.61 | 1.49 | <0.001 |
| CP + crude fat | 63.47 | 55.88 | 1.16 | <0.001 |

[1] For all variables measured, n = 20 per strain.

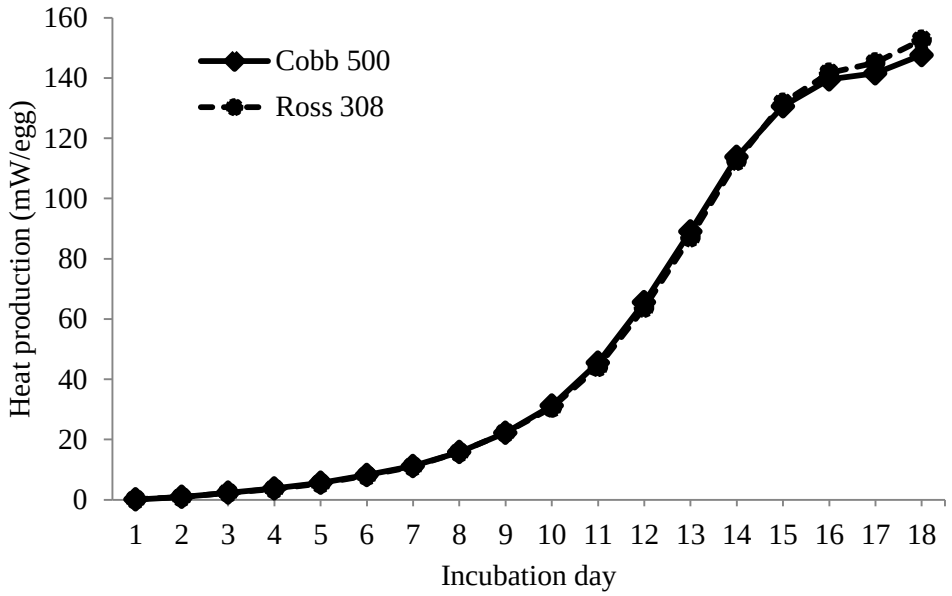


**Figure 1.** Heat production (HP; mW/egg) of Cobb 500 and Ross 308 embryos from E1 to E18 (strain × incubation day $P = 0.469$).

## DISCUSSION

The objective of this study was to examine influences of broiler strain on nutrient availability, embryonic development, and nutrient metabolism during incubation. To minimize confounding effects, Cobb 500 and Ross 308 eggs were obtained from the same breeder age and at the same egg weight range. According to standard breeder performance, an average egg during 43 to 46 weeks is weighing at 65.1 to 66.3 g for Cobb 500 fast feathering and 64.8 to 65.8 g for Ross 308. This means that selected eggs at weight range of 60 to 63 g were in a similar egg weight distribution range of both strains. Eggs of both strains were incubated at the same EST of 37.8ºC, which is shown to give optimal embryonic development (Lourens et al., 2005). The results showed that Cobb 500 and Ross 308 differed in nutrient availability, embryonic development, and nutrient metabolism during incubation.

Although eggs were selected at the same egg weight range, the eggs of Ross 308 had a higher energy content compared to Cobb 500. This can be

explained by a higher ratio of yolk: albumen in Ross 308 than in Cobb 500 eggs. Egg yolk functions as the main energy source providing approximately 90% of the total energy requirement of an embryo (Noble and Cocchi, 1990), whereas albumen has limited contribution to egg energy availability due to a low DM content and the almost absence of fat (Ar et al., 1987; Nangsuay et al., 2013). Similar to the results of Nangsuay et al. (2013), the present results indicate that the amount of energy in the eggs was determined mainly by the amount of energy in the yolk. The albumen weight was higher in Cobb 500 than in Ross 308, but the amount of energy in the albumen was similar. This was due to a similar amount of DM and CP content in the albumen of both strains. A one gram heavier yolk weight accompanied with a higher amount of DM and crude fat resulted in a higher amount of energy in the yolk of Ross 308 than Cobb 500 eggs. The amount of CP and energy derived from CP in the yolk did not differ between strains. In total, Ross 308 eggs had a higher DM and crude fat and thus a higher energy content than Cobb 500. It can be questioned if the commercial diets for both breeder strains have affected the egg compositions and egg energy content. Peebles et al. (2000) demonstrated that there is no effect of the diets containing ME in the ranges at 2,709 to 2,940 kcal/kg and crude fat at 2.26 to 5.28% on the yolk: albumen ratio of broiler breeder eggs of 31 to 47 weeks old hens. Since differences of commercial diets provided for breeders in the current study were minimal, it can be assumed that the nutrient availability for embryos at the start of incubation is influenced by broiler strains. However, we did not know the actual energy intake of the breeders which might have an influence on yolk: albumen ratio (Peebles et al., 2000).



During incubation, Cobb 500 and Ross 308 embryos showed differences in developmental pattern especially at the last stage of incubation. The YFBM at E11 and E14 did not differ, but at E18 Ross 308 embryos tended to have a heavier YFBM than Cobb 500 embryos. This might be caused by differences in growth rate between E14 and E18, where Ross 308 grew approximately 5% faster than Cobb 500. From E18 to hatch, the increase in YFBM was similar for both strains. However, at hatch Ross 308 chicks had a higher YFBM and a longer chick length than Cobb 500 chicks. Our findings are in agreement with Tona et al. (2010) who reported a faster development of Ross than Cobb strain during the second week of incubation. Although differences in embryonic development in the current study occurred only during the last stage of incubation, the results indicate an influence

of genetic background of broiler strains on developmental pattern during embryonic stages.

During the hatching process, the moments of EP and hatching were approximately 4 h earlier in Cobb 500 than in Ross 308, whereas the moment of IP was similar. A tendency of a lower eggshell conductance and egg weight loss at E18 in Cobb 500 than in Ross 308 might have an influence on respiration gas exchanges and consequently the hatching events might be affected. Visschedijk (1968) demonstrated that external pipping will be immediately or within a very short time followed by a sudden sharp rise of the total $O_2$ consumption and $CO_2$ production in the air cell. It is possible that after E18 Cobb 500 embryos had to deal with a higher magnitude of insufficient $O_2$ and an increase of $CO_2$ than the embryos of Ross 308. A trigger for an early EP and consequently early hatching of Cobb 500 embryos might occur by a limited supply of $O_2$ in the air cell. In addition, a higher hepatic glycogen in Cobb 500 than in Ross 308 might play a role in hatching process. It could mean that Cobb 500 embryos have more glycogen storage, which can provide a comparatively greater supply of glucose as energy source for a success of hatching in a shorter time. Our findings are in agreement with Tona et al. (2010) who reported approximately 2 h shorter in incubation duration of Cobb than Ross strain.

Cobb 500 and Ross 308 embryos differed in nutrient metabolism during incubation. This was shown in energy utilization, energy lost, and the efficiency of converting energy used to YFBM (EYFB). However, the observed differences in nutrient metabolism did not result in differences in metabolic blood parameters like glucose, lactate, and uric acid at 3 h after hatch. The differences in embryonic development pattern, nutrient availability, and gaseous exchanges might be reasons for the differences in nutrient metabolism. The available egg nutrients are catabolized and used by the embryos for the purposes of synthesis of new body tissues, maintenance of existing tissues and for muscular activity to sustain embryonic development through hatching (Vleck, 1991). Although we did not measure energy used before hatching, an increase in growth rate of Ross 308 embryos during E14 to E18 might have increased the use of energy for these embryos in this period compared to Cobb 500 embryos. As an increase in energy utilization requires availability of nutrients and gaseous exchange, a higher availability of nutrients in the eggs and a better gaseous exchange due to a thinner

egg shell and a tendency of higher conductance might permit Ross 308 embryos to utilize more energy than Cobb 500 embryos. An increase in growth rate and therefore a larger embryo in this period might increase the energy utilization for maintenance as well. After E18, the growth rate was similar for both strains, but the energy utilization was increased at hatch for Ross 308 compared to Cobb 500 chicks, which might be explained by a higher energy requirement for maintenance. At E18 and 3 h after hatch, YFBM was approximately 0.6 g higher for Ross 308 than for Cobb 500. To calculate the requirements for maintenance, we used the maintenance requirements for broilers of 435 kJ/kg/day (CVB, 2010), as maintenance requirements for embryos are not known to the author's knowledge. An expression of maintenance requirement based on direct weight of embryos instead of body weight to the power 0.75 as normally is used for broilers, has been proposed by Vleck et al. (1980) and Mortola and Cooney (2008). The calculations suggest that the requirement for maintenance of Ross 308 embryos at E18 and at 3 h after hatch was higher than of Cobb 500 embryos in the same period with approximately 260 to 270 Joule per day or 3 mW. These values are in agreement with the observed differences in HP at E18 between Ross 308 and Cobb 500. This might explain at least part of the higher energy usage and numerically higher embryonic HP at E18 for Ross 308 compared to Cobb 500.



From the total amount of energy used, Ross 308 embryo had approximately 8% lower EYFB than Cobb 500 embryos. It is possible that the embryos of both strains have different strategies for nutrient metabolism during the period of reaching limitation of gaseous exchanges or plateau stage. Dietz et al. (1998) suggested that during the plateau phase, embryos allocate the available energy in favor for maintenance by decreasing the development, increasing synthesis efficiency, and depressing the formation of glycogen. Cobb 500 embryos had less nutrients available in the eggs than Ross 308 embryos and they might have an earlier limitation of gaseous exchanges during incubation. Tona et al. (2010) demonstrated a similar partial pressure of $CO_2$ ($PCO_2$) and $O_2$ ($PO_2$) in the air cell at E18 for Cobb and Ross strains, but at the IP stage the $PO_2$ was lower, whereas $PCO_2$ was higher in Cobb than in Ross. Under these conditions, Cobb 500 embryos might have to slow down growth rate and a strategy to use the available resources at high synthesis efficiency might take place. On the other hand, Ross 308 embryos had more nutrients available in the eggs and maybe less limitation in gaseous

exchanges during the last stage of incubation. As a result, the embryonic development was able to continue. When the plateau stage is reached, Ross 308 embryos need to allocate more nutrients for maintenance due to a bigger YFBM. Furthermore, the prolonged incubation duration and the expanded time between IP and EP, and IP and hatch of 4 h for Ross 308 than for Cobb 500 might lead to a higher energy requirement for hatching activities. A higher energy lost in Ross 308 than in Cobb 500 could to a certain extent be a reflection of the differences in energy used for maintenance and for hatching activities. In agreement with the proposition of Dietz et al. (1998) we found that at 3 h after hatch Ross 308 chicks had a lower $E_{YFB}$ accompanied with a lower glycogen storage than Cobb 500. As hepatic glycogen levels are one of the indicators for energetic status of the hatchling, the differences of glycogen levels might have an influence on the early growth (Uni et al., 2005). The differences of EYFB demonstrated that embryos of different strains differ in converting energy used to YFB, however the significance for the hatchling in later life needs further research.

Our findings for energy utilization are consistent with Nangsuay et al. (2013, 2015) who demonstrated that yolk size and the availability of nutrients in the yolk can influence the amount of energy used by the embryos. The same authors proposed that embryonic HP increases with an increase of energy utilization. Although we did find a significant difference in energy lost, we found only a numerically higher HP in Ross 308 from E16 onwards. Although there was no EST control in the studies of Hamidu et al. (2007) and Tona et al. (2010), the current results of HP show a similar trend as found in those studies.

In conclusion, the current results indicate that genetic background of broiler strains influences embryonic development and nutrient metabolism during incubation. The differences occurred even with an identical EST. Taking into account the influence of genetic background on the results obtained, it can be questioned whether the optimal level of EST will be similar for Ross 308 and Cobb 500.

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# CHAPTER 6

# Effects of breeder age, broiler strain, and eggshell temperature on development and physiological status of embryos and hatchlings

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# ABSTRACT

Breeder age and broiler strain can influence the availability of nutrients and oxygen, particularly through differences in yolk size and shell conductance. We hypothesized that these egg characteristics might affect embryonic responses to changes in eggshell temperature (EST). This study aimed to investigate the effect of breeder age, broiler strain, and EST on development and physiological status of embryos. A study was designed as a 2 × 2 × 2 factorial arrangement using 4 batches of 1,116 hatching eggs of 2 flock ages at 29 to 30 wk (young) and 54 to 55 wk (old) of Ross 308 and Cobb 500. EST of 37.8 (normal) or 38.9ºC (high) was applied from incubation d 7 (E7) until hatching. The results showed that breeder age rather than broiler strain had an influence on yolk size ($P = 0.043$). The shell conductance was higher in Ross 308 than in Cobb 500 ($P < 0.001$). A high EST resulted in a higher yolk free body mass (YFBM) compared to the normal EST at E14 and E16, but at 3 h after hatch YFBM was lower when eggs were incubated at high EST compared to normal EST (all $P < 0.001$). Cobb 500 eggs yielded embryos with a lower YFBM at E14, E18, and 3 h after hatch (all $P < 0.05$) than Ross 308 eggs. Breeder age had no effect on YFBM, but the RSY weight was higher in embryos from the old flock compared to the young flock embryos at E14 and E16 (both $P < 0.05$). A 3-way interaction among breeder age, strain, and EST was found, especially for incubation duration, navel quality, and relative heart and stomach weights at 3 h after hatch (all $P < 0.05$). Based on the results obtained, we conclude that oxygen availability rather than nutrient availability determines embryonic development, and the egg characteristics affected embryonic responses to changes of EST, especially for variables related to chick quality.

**Key words:** breeder age, broiler strain, eggshell temperature, embryonic development

# INTRODUCTION

During incubation, energy for embryonic development and growth is derived exclusively from egg nutrients. To metabolize nutrients, embryos uptake oxygen and use it in the biochemical process for energy production. The availability of both egg nutrients and oxygen therefore plays a crucial role in energy production for embryonic development and growth. From the start of incubation until incubation day 18 (E18), chicken embryos act poikilothermic, which means that their nutrient metabolism and thus embryonic growth during this period can be influenced by temperature (Tazawa et al. (1988). Studies in recent year have shown the importance of eggshell temperature (**EST**) and a constant EST of 37.8ºC has been reported to be optimal for embryonic development and hatching success (Lourens et al., 2005; Lourens et al., 2007; Molenaar et al., 2010; 2011). An EST of 38.9ºC from d 8 to d 19 of incubation (E8 to E19) initially increased embryonic development until the second wk of incubation, which was reflected in a high heat production (Lourens et al., 2007). However, at the last stage of incubation, a high EST had a negative impact on embryonic development. At the same stage of incubation, an increase of the oxygen level (25%) resulted in improved embryonic development (Lourens et al., 2007; Molenaar et al., 2010; 2011). These studies of EST and oxygen levels indicate that embryos, which initially have been accelerated in growth rate by a high EST, might at the end of incubation be unable to maintain nutrient metabolism at the level required to have a sufficient energy supply for development and growth. In other words, they might encounter an imbalance between requirements and availability of nutrients and oxygen and subsequently decreased nutrient metabolism and embryonic development.



Although egg nutrients are deposited in both the albumen and yolk, yolk nutrients function as the main nutritional supply providing approximately 90% of the total energy requirement (Romanoff, 1967; Noble and Cocchi, 1990). An alteration of yolk size is influenced mainly by breeder age (O'Sullivan et al., 1991; Hamidu et al., 2007; Nangsuay et al., 2011) and the amount of energy in the egg increases with yolk size (Nangsuay et al., 2013). Differences in broiler strains also have an influence on yolk size and consequently on the nutrient availability

(Nangsuay et al., 2015). Yolk size does not only have an influence on the amount of yolk nutrients, but also on the capacity of the embryos to uptake yolk nutrients (Yadgary et al., 2013). Additionally, studies also have demonstrated that eggs from different breeder ages (O'Dea et al., 2004) and broiler strains (Nangsuay et al., 2015) tend to differ in eggshell conductance. As respiration gas exchanges occur across the eggshell, differences in eggshell conductance might affect the oxygen availability for the embryo, thereby affecting embryonic development and growth (Ar and Rahn, 1985). This means that hatching eggs originating from different breeder ages and strains could differ in oxygen and nutrient availability and this could affect nutrient metabolism and embryonic development during incubation. By integrating the available information from previous studies regarding the effects of EST and oxygen levels, it can be hypothesized that embryos from different breeder ages and broiler strains that differ in nutrient and oxygen availability, would react differently to an increase of EST. More specifically, it can be hypothesized that embryos from eggs with more nutrient availability and high eggshell conductance would cope better with an increased EST. Therefore, the objective of this study was to determine effects of breeder age, broiler strains, and EST on development and physiological status of embryos and hatchlings.

# MATERIALS AND METHODS

### *Experimental Design*

The experiment was designed as a 2 × 2 × 2 factorial arrangement with 2 breeder ages (young and old), 2 broiler strains (Ross 308 and Cobb 500), and 2 EST (37.8 and 38.9ºC). In all treatments, EST was applied from E7 until the eggs were transferred to the hatching baskets (E18). Thereafter the machine temperature was fixed corresponding to an EST of 37.8 or 38.9ºC. The experiment was performed in 4 consecutive batches and each treatment was repeated twice. Within each batch, eggs were obtained from the same breeder age of both strains and the eggs of each strain were incubated at 2 EST levels. The experimental protocol was approved by the Institutional Animal Care and Use Committee of Wageningen University, the Netherlands.

***Hatching Eggs and Egg Storage***

Four batches of 1,116 good quality hatching eggs of 2 flock ages at 29 to 30 wk (young; **Y**) and 54 to 55 wk (old; **O**) of Ross 308 (**R**) and Cobb 500 fast feathering (**C**) were obtained from commercial broiler breeder farms. The broiler breeders of all flocks received commercially available diets. The diets for each group were as follows; YC = 14.9% CP, 5.2% crude fat, 2,775 kcal/kg ME, and 1.9% linoleic acid; YR = 14.7% CP, 4.8% crude fat, 2,813 kcal/kg ME, and 1.8% linoleic acid; OC = 14.6% CP, 4.8% crude fat, 2,772 kcal/kg ME, and 1.8% linoleic acid; OR = 14.2% CP, 5.3% crude fat, 2,767 kcal/kg ME, and 2.0% linoleic acid. Within each batch, eggs of both strains were obtained from the same breeder age. To assess normal average and variation of egg weight of each breeder flock, 150 eggs of each flock were weighed individually during the egg collection day. The average egg weight and standard variation of each strain at a given age were as follows: YR = 56.35 ± 3.77 g, OR = 68.75 ± 5.16 g, YC = 57.41 ± 4.10 g, and OC = 70.84 ± 5.52 g. Thereafter, for each batch, a total of 1,116 eggs (558 eggs of each strain) were selected at an egg weight range between 60 and 63 g. Per batch, 30 eggs of each strain and breeder age were used to determine egg composition. The remaining eggs of each strain were placed on 6 setter trays (88 eggs per tray) and were stored at 20ºC and 55 to 60% relative humidity for 2 to 3 d.



***Incubation***

Incubation E0 to E7: Prior to the start of incubation, average egg weight of each tray was determined. All 12 setter trays (6 trays of each strain) of the 2 different strains were alternately placed in the same setter with a maximum setting capacity of 1,408 eggs (HatchTech B.V., Veenendaal, the Netherlands). From E0 to E7, EST was maintained at 37.8ºC and the relative humidity at 55 to 60%. The EST was measured by 4 sensors (NTC Thermistors: type DC 95; Thermometrics, Somerset, UK). The sensors were attached to 4 individual eggs, 2 eggs of each strain. The EST sensors were attached with a small piece of tape (Tesa BV, Almere, The Netherlands) in heat conducting paste (Schaffner Holding AG, Luterbach, Switzerland) to the eggshell at the equator of the egg. At E7, all eggs were candled and infertile and cracked eggs were removed from the trays.

Thereafter weights of the fertile eggs plus tray and weight of the empty tray were measured. The average egg weight of the fertile eggs of each tray was used to calculate egg weight loss at E7; average egg weight of each tray at E0 minus the average egg weight of the fertile eggs of each tray at day E7. This egg weight loss was used in later calculations to determine eggshell conductance.

Incubation E7 to E18: After candling at E7, eggs of each strain were divided into a control and a treatment group and were incubated separately in one of 4 open circuit climate respiration chambers (**CRC**). The CRC consisted of 2 CRC with a size of 267 L (small; Lourens et al., 2006) and 2 CRC with a size of 1,800 L (large; Verstegen and Henken, 1987). Each treatment was repeated twice, one replicate in a small CRC and the other replicate in a large CRC. Due to difference in CRC volume, approximately 70% of the eggs of each strain within each batch were incubated in the large CRC and the remaining eggs were incubated in the small CRC. All 4 CRC were equipped with the same system for egg turning, ventilation, temperature, and humidity control as described by Lourens et al. (2006). Each CRC has one Vaisala HMT330 series combi sensor (Vaisala Company, Vantaa, Finland) to measure machine temperature and relative humidity. The EST was measured by 5 sensors (Pt-100, Sensor Data BV, Rijswijk, The Netherlands) per CRC, which were attached to 5 individual fertile eggs in each CRC. The EST sensors were attached with tape as explained above. Between E7 to E18, EST of the control group was maintained at 37.8ºC and at 38.9ºC for the treatment group. The relative humidity was maintained at 55 to 60% for both the control and treatment groups. During incubation, EST from the 5 individual fertile eggs was measured continuously and the machine temperature was adjusted automatically every 5 min if the median EST differed from the target EST.

At E18, all eggs were candled and thereafter fertile eggs were transferred to hatching baskets. The machine temperature set point was set at a constant value that corresponded to an EST just before transfer of 37.8ºC for the control group and 38.9ºC for the treatment group. For the remaining time until hatching, machine temperature was fixed and EST was allowed to change.

### *Fresh Egg Composition*

Fresh egg composition was measured to investigate nutrient availability in the eggs. In each batch, 30 eggs of each strain were boiled for 10 min and thereafter albumen and yolk were separated and weighed. The eggshell, excluding shell membranes, was dried for 24 h at room temperature and weighed. Albumen and yolk were stored at −20ºC for further analyses.

### *Eggshell Conductance*

Eggshell conductance influences diffusion rate of $O_2$ across the eggshell and thus oxygen availability for embryos. The eggshell conductance was calculated by using egg weight loss of fertile eggs between E0 and E7. Because the egg weight loss is a function of time, eggshell conductance and the water vapor pressure deficit ($\Delta P_{H2O}$) across the egg shell, Meijerhof and van Beek (1993) demonstrated the eggshell conductance can be calculated by determination of egg weight loss under known $\Delta P_{H2O}$. The water vapor pressure ($P_{H2O}$) is a function of temperature and relative humidity. To determine $P_{H2O}$, the average temperature and relative humidity between E0 and E7 was determined. The calculation for eggshell conductance was made as follows; egg weight loss / $\Delta P_{H2O}$; where egg weight loss (mg/h) = egg weight loss of fertile eggs at E7/ (7 × 24) and $\Delta P_{H2O}$ (kPa) = average vapor pressure deficit between E0 to E7. To calculate $\Delta P_{H2O}$, water vapor pressure inside the eggs was determined as the saturation vapor pressure at 37.8ºC and 100% relative humidity. The water vapor pressure outside of the eggs was calculated based on average temperature (ºC) and humidity (%) of the machine between E0 to E7.



### *Embryonic Development*

To investigate an influence of breeder age, broiler strain, and EST on embryonic development, the measurements were made during incubation. At E7, 15 fertile eggs of each strain from each batch were randomly sampled. The eggs were opened and the embryo yolk free body was separated, dipped on tissue paper to dry, and weighed to obtain yolk free body mass **(YFBM)**. At E14, 30 eggs, and

at E16, 15 eggs per treatment per batch were sampled. The eggs were opened and embryo YFBM and residual yolk (**RSY**) were separated and weighed. At E18, 22 to 25 fertile eggs of each treatment per batch were randomly sampled. Blood samples were withdrawn from the jugular vein of the embryos and thereafter embryo YFBM was determined. After decapitation, the liver was removed, weighed, and immediately stored in liquid nitrogen. The RSY and heart were removed and weighed. The YFBM of embryos at E18 was stored at −20ºC for further analyses.

All other eggs that were transferred to hatching baskets were checked every 3 h for the hatching moment (chicks completely emerged from the eggshell) starting at 463 h after the start of incubation. The incubation duration was defined as the time between start of incubation and the hatching moment. At 3 h after hatch, chicks were taken out of the chambers and then cloacal temperature was immediately measured by using an infrared ThermoScan 4520 ExacTemp (Braun GmbH, Kronberg, Germany). The navel quality was assessed as 1 = good (nothing left outside the navel), 2 = moderate (a little black string or little black button smaller than 2 mm left outside the navel) or 3 = poor (a black button exceeding 2 mm left outside the navel). Thereafter, chick weight was measured and blood was collected after decapitation (0.5 to one mL). Chicks were opened and the liver was removed, weighed, and immediately stored in liquid nitrogen. The RSY and heart were removed and weighed. Chick YFBM was determined as chick weight minus RSY weight. Chick YFBM and RSY were stored at −20ºC for further analyses. The stomach and intestines of the embryos at E18 and chicks at 3 h after hatch were weighed after defrosting the YFBM at room temperature for 24 h. All organ weights are expressed as percentage of YFBM. Hatch of fertile was calculated as (number of hatched chick/number of fertile eggs that were transferred to hatching basket) x 100.

***Plasma Metabolite and Hepatic Glycogen Determination***

Plasma metabolite and hepatic glycogen are indications for physiological status of the embryos, which might be different due to variation of nutrients and oxygen availability. At E18, the sampled fertile eggs were opened and blood was withdrawn from the jugular vein using a 30-gauge needle and 1-mL syringe,

flushed with 10% heparin. Blood was collected for approximately 0.3 to 0.5 mL per embryo in a 1.5 mL Eppendorfs containing 0.01 mL of 10% heparin. Blood was centrifuged at 12,000 g for one min at room temperature and plasma was stored at −20ºC for further analyses. At 3 h after hatch, chicks were decapitated and blood was collected in a 4-mL blood tube containing 10 mg of sodium fluoride and 8 mg of potassium oxalate (BD Vacutainer, Franklin Lakes, NJ). An extra 0.02 mL of 10% heparin was added and mixed into the tube before sampling. Blood was centrifuged at 2,000 × g for 10 min at room temperature and plasma was stored at −20ºC until further analyses. Plasma glucose, lactate, and uric acid concentrations of embryos at E18 and at 3 h after hatch were determined with commercially available enzymatic photometric kits (DiaSys Diagnostic Systems International, Holzheim, Germany).

Livers of embryos at E18 and chicks at 3 h after hatch were stored at −80ºC until further analysis. The hepatic glycogen determination was performed as described by Molenaar et al. (2010). All procedures for hepatic glycogen determination were carried out on ice. The whole liver was homogenized with a glass stirring spoon after the addition of one $\mu$L of 7% HCLO4/mg of wet tissue. The suspension was centrifuged (2,900 ×$g$) at 4ºC for 15 min. The supernatant was decanted, cleaned with one mL petroleum ether, and frozen at −80ºC until further analysis. The supernatant was defrosted, centrifuged, and decanted again. Hepatic glycogen was determined by the iodine binding assay using an iodine (I2-KI) solution containing saturated CaCl2 and absorbance was measured by using microtiter plate reader at 450 nm (Dreiling et al., 1987). Hepatic bovine glycogen (Type IX, Sigma Aldrich Chemie GmbH, Steinheim, Germany) was used as a standard.



***Statistical Analyses***

The setter tray was used as the experimental unit for egg weight loss and eggshell conductance at E7. For other variables, egg and chick were used as the experimental unit in the statistical analyses. Distributions of means and residuals were examined to verify model assumptions. Lactate and uric acid at E18 were transformed to log10 and uric acid at 3 h after hatch was transformed to square-root data to obtain normal distribution. All data, except navel quality and hatch of fertile

were analyzed using PROC MIXED in the statistical software package SAS 9.3 (SAS Institute Inc. 2002-2010, Cary, NC). The model used for the statistical analyses of fresh egg composition, egg weight loss at E7, and eggshell conductance was

$$Y_{ijk} = \mu + A_i + B_j + AB_{ij} + C(A_i) + e_{ijk} \quad (1)$$

where $Y_{ijk}$ was the dependent variable, $\mu$ was the overall mean, $A_i$ was the breeder age (i = young or old), $B_j$ was the strain (j = Cobb 500 or Ross 308), $AB_{ij}$ was the interaction between breeder age and strain, $C(A_i)$ was the breeder age nested within 4 batches and this term was used as an random effect, and $e_{ijk}$ was the error term. Variables measured after E7, when the EST treatment was applied, were analyzed using PROC MIXED in the statistical software package SAS 9.3 (SAS Institute Inc. 2002–2010, Cary, NC) using model [1] extended with the EST ($D_l$; l = 37.8 or 38.9) and interactions of the other factors with EST (model 2). Type of climate respiration chambers (**CRC**; small and large) was initially included in model [2], but statistic results showed no influence of CRC on results obtained and thereafter this factor was excluded from the analysis. The navel quality scores were converted to a binary variable, where score 1 and score 2 were converted to 0 and score 3 was converted to 1. The statistical analyses for navel quality and hatch of fertile were performed using PROC GLIMMIX in the statistical software package SAS 9.3 (SAS Institute Inc. 2002-2010, Cary, NC) using model 2. Least square means were compared using Bonferroni adjustments for multiple comparisons. Values are expressed as LS means. For lactate and uric at E18 and uric acid at 3 h after hatch, LS means of original data are presented, combined with *P*-values of the transform data. In all cases, a difference was considered significant at $P \leq 0.05$.

# RESULTS

### *Fresh Egg Composition, Egg Weight Loss, and Eggshell Conductance*

A 2-way interaction between breeder age and strain was found for yolk weight ($P = 0.043$; Table 1). Yolk weight was higher in eggs of old breeder flocks than that of young breeder flocks in both strains, but the difference in Cobb 500 was smaller than in Ross 308 (3.20 and 3.81 g, respectively).

Although we selected on egg weight range, egg weight was higher in eggs from old breeder flocks than from young breeder flocks ($\Delta = 0.5$ g; $P = 0.048$). Egg weight of Cobb 500 and Ross 308 did not differ. Albumen weight was lower in eggs of old breeder flocks than that of young breeder flocks ($\Delta = 2.95$ g; $P = 0.003$), but did not differ between strains. Shell weight did not differ between breeder ages and strains. The ratio of yolk to albumen was higher in eggs of old breeder flocks than that of young breeder flocks ($\Delta = 0.14$; $P = 0.002$), whereas the ratio of yolk to albumen did not differ between strains. At E7, egg weight loss ($\Delta = 0.15\%$; $P < 0.001$) and eggshell conductance ($\Delta = 0.21$ mg/h/kPa; $P < 0.001$) were higher in Ross 308 than in Cobb 500, but did not differ between breeder ages.



### *Yolk Free Body Mass (YFBM), Residual Yolk (RSY), Hatch of Fertile Egg (HOF), Incubation Duration, and Navel Quality*

YFBM of embryos at E7, E14, E16, E18, and at 3 h after hatch and chick weight at 3 h after hatch did not differ between breeder ages (Table 2). YFBM of embryos at E7 and E16 did not differ between strains, but at E14 ($\Delta = 0.19$ g; $P < 0.026$), E18 ($\Delta = 0.59$ g; $P = 0.005$) and at 3 h after hatch ($\Delta = 0.59$ g; $P < 0.001$) YFBM was higher in Ross 308 embryos than in Cobb 500 embryos. At E14 ($\Delta = 1.11$ g; $P < 0.001$) and E16 ($\Delta = 0.94$ g; $P < 0.001$) the YFBM was higher in embryos at EST of 38.9◦ than at 37.8ºC, but at 3 h after hatch the YFBM was lower ($\Delta = 0.83$ g; $P < 0.001$) in embryos at EST of 38.9 than 37.8ºC. Chick weight at 3 h after hatch did not differ between strains and EST.

A 2-way interaction between breeder age and strain was found for RSY weight at 3 h after hatch ($P = 0.048$; Table 3). The RSY weight was lower in both

young and old flocks of Ross 308 chicks than in Cobb 500 chicks, but this difference was more pronounced in the young flocks (Δ = 0.93 g) than in the old flocks (Δ = 0.48 g).

**Table 1.** Fresh egg, yolk, albumen, and shell weights (g), ratio of yolk to albumen, egg weight loss at incubation day (E) 7 (%), and eggshell conductance (mg/h/kPa) of Cobb 500 (C) and Ross 308 (R) eggs from young (Y; 29 to 30 wk) and old (O; 54 to 55 wk) flock (LSmeans).[1]

| Effect | Egg, g | Yolk, g | Albumen, g | Shell, g | Yolk: Albumen | Egg weight loss, %[2] | Eggshell conductance, mg/h/kPa[2] |
|---|---|---|---|---|---|---|---|
| Breeder age x strain | | | | | | | |
| Y C | 61.02 | 17.13[b] | 38.39 | 5.50 | 0.45 | 3.05 | 4.04 |
| Y R | 61.19 | 16.89[b] | 38.61 | 5.68 | 0.44 | 3.26 | 4.31 |
| O C | 61.56 | 20.33[a] | 35.69 | 5.55 | 0.57 | 3.30 | 4.41 |
| O R | 61.64 | 20.70[a] | 35.42 | 5.53 | 0.59 | 3.41 | 4.55 |
| SEM | 0.11 | 0.15 | 0.17 | 0.06 | 0.01 | 0.11 | 0.15 |
| Breeder age (wk) | | | | | | | |
| Y | 61.10[b] | 17.01 | 38.50[a] | 5.59 | 0.44[b] | 3.16 | 4.18 |
| O | 61.60[a] | 20.51 | 35.55[b] | 5.54 | 0.58[a] | 3.36 | 4.48 |
| SEM | 0.08 | 0.10 | 0.12 | 0.05 | 0.004 | 0.11 | 0.15 |
| Strain | | | | | | | |
| C | 61.29 | 18.73 | 37.04 | 5.52 | 0.51 | 3.18[b] | 4.22[b] |
| R | 61.41 | 18.79 | 37.02 | 5.60 | 0.51 | 3.33[a] | 4.43[a] |
| SEM | 0.08 | 0.10 | 0.13 | 0.04 | 0.004 | 0.08 | 0.11 |
| Source of variation | | | | *P*-values | | | |
| Breeder age x strain | 0.724 | 0.043 | 0.152 | 0.079 | 0.071 | 0.141 | 0.137 |
| Breeder age | 0.048 | 0.002 | 0.003 | 0.508 | 0.002 | 0.329 | 0.287 |
| Strain | 0.273 | 0.639 | 0.887 | 0.164 | 0.697 | <.0001 | <.0001 |

[1] n = 60 for treatment combination of breeder age x strain. [2] n = 12 for treatment combination of breeder age x strain. [a,b] LSmeans lacking a common superscript within a column and factor differ ($P \leq 0.05$).

**Table 2.** Yolk free body mass (YFBM, g) at E7, E14, E16, E18, and at hatch (3 h after hatch), chick weight at 3 h after hatch (g), and incubation duration (h) of Cobb 500 (C) and Ross 308 (R) eggs from young (Y; 29 to 30 wk) and old (O; 54 to 55 wk) flock incubated at an eggshell temperature (EST) of 37.8 or 38.9ºC from E7 until hatching (LSmeans).[1]

| Effect[2] | YFBM, g | | | | | Chick weight, g | Incubation duration, h |
|---|---|---|---|---|---|---|---|
| | E7[3] | E14 | E16 | E18 | Hatch | | |
| Breeder age x strain | | | | | | | |
| Y C | 0.75 | 12.78 | 20.23 | 28.56 | 37.85 | 45.26 | 492 |
| Y R | 0.76 | 12.96 | 20.03 | 29.25 | 38.52 | 45.00 | 497 |
| O C | 0.79 | 12.93 | 19.82 | 28.29 | 37.74 | 45.38 | 488 |
| O R | 0.78 | 13.15 | 20.11 | 28.79 | 38.25 | 45.38 | 493 |
| SEM | 0.01 | 0.23 | 0.17 | 0.30 | 0.13 | 0.14 | 0.93 |
| Breeder age x EST | | | | | | | |
| Y 37.8 | | 12.34 | 19.61 | 28.77 | 38.71 | 45.17 | 499 |
| Y 38.9 | | 13.40 | 20.65 | 29.04 | 37.67 | 45.09 | 490 |
| O 37.8 | | 12.46 | 19.54 | 28.47 | 38.31 | 45.21 | 496 |
| O 38.9 | | 13.63 | 20.38 | 28.61 | 37.69 | 45.54 | 485 |
| SEM | | 0.19 | 0.17 | 0.30 | 0.13 | 0.14 | 0.93 |
| Strain x EST | | | | | | | |
| C 37.8 | | 12.34 | 19.57 | 28.17 | 38.27 | 45.32 | 496 |
| C 38.9 | | 13.38 | 20.48 | 28.68 | 37.33 | 45.32 | 483 |
| R 37.8 | | 12.46 | 19.58 | 29.07 | 38.74 | 45.06 | 499 |
| R 38.9 | | 13.64 | 20.55 | 28.97 | 38.03 | 45.32 | 491 |
| SEM | | 0.17 | 0.17 | 0.26 | 0.13 | 0.13 | 0.78 |
| Breeder age | | | | | | | |
| Y | 0.76 | 12.87 | 20.13 | 28.91 | 38.19 | 45.13 | 494 |
| O | 0.78 | 13.04 | 19.96 | 28.54 | 37.99 | 45.38 | 490 |
| SEM | 0.01 | 0.22 | 0.12 | 0.26 | 0.09 | 0.11 | 0.83 |
| Strain | | | | | | | |
| C | 0.77 | 12.86[b] | 20.02 | 28.43[b] | 37.80[b] | 45.32 | 490 |
| R | 0.77 | 13.05[a] | 20.07 | 29.02[a] | 38.39[a] | 45.19 | 495 |
| SEM | 0.01 | 0.16 | 0.12 | 0.21 | 0.09 | 0.10 | 0.65 |
| EST | | | | | | | |
| 37.8 | | 12.40[b] | 19.58[b] | 28.62 | 38.51[a] | 45.19 | 498 |
| 38.9 | | 13.51[a] | 20.52[a] | 28.82 | 37.68[b] | 45.32 | 487 |
| SEM | | 0.16 | 0.12 | 0.21 | 0.09 | 0.10 | 0.65 |
| Source of variation | | | | *P*-values | | | |
| Breeder age x strain | 0.333 | 0.790 | 0.160 | 0.671 | 0.535 | 0.264 | 0.302 |
| Breeder age x EST | | 0.542 | 0.574 | 0.776 | 0.110 | 0.077 | 0.112 |
| Strain x EST | | 0.433 | 0.866 | 0.154 | 0.370 | 0.274 | <.001 |
| Breeder age | 0.187 | 0.627 | 0.448 | 0.420 | 0.276 | 0.248 | 0.073 |
| Strain | 0.933 | 0.026 | 0.802 | 0.005 | <.001 | 0.272 | <.001 |
| EST | | <.001 | <.001 | 0.340 | <.001 | 0.272 | <.001 |

[1] n for each treatment combination of breeder age x strain, breeder age x EST, and strain x EST at E14 = 120, E16 = 60, E18 = 88 to 100. n at hatch for chick weight and incubation duration = 120 to 132. [2] Significant interaction between breeder age x strain x EST was found only for incubation duration and the results are presented in Table 5. [3] n = 30 for treatment combination of breeder age x strain. [a,b] LSmeans lacking a common superscript within a column and factor differ ($P \leq 0.05$).

At E14 (Δ = 3.59 g; $P = 0.002$) and E16 (Δ = 3.25 g; $P = 0.028$) the RSY weight was higher in old flock embryos than in young flock embryos, but these effects disappeared at E18 and at 3 h after hatch (Table 3). At E14, E16, and at 3 h after hatch the RSY weight did not differ between strains, but at E18 the RSY weight was higher (Δ = 0.46 g; $P = 0.021$) in Cobb 500 embryos than in Ross 308 embryos. At E14, E16, and E18, RSY weight did not differ between EST, but at 3 h after hatch, the RSY weight was 0.97 g higher in embryos incubated at an EST of 38.9 than 37.8ºC ($P < 0.001$).

Percentage of hatch of fertile eggs did not differ among breeder ages, strains, and EST (Table 3).

A 3-way interaction among breeder age, strain, and EST was found for incubation duration ($P = 0.002$) and navel quality ($P = 0.009$) (Table 5). In young flocks, incubation duration in both EST was approximately 5 to 7 h shorter in Cobb 500 than in Ross 308. In old flocks, incubation duration at an EST of 37.8ºC did not differ between strains; but at an EST of 38.9ºC Cobb 500 chicks hatched approximately 9 h earlier than Ross 308 chicks. Navel quality expressed as percentage of chicks with navel score 3 (poor quality) was highest (35%) in Cobb 500 chicks at an EST at 38.9ºC, whereas the other treatment groups were not different from each other.

***Relative Organ Weights***

A 3-way interaction between breeder age, strain, and EST was found for relative weights of heart ($P = 0.001$) and stomach ($P = 0.011$) 3 h after hatch (Table 5). At an EST of 37.8ºC, relative heart weight did not differ between young and old flock chicks of either strain. An EST of 38.9ºC led to a lower relative heart weight of chicks from both breeder ages and strains and this effect was more pronounced in old Cobb 500 (Δ = 0.21%) than in the other breeder age x strain combinations (Δ = 0.11 to 0.19%). Relative stomach weight of all groups did not differ between EST, but there were differences within each EST. At an EST of 37.8ºC, relative stomach weight was higher in old Cobb 500 chicks than old Ross 308 chicks (Δ = 0.41%), whereas at an EST of 38.9ºC, relative stomach weight was lower in young Ross 308 chicks than young Cobb 500 chicks (Δ = 0.30%).

**Table 3.** Residual yolk (RSY) at E14, E16, E18, and at hatch (3 h after hatch, g), navel quality (% of navel score 3), and hatch of fertile eggs (HOF, %) of Cobb 500 (C) and Ross 308 (R) eggs from young (Y; 29 to 30 wk) and old (O; 54 to 55 wk) breeder eggs incubated at an eggshell temperature (EST) of 37.8 or 38.9ºC from E7 until hatching (LSmeans).[1]

| Effect[4] | RSY, g | | | | Navel quality, %[2] | HOF, %[3] |
|---|---|---|---|---|---|---|
| | E14 | E16 | E18 | Hatch | | |
| Breeder age x strain | | | | | | |
| Y C | 14.17 | 13.29 | 12.32 | 7.40$^{ab}$ | 15.9 | 93.18 |
| Y R | 13.92 | 13.91 | 11.72 | 6.47$^{c}$ | 9.6 | 94.56 |
| O C | 17.58 | 16.64 | 13.48 | 7.63$^{a}$ | 19.1 | 95.00 |
| O R | 17.68 | 17.06 | 13.15 | 7.15$^{b}$ | 6.3 | 96.67 |
| SEM | 0.14 | 0.44 | 0.37 | 0.11 | | |
| Breeder age x EST | | | | | | |
| Y 37.8 | 14.19 | 13.73 | 11.99 | 6.46 | 7.3 | 94.78 |
| Y 38.9 | 13.89 | 13.48 | 12.05 | 7.41 | 16.3 | 93.59 |
| O 37.8 | 17.69 | 17.09 | 13.48 | 6.90 | 5.2 | 96.67 |
| O 38.9 | 17.57 | 16.61 | 13.14 | 7.89 | 20.7 | 95.00 |
| SEM | 0.14 | 0.44 | 0.37 | 0.11 | | |
| Strain x EST | | | | | | |
| C 37.8 | 15.99 | 15.29 | 13.04 | 7.04 | 7.3 | 96.85 |
| C 38.9 | 15.76 | 14.64 | 12.75 | 7.99 | 24.4 | 92.36 |
| R 37.8 | 15.89 | 15.52 | 12.43 | 6.32 | 5.1 | 94.49 |
| R 38.9 | 15.71 | 15.45 | 12.44 | 7.31 | 10.6 | 95.47 |
| SEM | 0.14 | 0.36 | 0.30 | 0.11 | | |
| Breeder age | | | | | | |
| Y | 14.04$^{b}$ | 13.60$^{b}$ | 12.02 | 6.94 | 12.9 | 93.85 |
| O | 17.63$^{a}$ | 16.85$^{a}$ | 13.31 | 7.39 | 12.8 | 95.83 |
| SEM | 0.09 | 0.39 | 0.34 | 0.08 | | |
| Strain | | | | | | |
| C | 15.87 | 14.96 | 12.89$^{a}$ | 7.52 | 17.2 | 93.69 |
| R | 15.80 | 15.49 | 12.43$^{b}$ | 6.81 | 8.2 | 95.17 |
| SEM | 0.09 | 0.31 | 0.26 | 0.08 | | |
| EST | | | | | | |
| 37.8 | 15.94 | 15.41 | 12.74 | 6.68$^{b}$ | 6.3 | 95.67 |
| 38.9 | 15.73 | 15.04 | 12.60 | 7.65$^{a}$ | 17.9 | 93.88 |
| SEM | 0.09 | 0.31 | 0.26 | 0.08 | | |
| Source of variation | | | | *P*-values | | |
| Breeder age x strain | 0.211 | 0.707 | 0.505 | 0.048 | 0.912 | 0.476 |
| Breeder age x EST | 0.548 | 0.678 | 0.308 | 0.883 | 0.810 | 0.920 |
| Strain x EST | 0.856 | 0.278 | 0.444 | 0.819 | 0.138 | 0.179 |
| Breeder age | 0.002 | 0.028 | 0.114 | 0.054 | 0.716 | 0.510 |
| Strain | 0.589 | 0.057 | 0.021 | <.0001 | 0.029 | 0.863 |
| EST | 0.136 | 0.175 | 0.494 | <.001 | 0.001 | 0.432 |

[1] n for each treatment combination of breeder age x strain, breeder age x EST, and strain x EST at E14 = 120, E16 = 60, E18 = 88 to 100. n at hatch for chick weight, navel quality, and HOF = 120 to 132. [2] The values are the percentage of chicks with navel score 3 (poor). [3] The values are means of percentage of hatch of fertile eggs. [4] Significant interaction between breeder age x strain x EST was found only for navel quality and the results are presented in Table 5. [a,b,c] LSmeans lacking a common superscript within a column and factor differ ($P \leq 0.05$).

**Table 4.** Relative weights of liver, heart, stomach, and intestines (% of YFBM) of the embryos at E18 and chicks at 3 h after hatch of Cobb 500 (C) and Ross 308 (R) eggs from young (Y; 29 to 30 wk) and old (O; 54 to 55 wk) flock incubated at an eggshell temperature (EST) of 37.8 or 38.9ºC from E7 until hatching (LSmeans).[1]

| Effect[2] | Relative weight at E18, % | | | | Relative weight at 3 h after hatch,% | | | |
|---|---|---|---|---|---|---|---|---|
| | Liver | Heart | Stomach | Intestines | Liver | Heart | Stomach | Intestines |
| Breeder age x strain | | | | | | | | |
| Y C | 1.94 | 0.60 | 4.18 | 1.69 | 2.19 | 0.81 | 5.60 | 3.63 |
| Y R | 1.93 | 0.62 | 4.09 | 1.64 | 2.23 | 0.83 | 5.42 | 3.87 |
| O C | 1.96 | 0.75 | 4.07 | 1.85 | 2.31 | 0.81 | 5.68 | 3.64 |
| O R | 1.97 | 0.75 | 4.08 | 1.97 | 2.33 | 0.84 | 5.41 | 3.88 |
| SEM | 0.03 | 0.07 | 0.07 | 0.10 | 0.03 | 0.01 | 0.05 | 0.09 |
| Breeder age x EST | | | | | | | | |
| Y 37.8 | 2.01$^{a}$ | 0.66$^{ab}$ | 4.01 | 1.54 | 2.27 | 0.91 | 5.59 | 3.74 |
| Y 38.9 | 1.86$^{b}$ | 0.57$^{c}$ | 4.25 | 1.80 | 2.16 | 0.74 | 5.43 | 3.77 |
| O 37.8 | 1.98$^{a}$ | 0.84$^{a}$ | 4.00 | 1.79 | 2.37 | 0.91 | 5.61 | 3.77 |
| O 38.9 | 1.94$^{ab}$ | 0.66$^{bc}$ | 4.15 | 2.00 | 2.26 | 0.74 | 5.49 | 3.76 |
| SEM | 0.03 | 0.07 | 0.07 | 0.10 | 0.03 | 0.01 | 0.05 | 0.09 |
| Strain x EST | | | | | | | | |
| C 37.8 | 2.01 | 0.76$^{a}$ | 4.03 | 1.63 | 2.32 | 0.90 | 5.72 | 3.69$^{bc}$ |
| C 38.9 | 1.90 | 0.60$^{b}$ | 4.22 | 1.92 | 2.19 | 0.73 | 5.57 | 3.59$^{c}$ |
| R 37.8 | 1.98 | 0.75$^{a}$ | 3.98 | 1.70 | 2.32 | 0.91 | 5.48 | 3.81$^{ab}$ |
| R 38.9 | 1.91 | 0.63$^{b}$ | 4.18 | 1.88 | 2.23 | 0.76 | 5.35 | 3.94$^{a}$ |
| SEM | 0.03 | 0.05 | 0.07 | 0.07 | 0.02 | 0.01 | 0.05 | 0.08 |
| Breeder age | | | | | | | | |
| Y | 1.97 | 0.61 | 4.13 | 1.67 | 2.21 | 0.82 | 5.51 | 3.75 |
| O | 1.94 | 0.75 | 4.07 | 1.90 | 2.32 | 0.82 | 5.55 | 3.76 |
| SEM | 0.02 | 0.07 | 0.06 | 0.09 | 0.02 | 0.01 | 0.03 | 0.09 |
| Strain | | | | | | | | |
| C | 1.95 | 0.68 | 4.12 | 1.77 | 2.25 | 0.81 | 5.64 | 3.64 |
| R | 1.95 | 0.69 | 4.08 | 1.79 | 2.28 | 0.83 | 5.41 | 3.88 |
| SEM | 0.02 | 0.05 | 0.05 | 0.07 | 0.02 | 0.01 | 0.03 | 0.07 |
| EST | | | | | | | | |
| 37.8 | 1.99 | 0.75 | 4.01$^{b}$ | 1.66$^{b}$ | 2.32$^{a}$ | 0.91 | 5.59 | 3.75 |
| 38.9 | 1.91 | 0.61 | 4.20$^{a}$ | 1.90$^{a}$ | 2.21$^{b}$ | 0.74 | 5.46 | 3.76 |
| SEM | 0.02 | 0.05 | 0.05 | 0.07 | 0.02 | 0.01 | 0.03 | 0.07 |
| Source of variation | *P*-values | | | | | | | |
| Breeder age x strain | 0.808 | 0.485 | 0.419 | 0.099 | 0.748 | 0.930 | 0.352 | 0.996 |
| Breeder age x EST | 0.012 | <.001 | 0.407 | 0.546 | 0.887 | 0.763 | 0.679 | 0.724 |
| Strain x EST | 0.355 | 0.048 | 0.923 | 0.165 | 0.305 | 0.334 | 0.870 | 0.032 |
| Breeder age | 0.489 | 0.287 | 0.563 | 0.227 | 0.091 | 0.988 | 0.514 | 0.938 |
| Strain | 0.798 | 0.255 | 0.538 | 0.692 | 0.131 | 0.029 | <.001 | <.001 |
| EST | <.001 | <.001 | 0.002 | <.001 | <.001 | <.001 | 0.004 | 0.788 |

[1] n for each treatment combination of breeder age x strain, breeder age x EST, and strain x EST at E18 = 88 to 100, and at 3 h after hatch = 120 to 132. [2] Significant interaction between breeder age x strain x EST was found for relative heart and stomach weight at 3 h after hatch and the results are presented in Table 5. [a,b,c] LSmeans lacking a common superscript within a column and factor differ ($P \leq 0.05$).

In embryos at E18, a 2-way interaction between breeder age and EST was found for relative weight of liver ($P$ = 0.012) and heart ($P$ < 0.001) (Table 4). At an EST of 37.8ºC, relative liver weight of embryos at E18 did not differ between breeder ages, whereas at this moment in incubation an EST at 38.9ºC led to a lower relative liver weight only in the young flock (Δ = 0.15%). At an EST of 37.8ºC, relative heart weight of embryos at E18 did not differ between breeder ages, whereas an EST at 38.9ºC led to a lower relative heart weight in both young (Δ = 0.09%) and old flocks (Δ = 0.18%). A 2-way interaction between strain and EST was found for relative heart weight of embryos at E18 ($P$ = 0.048) and relative intestinal weight of chicks at 3 h after hatch ($P$ = 0.032). At an EST of 37.8ºC, relative heart weight of embryos at E18 did not differ between strains, whereas an EST at 38.9ºC led to a lower relative heart weight in both Cobb 500 (Δ = 0.16%) and Ross 308 (Δ = 0.12%). At an EST of 37.8ºC, relative intestinal weight of chicks at 3 h after hatch did not differ, whereas at an EST of 38.9ºC Ross 308 chicks had a higher relative intestinal weight than Cobb 500 chicks (Δ = 0.35%).



**Table 5.** Incubation duration (h), navel quality of chicks at 3 h after hatch (% of chicks with score 3), heart, and stomach weight (% YFBM) at 3 h after hatch of Cobb 500 (C) and Ross 308 (R) from young (Y; 29 to 30 wk) and old (O; 54 to 55 wk) flock incubated at an eggshell temperature (EST) of 37.8 and 38.9ºC (LSmeans).[1]

| Effect | Incubation h | Navel % of score | Heart, % | Stomach, % |
|---|---|---|---|---|
| Breeder age x strain x EST | | | | |
| Y C 37.8 | 497$^{b}$ | 10.80$^{ab}$ | 0.89$^{a}$ | 5.62$^{ab}$ |
| Y C 38.9 | 486$^{d}$ | 18.90$^{ab}$ | 0.74$^{bc}$ | 5.58$^{ab}$ |
| Y R 37.8 | 502$^{a}$ | 3.40$^{b}$ | 0.93$^{a}$ | 5.56$^{abc}$ |
| Y R 38.9 | 493$^{c}$ | 13.40$^{ab}$ | 0.74$^{bc}$ | 5.28$^{c}$ |
| O C 37.8 | 496$^{bc}$ | 3.40$^{b}$ | 0.92$^{a}$ | 5.81$^{a}$ |
| O C 38.9 | 480$^{e}$ | 35.10$^{a}$ | 0.71$^{c}$ | 5.56$^{abc}$ |
| O R 37.8 | 496$^{bc}$ | 6.90$^{b}$ | 0.89$^{a}$ | 5.40$^{bc}$ |
| O R 38.9 | 489$^{d}$ | 5.60$^{b}$ | 0.78$^{b}$ | 5.42$^{bc}$ |
| SEM | 1.10 | | 0.02 | 0.07 |
| Source of variation | | *P*-values | | |
| Breeder age x strain x EST | 0.002 | 0.009 | 0.001 | 0.011 |

[1] n for incubation duration, navel quality, heart, and stomach weight at 3 h after hatch = 60 to 66. [2] The values are the percentage of chicks with navel score 3 (poor). [a,b,c,d,e] LSmeans lacking a common superscript within a column differ ($P \leq 0.05$).

**Table 6.** Total hepatic glycogen at E18 and at 3 h after hatch (mg) of Cobb 500 (C) and Ross 308 (R) eggs from young (Y; 29 to 30 wk) and old (O; 54 to 55 wk) flock incubated at an eggshell temperature (EST) of 37.8 or 38.9ºC from E7 until hatching (LSmeans).[1]

| Effect[2] | Total hepatic glycogen, mg | |
|---|---|---|
| | E18 | At 3 h after hatch |
| Breeder age x strain | | |
| Y C | 20.42 | 20.60 |
| Y R | 17.55 | 17.65 |
| O C | 18.20 | 24.14 |
| O R | 16.28 | 23.65 |
| SEM | 2.88 | 2.15 |
| Breeder age x EST | | |
| Y 37.8 | 20.97 | 18.91 |
| Y 38.9 | 16.99 | 19.34 |
| O 37.8 | 19.49 | 23.60 |
| O 38.9 | 14.98 | 24.20 |
| SEM | 2.83 | 2.15 |
| Strain x EST | | |
| C 37.8 | 20.57 | 22.08 |
| C 38.9 | 18.04 | 22.67 |
| R 37.8 | 19.89 | 20.42 |
| R 38.9 | 13.93 | 20.87 |
| SEM | 2.27 | 1.74 |
| Breeder age | | |
| Y | 18.98 | 19.12 |
| O | 17.23 | 23.89 |
| SEM | 2.62 | 1.97 |
| Strain | | |
| C | 19.31 | 22.37 |
| R | 16.91 | 20.65 |
| SEM | 2.00 | 1.52 |
| EST | | |
| 37.8 | 20.23 | 21.25 |
| 38.9 | 15.99 | 21.77 |
| SEM | 2.00 | 1.52 |
| Source of variation | *P*-values | |
| Breeder age x strain | 0.754 | 0.308 |
| Breeder age x EST | 0.859 | 0.667 |
| Strain x EST | 0.262 | 0.953 |
| Breeder age | 0.684 | 0.229 |
| Strain | 0.118 | 0.153 |
| EST | 0.006 | 0.667 |

[1] n for each treatment combination of breeder age x strain, breeder age x EST, and strain x EST for total hepatic glycogen at E18 and at 3 h after hatch = 40. [2] Significant interaction between breeder age x strain x EST was found for total hepatic glycogen at E18 and the results are presented in Table 8.

**Table 7.** Blood plasma glucose, lactate, and uric acid (mmol/L) at E18 and at 3 h after hatch of Cobb 500 (C) and Ross 308 (R) eggs from young (Y; 29 to 30 wk) and old (O; 54 to 55 wk) flock incubated at an eggshell temperature (EST) of 37.8 or 38.9ºC from E7 until hatching (LSmeans).[1]

| Effect[2] | E18, mmol/L | | | At 3 h after hatch,mmol/L | | |
|---|---|---|---|---|---|---|
| | Glucose | Lactate | Uric acid | Glucose | Lactate | Uric acid |
| Breeder age x strain | | | | | | |
| Y C | 9.82 | 1.65 | 0.15 | 11.12 | 2.86 | 0.12[ab] |
| Y R | 9.00 | 1.50 | 0.16 | 10.82 | 2.62 | 0.14[a] |
| O C | 10.02 | 2.16 | 0.14 | 11.70 | 2.88 | 0.11[ab] |
| O R | 9.54 | 1.87 | 0.13 | 11.53 | 2.50 | 0.09[b] |
| SEM | 0.57 | 0.34 | 0.01 | 0.19 | 0.15 | 0.01 |
| Breeder age x EST | | | | | | |
| Y 37.8 | 9.93 | 1.62 | 0.16 | 11.22 | 2.92 | 0.13 |
| Y 38.9 | 8.89 | 1.53 | 0.15 | 10.72 | 2.55 | 0.13 |
| O 37.8 | 10.03 | 2.00 | 0.13 | 11.80 | 2.86 | 0.10 |
| O 38.9 | 9.53 | 2.03 | 0.14 | 11.42 | 2.51 | 0.11 |
| SEM | 0.57 | 0.34 | 0.01 | 0.19 | 0.15 | 0.01 |
| Strain x EST | | | | | | |
| C 37.8 | 10.31 | 1.99 | 0.14 | 11.66 | 2.95 | 0.12 |
| C 38.9 | 9.53 | 1.82 | 0.15 | 11.16 | 2.78 | 0.11 |
| R 37.8 | 9.65 | 1.63 | 0.14 | 11.36 | 2.83 | 0.12 |
| R 38.9 | 8.89 | 1.74 | 0.14 | 10.99 | 2.29 | 0.12 |
| SEM | 0.42 | 0.25 | 0.01 | 0.15 | 0.14 | 0.01 |
| Breeder age | | | | | | |
| Y | 9.41 | 1.58 | 0.15 | 10.97 | 2.74 | 0.13 |
| O | 9.78 | 2.02 | 0.13 | 11.61 | 2.69 | 0.10 |
| SEM | 0.56 | 0.34 | 0.01 | 0.17 | 0.12 | 0.01 |
| Strain | | | | | | |
| C | 9.92 | 1.91[a] | 0.15 | 11.41 | 2.87[a] | 0.12 |
| R | 9.27 | 1.68[b] | 0.14 | 11.17 | 2.56[b] | 0.12 |
| SEM | 0.41 | 0.24 | 0.01 | 0.13 | 0.10 | 0.01 |
| EST | | | | | | |
| 37.8 | 9.98 | 1.81 | 0.14 | 11.51 | 2.89[a] | 0.12 |
| 38.9 | 9.21 | 1.78 | 0.14 | 11.07 | 2.53[b] | 0.12 |
| SEM | 0.41 | 0.24 | 0.01 | 0.13 | 0.10 | 0.01 |
| Source of variation | | | *P*-values | | | |
| Breeder age x strain | 0.255 | 0.638 | 0.646 | 0.539 | 0.582 | 0.031 |
| Breeder age x EST | 0.059 | 0.205 | 0.449 | 0.584 | 0.940 | 0.499 |
| Strain x EST | 0.959 | 0.067 | 0.266 | 0.533 | 0.131 | 0.239 |
| Breeder age | 0.726 | 0.501 | 0.176 | 0.120 | 0.791 | 0.262 |
| Strain | <.0001 | 0.003 | 0.729 | 0.027 | 0.013 | 0.671 |
| EST | <.0001 | 0.817 | 0.961 | <.001 | 0.004 | 0.795 |

[1] n for each treatment combination of breeder age x strain, breeder age x EST, and strain x EST for glucose, lactate, and uric acid at E18 and at 3 h after hatch = 40. [2] Significant interaction between breeder age x strain x EST was found for glucose at E18 and glucose at 3 h after hatch and the results are presented in Table 8. [a,b] LSmeans lacking a common superscript within a column and factor differ ($P \leq 0.05$).

Relative stomach weight at E18 was higher in embryos from an EST of 38.9 than 37.8ºC (Δ = 0.19%; P = 0.002; Table 4). Relative intestinal weight at E18 was higher in embryos from an EST of 38.9 than 37.8ºC (Δ = 0.24%; P < 0.001). Relative stomach and intestinal weights of embryos at E18 did not differ between breeder ages and strains. Relative liver weight of chicks at 3 h after hatch was higher at an EST of 37.8 than at 38.9ºC (Δ = 0.11%; P < 0.001). Breeder age and strain had no effect on relative liver weight of chicks at 3 h after hatch. Breeder age had no effect on relative intestinal weight of chicks at 3 h after hatch.

***Total Hepatic Glycogen and Blood Metabolites***

A 3-way interaction among breeder age, strain, and EST was found for total hepatic glycogen of embryos at E18 ($P$ = 0.029), plasma glucose concentration of embryos at E18 ($P$ = 0.005), and plasma glucose concentration of chicks at 3 h after hatch ($P$ = 0.023) (Table 8). Total hepatic glycogen at E18 was higher in young Cobb 500 embryos at an EST of 37.8ºC than in old Ross 308 embryos at an EST of 38.9ºC (Δ = 11.73 mg), whereas other groups were intermediate and not different from each other. At an EST of 37.8ºC, plasma glucose concentration of embryos at E18 did not differ between young and old flocks of either strain, whereas an EST at 38.9ºC led to a lower glucose concentration only in young Ross 308 embryos (Δ = 1.43 mmol/L). At an EST of 37.8ºC, plasma glucose concentration of chicks at 3 h after hatch was higher in old Cobb 500 than other groups, which were not different from each other. An EST at 38.9ºC led to a lower glucose concentration only in old Cobb 500 chicks (Δ = 0.68 mmol/L).

A 2-way interaction between breeder age and strain was found for uric acid concentration in plasma of chicks at 3 h after hatch ($P$ = 0.031; Table 7). Uric acid in plasma of Cobb 500 chicks did not differ between breeder ages, whereas old Ross 308 chicks had a lower uric acid concentration than young Ross 308 chicks (Δ = 0.05 mmol/L).

Total hepatic glycogen of chicks at 3 h after hatch did not differ between breeder ages, strains, and EST (Table 6). Lactate in plasma of embryos at E18 (Δ = 0.23 mmol/L; $P$ = 0.003) and chicks at 3 h after hatch (Δ = 0.31 mmol/L; $P$ = 0.013) was higher in Cobb 500 than in Ross 308 (Table 7). Lactate in plasma of

embryos at E18 did not differ between breeder ages and EST. Lactate in plasma of chicks at 3 h after hatch was higher in an EST at 37.8 than 38.9ºC ($\Delta$ = 0.36 mmol/L; $P$ = 0.004). Lactate of chicks at 3 h after hatch did not differ between breeder ages. Uric acid in plasma of embryos at E18 did not differ among breeder age, strains, and EST, and of chicks at 3 h after hatch did not differ between EST.

**Table 8.** Total hepatic glycogen (mg) at E18, glucose at E18 and at 3 h after hatch (mmol/L) of Cobb 500 (C) and Ross 308 (R) from young (Y; 29 to 30 wk) and old (O; 54 to 55 wk) flock incubated at an eggshell temperature (EST) of 37.8 and 38.9ºC (LSmeans) [1]

| Effect | Total hepatic glycogen mg | Glucose mmol/L | Glucose at 3 h after mmol/L |
|---|---|---|---|
| Breeder age x strain x EST | | | |
| Y C 37.8 | 23.22[a] | 10.14[ab] | 11.28[b] |
| Y C 38.9 | 17.62[ab] | 9.50[b] | 10.96[bc] |
| Y R 37.8 | 18.72[ab] | 9.72[ab] | 11.15[bc] |
| Y R 38.9 | 16.37[ab] | 8.29[c] | 10.48[c] |
| O C 37.8 | 17.92[ab] | 10.48[a] | 12.04[a] |
| O C 38.9 | 18.47[ab] | 9.57[ab] | 11.36[b] |
| O R 37.8 | 21.07[ab] | 9.58[ab] | 11.56[ab] |
| O R 38.9 | 11.49[b] | 8.29[b] | 11.49[ab] |
| SEM | 3.22 | 0.62 | 0.26 |
| Source of variation | | *P*-values | |
| Breeder age x strain x EST | 0.029 | 0.005 | 0.023 |

[1] n for total hepatic glycogen at E18, glucose at E18, and glucose at 3 h after hatch = 20.

[a,b,c] LSmeans lacking a common superscript within a column differ ($P \leq 0.05$).

# DISCUSSION

Chicken embryos uptake nutrients from albumen and yolk and these nutrients are oxidized to provide energy for development and growth. Although both albumen and yolk are used for energy production, egg yolk functions as the main nutritional supply accounting for approximately 90% of total energy requirement (Romanoff, 1967). An alteration of yolk size, due to changes in breeder age (Nangsuay et al., 2013) or strain (Nangsuay et al., 2015) has been shown to noticeably differentiate nutrient availability in the eggs, which might have an influence on embryonic development and nutrient metabolism during incubation. In the current study an attempt was made to confirm and extend previous findings regarding the composition of young and old flock eggs of Cobb 500 and Ross 308 strains. The average weight of the selected eggs in the current study was 0.5 g higher in old flocks than in young flocks, but we do not expect this relatively small difference to have a biological effect on results obtained.

A significant interaction between breeder age and strain for yolk weight indicated a pronounced effect of breeder age. Yolk weight was approximately 3.2 and 3.8 g higher in the old flocks of Cobb 500 and Ross 308, respectively, than in the young flocks of both strains. On the other hand, yolk weight did not differ for eggs of the same breeder age originating from different strains. As a consequence of changes of yolk weight with breeder age, ratio of yolk to albumen was higher in old flock eggs than young flock eggs. The current results confirm previous studies that with an increase in breeder age, the yolk size increases as well (Ahn et al., 1997; Nangsuay et al., 2011, 2013). In contrast to Nangsuay et al. (2015), Cobb 500 and Ross 308 did not differ in yolk weight. The inconsistency among studies for egg compositions and especially for yolk weight of Cobb 500 and Ross 308 could be due to the difference in distribution of the selected eggs compared to the flock average egg weight of each breeder age, which might have influenced the yolk-to-albumen ratio (Nangsuay et al., 2011). As the eggs in this experiment were selected on a specific weight range for both age groups and strains, weights of selected eggs were not fully representative for the normal egg weight distribution of the flocks. Another effect on yolk-to-albumen ratio of eggs might have been the energy intake of the breeders, which can be influenced by diet composition and

feed quantity (Peebles et al., 2000). As the eggs were obtained from different commercial flocks using different diet compositions, an influence of diet and energy intake on the yolk size and yolk composition and therefore on nutrient availability cannot be totally excluded. Although both these factors might have slightly influenced the results, the current study suggests that due to alteration of yolk size the breeder age rather than strain influences nutrient availability in hatching eggs.

***Embryonic Development and Hatchling***

During incubation, the developing embryos require nutrients and oxygen for development and growth. The requirements for nutrients and oxygen are progressively increased with embryonic growth rate. Tazawa et al. (1988) demonstrated that embryos before E18 act as poikilothermic, which means that embryonic development and growth during this period are temperature dependent. In agreement with this observation, the current results showed that embryos at an EST of 38.9ºC had a higher YFBM at E14 and E16 than that at an EST of 37.8ºC. However, these effects disappeared at later stages of incubation and at 3 h after hatch chick YFBM was lower at a high EST than at a normal EST. These embryonic growth patterns manifest embryonic responses to EST, which might be associated with the propositions made by Tazawa et al. (1988) and Whittow and Tazawa. (1991). According to these authors, a higher embryonic growth at E14 and E16 might be explained by the temperature-dependent Arrhenius limited stage, whereas a lower growth rate at a later stage could be explained by an oxygen-conductance limited stage. As the Arrhenius-limited stage is characterized by a metabolic rate related to changes in temperature, a high EST at 38.9ºC will have accelerated nutrient metabolism, thereby enhancing embryonic growth rate. Consequently, the demand for energy for maintenance of existing embryonic tissues and the continuing synthesis of new body tissues are progressively increased. To meet the embryonic demands in this stage of development, nutrient and oxygen availability should be increased as well. A similar fresh yolk weight suggests that embryos of both EST had an equal nutrient availability at the start of incubation. However, acceleration of YFBM at E14 and E16 by an increase in EST implied a necessity for a higher nutrient metabolism to sustain growth. It can be

expected that an accelerated growth rate at high EST resulting in an increased oxygen requirement has resulted in embryos reaching an oxygen-conductance-limited stage. Although sufficient nutrients are available, yolk utilization will be decreased due to limited oxygen availability on tissue level and subsequently yolk uptake will be affected. A higher RSY of chicks from high EST compared to normal EST could be a reflection of limited oxygen availability to metabolize yolk nutrients.

Oxygen uptake by embryos is influenced by eggshell conductance. The eggshell conductance determines the diffusion rate of oxygen and this characteristic is fixed once the shell is formed (Ackerman and Rahn, 1981). Ar and Rahn (1985) demonstrated that eggshell water vapor conductance and oxygen consumption rate at the pre-internal pipping stage are directly proportional to absolute mean growth rate of embryos (g per day). In agreement with a previous study (Nangsuay et al., 2015), the current results show that Cobb 500 eggs, which had a lower eggshell conductance than Ross 308 eggs, yielded a lower YFBM at E14, E18, and at 3 h after hatch. Since the embryos of both strains were equal in yolk size, which indicates similar nutrient availability at the start of incubation, it might be possible that oxygen limitation is a reason for the lower YFBM. Cobb 500 embryos might experience an imbalance between requirement and availability of oxygen already at E14. This condition might be continued until the moment of external pipping and as a result nutrient uptake from the yolk in Cobb 500 embryos will be lower than in Ross 308 embryos. Comparable observations were made in a study in chicks 3 d after hatch by Pulikanti et al. (2012). These authors reported that a specific eggshell water vapor conductance (eggshell water vapor conductance adjusted to 100 g of set egg weight basis) was positively correlated with chick carcass weight relative to egg weight, but was negatively correlated with yolk sac weight as a percentage of chick weight. Furthermore, Tona et al. (2010) reported that at the internal pipping stage Cobb eggs had a lower partial oxygen pressure (pO2) in the air cell than Ross eggs, suggesting that Cobb embryos were experiencing limited oxygen availability, and consequently yolk nutrient utilization and yolk uptake might be affected. A higher RSY of embryos at E18 and chicks at 3 h after hatch in Cobb 500 compared to Ross 308 for both age groups might indicate a condition in which oxygen availability was limited for yolk nutrient metabolism. It could be implied that due to a lower eggshell conductance

an earlier and probably a higher magnitude of oxygen-conductance limited stage is induced in Cobb 500 compared to Ross 308. Consequently less oxygen is available for nutrient metabolism and as a result embryonic growth rate was decreased. Although both nutrient and oxygen availability are essential factors, the current results suggest that oxygen rather than nutrient level was limited for energy production to sustain embryonic growth. The current observation is supported by studies of Lourens et al. (2007) and Molenaar et al. (2010) who reported an increase of YFBM and a decrease of RSY of embryos subjected to a high oxygen level (25%) between E7 and E19.

The results obtained from eggs varying in yolk size, which suggested differences in nutrient availability due to breeder age, confirm the above suggestion. Eggs obtained from young and old flocks did not differ in eggshell conductance, which suggests equal oxygen availability for embryos of both breeder ages. The YFBM of embryos and chicks originating from young and old flock eggs, which varied in yolk size, were comparable at all measurement d. Although yolk utilization, reflected in RSY at 3 h after hatch, was higher in old flocks than in young flocks, the YFBM at hatch did not differ between either flock age. The current results disagree with the previous suggestions regarding yolk utilization as a determinant factor for embryonic growth (Byerly, 1932; Wilson, 1991). Similar results were demonstrated by Nangsuay et al. (2011) who reported a higher yolk utilization of embryos from old flocks than of young flocks, but the differences in yolk utilization had no effect on YFBM at hatch.



Differences in embryonic development in response to high EST also were shown in the internal organ weights relative to YFBM. These findings might be related to differentiation of internal organ growth in relation to embryonic developmental stages and nutrient metabolism, which in turn are related to availability of nutrients and oxygen. During incubation, development patterns of internal organs relative to embryonic weight differ; for example, the heart reaches maximum weight relative to embryonic weight at E5, whereas the liver, intestine, and gizzard reach this at E21 (Romanoff, 1960). In a condition in which there is an alteration of nutrient metabolism by a high EST and the trajectory of embryonic growth is changed, development of demand (e.g., skeleton and muscle) and supply organs (e.g. heart and liver) might subsequently be affected. The effects could be different for embryos of different breeder ages, which vary in yolk nutrient

availability and for strains that differ in oxygen availability. In the current results, relative liver weight at E18 was lower in high EST than normal EST for embryos of both young and old flocks, but the effect was only statistically significant in the young flock. This might be related to a low yolk nutrient availability. As a result young flock embryos might have low nutrient metabolism and experience a high magnitude of limited energy supply. Therefore the immediate available energy might be prioritized to the demand organ growth in expense of retardation of the liver, which has not reached maximum growth. The same phenomenon also could explain the results at 3 h after hatch, where relative liver weight was lower in high EST than normal EST. These findings are in agreement with Romanoff (1960) who indicated that incubation temperature at 38.5◦C resulted in a retardation of liver growth especially during the last d of incubation.

The heart development showed consistent negative response to a high EST, which was found in previous studies (Leksrisompong et al., 2007; Lourens et al., 2007; Molenaar et al., 2011; Maatjens et al., 2014) as well as in the current study. The current results show that relative heart weight in embryos at E18 of both young and old flocks was lower in high EST than normal EST. A similar effect also was found for the embryos of both strains, which had a lower relative heart weight in high EST than normal EST. At 3 h after hatch, a 3-way interaction showed that embryos of all groups had a lower relative heart weight in high EST than normal EST and this effect was more pronounced in old Cobb 500 chicks. The plausible explanations of prioritizing limited immediate available energy under high EST for the demand organs might be applicable also for a lower relative heart weight. However, at this moment it is not clear which underlying mechanism is responsible for a higher magnitude in response to high EST of old Cobb 500 chicks.

Development of the gastrointestinal tract, stomach, and intestinal development is inconsistent in response to EST. Stomach and intestinal weights relative to YFBM of embryos at E18 was higher in high EST than normal EST. At 3 h after hatch, there were 2-way interactions between strain and EST for relative intestinal weight and 3-way interactions among breeder age, strain, and EST for relative stomach weight, but these interactions were relatively weak.

Although changes of EST had no effect on hatch of fertile, incubation duration and navel quality revealed that embryos of eggs from different breeder age and strains responded differently to changes of EST. In agreement with previous

findings of Nangsuay et al. (2015), at EST of 37.8ºC the incubation duration in Cobb 500 was approximately 4 h shorter than Ross 308, especially in the young flocks. This might be explained by a lower eggshell conductance in Cobb 500 than in Ross 308. A low eggshell conductance could lead to a combination of an earlier oxygen-limited stage as previously explained and the difficulty to get rid of $CO_2$. Consequently at the internal pipping stage the partial pressure of $CO_2$ ($pCO_2$) in the air cell is increased (Tona et al., 2010). A high $pCO_2$ in the air cell at the internal pipping stage is proposed to be a trigger for the hatching process (Visschedijk, 1968). These effects of eggshell conductance were more pronounced at an EST of 38.9ºC than at an EST of 37.8ºC. The effects of an increased EST to 38.9ºC on incubation duration were clearly shown in both young and old flocks of Cobb 500 and Ross 308, but varied in degree of the effect. An EST at 38.9◦C in the young flock resulted in a shorter incubation duration of 11 h in Cobb 500 and 9 h in Ross 308, whereas in the old flock incubation duration was shorter by 7 h in Ross 308 and 16 h for Cobb 500. It is not clear why old Cobb 500 chicks appears to be the most sensitive for higher EST in regards to incubation duration. Comparable results were observed for the navel quality. Old Cobb 500 chicks had the highest percentage of chicks with navel score 3, characterized as a black button exceeding 2 mm left outside the navel. A possible reason could be that old Cobb 500 embryos demand more oxygen for nutrient metabolism than young flock embryos due to a large yolk size, whereas the oxygen supply might be less and the $CO_2$ accumulation in the air cell could be more than old Ross 308. As a result, more yolk nutrient remained unabsorbed, while a high $pCO_2$ in the air cell triggers the chicks to hatch. A short incubation duration in combination with a high level of navel score 3 suggests that old Cobb 500 chicks precede the hatching, while the yolk has not been completely absorbed.



***Hepatic Glycogen and Blood Metabolites***

The measurements of total hepatic glycogen and glucose indicate some responses of embryos to changes of EST, but the pattern of responses was not clear to explicitly explain an influence of strains and breeder ages. Molenaar et al. (2013) demonstrated that a high EST at 38.9ºC from E10.5 onward caused an increase in glucose oxidation and decreased hepatic glycogen prior to the hatching

process. Nangsuay et al. (2015) reported that at 3 h after hatch Cobb 500 had a higher hepatic glycogen than Ross 308.The current results showed a 3-way interaction among breeder age, strain, and EST for total hepatic glycogen at E18. The results showed that young Cobb500 embryos at normal EST had a higher total hepatic glycogen at E18 than old Ross 308 embryos at high EST, whereas the other groups were similar. There was an indication that a high EST at 38.9ºC led to numerically low hepatic glycogen at E18, but the same observation did not occur at 3 h after hatch. The current observations regarding high EST suggesting a higher glucose oxidation and lower hepatic glycogen than in a normal EST are in the same direction with Molenaar et al. (2013), but the patterns and mechanisms underlying the responses of embryos from different strains and breeder ages cannot be explicitly explained. An increase in glucose oxidation with high EST might lead to a reduction of substrate for anaerobic glycolysis, which occurs prior to hatching. The current results show that high EST compared to normal EST resulted in a lower plasma lactate in chicks at 3 h after hatch. A similar mechanism of a higher glucose oxidation also could be attributed to a lower plasma lactate in Ross 308 than in Cobb 500. A higher eggshell conductance in Ross 308than in Cobb 500 can facilitate more oxygen for nutrient metabolism and thereby a reduced glucose availability for anaerobic glycolysis. As a result, plasma lactate of Ross 308 is higher than Cobb 500 at E18 and at 3 h after hatch.

In conclusion, the current results indicate that breeder age has an influence on yolk size, whereas broiler strain has an influence on oxygen availability through eggshell conductance. By incubating eggs of different breeder ages and strains with a normal EST at 37.8ºC and high EST at 38.9ºC from E7 until hatching, we concluded that oxygen rather than nutrient availability limits embryonic development and growth. Oxygen availability has an influence also in incubation duration and navel quality. The differences in response to EST of embryos from different breeder ages and strains might be related to eggshell conductance. The current results indicate a crucial role of eggshell conductance, which determines embryonic development and growth and influences the magnitude of responses to changes in EST of embryos from different breeder ages and strains. These results suggest that, due to differences in eggshell conductance, embryos from different breeder ages and strains might require different EST to maintain a balance between

the availability of oxygen and nutrients, to be able to optimize embryonic development and growth, and good chick quality.

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# CHAPTER 7

# Effects of breeder age, strain, and eggshell temperature on nutrient metabolism of broiler embryos

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# ABSTRACT

Breeder age and broiler strain influence the availability of nutrients and oxygen through yolk size and eggshell conductance, and the effects of these egg characteristics on nutrient metabolism might be influenced by eggshell temperature (EST). This study aims to determine effects of breeder age, strain, and EST on nutrient metabolism of embryos. A study was designed as 2 x 2 x 2 factorial arrangement using four batches of in total 4,464 hatching eggs of 2 flock ages at 29 to 30 wk (young) and 54 to 55 wk (old) of Ross 308 and Cobb 500. EST of 37.8 (normal) or 38.9°C (high) was applied from incubation d 7 (E7) until hatching. Wet yolk weight was determined mainly by breeder age ($P$ = 0.043).Energy content in yolk ($P$ = 0.004) and albumen plus yolk ($P$ = 0.005) was higher in old flock eggs than in young flock eggs, but did not differ between broiler strains. Eggshell conductance was higher in Ross 308 eggs than in Cobb 500 eggs ($P$< 0.001). Old flock embryos used more energy ($P$ = 0.046) and accumulated more energy into yolk free body mass (YFBM; $P$ = 0.030) than young flock embryos, whereas heat production (HP), energy lost, and efficiency of converting energy used to YFBM ($E_{YFB}$) did not differ. Ross 308 embryos used more energy ($P$ = 0.006), had a higher energy lost ($P$ = 0.010), and a higher HP between E15 to E18($P$ < 0.05) than Cobb 500 embryos. Energy content in YFBM did not differ between strains and $E_{YFB}$ ($P$ = 0.024) was lower in Ross 308 than in Cobb 500. High EST resulted in higher HP than low EST from E11 to E15 ($P$ < 0.05), but not after E15. Amount of energy used ($P$ = 0.006) and energy accumulated in the YFBM ($P$ < 0.001) was lower for embryos incubated at an EST of 38.9 than that of 37.8ºC, whereas $E_{YFB}$ did not differ. In conclusion, breeder age, broiler strain, and EST differentially influence embryonic metabolism and particularly the availability of oxygen could have contributed to these differences.

**Key words:** breeder age, broiler strain, eggshell temperature, embryonic development, nutrient metabolism

# INTRODUCTION

Egg content is the only nutritional source available to sustain embryonic development and growth during incubation. The metabolism of available egg nutrients requires $O_2$, whereas $CO_2$, metabolic water, and metabolic heat are produced as by-products. This means that factors which influence the availability of egg nutrients or $O_2$ may alter nutrient metabolism and heat production (**HP**). Although egg nutrients are deposited in both albumen and yolk, it has been shown that embryos obtain approximately 90% of the total energy requirement from the yolk (Romanoff, 1967; Noble and Cocchi, 1990). The amount of yolk can be influenced by e.g. breeder age (Nangsuay et al., 2013) and broiler strain (Nangsuay et al., 2015) and those studies showed that eggs with a bigger yolk also had more energy available in the eggs than eggs with a smaller yolk. Furthermore, it has been shown that both breeder age (Peebles et al., 1998; O'Dea et al., 2004) and broiler strain (Nangsuay et al., 2015) also influence eggshell conductance, which plays a crucial role in exchanges of $O_2$ and $CO_2$ during embryonic development. Differences in eggshell conductance can therefore be an indicator for differences in oxygen availability. It can be expected that eggs of different breeder ages and broiler strains, which differ in yolk size and eggshell conductance, might yield embryos that differ in nutrient metabolism and thus embryonic development and growth.



During the period from the onset of incubation to incubation d 18 (E18), embryos act poikilothermic, which means that the nutrient metabolism and embryonic growth during this period are temperature dependent (Tazawa et al. (1988). Lourens et al. (2007) showed that incubation at an eggshell temperature (**EST**) of 38.9ºC between E8 and E19 resulted in a higher embryonic development compared to incubation at an EST of 37.8ºC. This was reflected in a higher HP from E9 until E15. However, the same authors reported that a high EST resulted in retarded embryonic development at 21.5 day of incubation. In a later study, Molenaar et al. (2010) reported comparable results including an observation that the efficiency of converting egg protein energy into yolk free body mass (**YFBM**) was lower with an EST at 38.9ºC. The combined results of Lourens et al. (2007) and Molenaar et al. (2010) indicate that at later incubation stages, embryos

incubated at an EST of 38.9ºC experienced limitations in nutrient metabolism to sustain development and growth. When $O_2$ level was increased to 25%, Molenaar et al. (2010) showed that chick YFBM development at 38.9ºC was partly restored and HP between E15 to E18 increased, which indicates the importance of $O_2$ availability to sustain nutrient metabolism and embryonic development. Although availability of both oxygen and nutrients is essential for nutrient metabolism of embryos, it is still unclear whether $O_2$ availability interacts with the amount of available nutrient in the eggs.

Since eggs originate from different breeder ages and broiler strains, providing a possibility to vary nutrients and oxygen availability through egg content and shell conductance, it can be hypothesized that during incubation, embryos of these eggs differ in nutrient metabolism and development. In addition, due to variation in nutrient and oxygen availability, it might be possible that embryos of different breeder ages and broiler strains would differentially react to changes of EST during incubation. Therefore, the objective of this study was to investigate effects of breeder age, strain, and EST on nutrient metabolism of broiler embryos.

## MATERIALS AND METHODS

### *Experimental Design*

The experiment was designed as a 2 x 2 x 2 factorial arrangement with 2 breeder ages (young and old), 2 broiler strains (Ross 308 and Cobb 500), and 2 EST (37.8 and 38.9ºC). In all treatments, EST was applied from E7 until the eggs were transferred to the hatching baskets (E18) and thereafter the machine temperature was fixed, corresponding to an EST of 37.8 or 38.9ºC. The experiment was performed in four consecutive batches and each treatment was repeated twice. Eggs were obtained from the same breeder age of both strains within each batch, but breeder age differed among batches. The eggs of each strain within each batch were incubated at 2 EST. The experimental protocol was approved by the Institutional Animal Care and Use Committee of Wageningen University, the Netherlands.

***Hatching Eggs and Egg Storage***

Four batches of 1,116 good quality hatching eggs of 2 flock ages at 29 to 30 wk (young; **Y**) and 54 to 55 wk (old; **O**) of Ross 308 (**R**) and Cobb 500 fast feathering (**C**) were obtained from commercial broiler breeder farms. The broiler breeders of all flocks received commercially available diets. The diets for each group were as follows; YC = 14.9% CP, 5.2% crude fat, 2,775 kcal/kg ME, and 1.9% linoleic acid; YR = 14.7% CP, 4.8% crude fat, 2,813 kcal/kg ME, and 1.8% linoleic acid; OC = 14.6% CP, 4.8% crude fat, 2,772 kcal/kg ME, and 1.8% linoleic acid; OR = 14.2% CP, 5.3% crude fat, 2,767 kcal/kg ME, and 2.0% linoleic acid. Within each batch, eggs of both strains were obtained from the same breeder age. To assess normal average and standard deviation (**SD**) of egg weight of each breeder flock, 150 randomly taken eggs of each flock were weighed individually during the egg collection day. The average egg weight and SD of each strain at a given age were as follows: YR = 56.35 ± 3.77 g, OR = 68.75 ± 5.16 g, YC = 57.41 ± 4.10, g and OC = 70.84 ± 5.52 g. Thereafter, for each batch, a total of 1,116 eggs (558 eggs of each strain) were selected at an egg weight range between 60.0 and 63.0 g. Per batch, 30 eggs of each strain and breeder age were used to determine egg composition. The remaining eggs of each strain and breeder age were placed on 6 setter trays (88 eggs per tray) and were stored at 20ºC and 55 to 60 % RH for 2 to 3 d.



***Incubation***

Incubation E0 to E7: Prior to the start of incubation, average egg weight of each tray was determined. All 12 setter trays (6 trays of each strain) of the two different strains were alternately placed in the same setter with a maximum setting capacity of 1,408 eggs (HatchTech B.V., Veenendaal, the Netherlands). From E0 to E7, EST was maintained at 37.8°C and the RH at 55 to 60%. The EST was measured by 4 sensors (NTC Thermistors: type DC 95; Thermometrics, Somerset, UK), which were attached to 4 individual eggs (two eggs of each strain). The EST sensors were attached with a small piece of tape (Tesa BV, Almere, The Netherlands) in heat conducting paste (Schaffner Holding AG, Switzerland) to the eggshell at the equator of the egg. At E7, all eggs were candled and infertile and

cracked eggs were removed from the trays. Thereafter, weights of the fertile eggs plus tray and weight of the empty tray were measured. The average egg weight of the fertile eggs of each tray was used to calculate egg weight loss at E7; average egg weight of each tray at E0 minus the average egg weight of the fertile eggs of each tray at day E7. This egg weight loss was used in later calculations to determine eggshell conductance.

Incubation E7 to hatching: After candling at E7, eggs of each strain were divided in a control and a treatment group and were incubated separately in 1 of 4 open-circuit climate respiration chambers (**CRC**). The CRC consisted of 2 CRC with a size of 267 L (small; Lourens et al., 2006) and 2 CRC with a size of 1,800 L (large; Verstegen and Henken, 1987). Each treatment was repeated twice, one replicate in a small CRC and the other replicate in a large CRC. Due to difference in CRC volume, approximately 70% of the eggs of each strain within each batch were incubated in the large CRC and the remaining eggs were incubated in the small CRC. All 4 CRC were equipped with the same system for egg turning, ventilation, and temperature and humidity control as described by Lourens et al. (2006). Each CRC had one Vaisala HMT330 series combi sensor (Vaisala company, Vantaa, Finland) to measure machine temperature (**MT**) and relative humidity. The EST was measured by 5 sensors (Pt-100, Sensor Data BV, Rijswijk, The Netherlands) per CRC, which were attached to 5 individual fertile eggs in each CRC. The EST sensors were attached to the eggshell with tape as described above. Between E7 to E18, EST of the control group was maintained at 37.8ºC and at 38.9ºC for the treatment group. The RH was maintained at 55 to 60% for both the control and treatment groups. During incubation, EST from the 5 individual fertile eggs were measured continuously and the MT was adjusted automatically every 5 min if the median EST differed from the target EST.

At E18, all eggs were candled and thereafter fertile eggs were transferred to hatching baskets. The MT was set at a constant value that corresponded to a constant EST of 37.8ºC for the control groups and 38.9ºC for the treatment groups. For the remaining time until hatching, MT was fixed and EST was allowed to change. The hatching moment, which was defined as the moment that chicks completely emerged from the eggshell was checked every 3 h starting at 463 h after the start of incubation.

***Heat Production (HP)***

To determine HP from E7 to E18, $O_2$and $CO_2$ concentrations were measured each 7.5 min in all CRC and in fresh air. $O_2$concentration was measured with a paramagnetic $O_2$ analyser and $CO_2$ concentration was measured with a non-dispersive infrared $CO_2$analyser (both ABB, type Advance Optima Uras 26; ABB Automation GmbH, Frankfurt am Main, Germany). The refreshed air volume set point was 6 L/min for the small CRC and 24 L/min for the large CRC throughout the incubation period. The exact air volumes were measured with a Schlumberger G1.6 and G4 dry gas meter (Itron; former Schlumberger, The Netherlands) for the small and large CRC, respectively. Eggs that were removed after candling at E7 and E18 and non-hatched eggs were opened to determine true fertility and timing of embryonic mortality as described by Lourens et al. (2006). The HP per living, fertile embryo (egg) was calculated from oxygen consumption and carbon dioxide production (Romijn and Lokhorst, 1961) and adjusted for fertility and day of embryo mortality.

***Eggshell Conductance***

The eggshell conductance was calculated by using egg weight loss of fertile eggs between E0 and E7. Because the egg weight loss is a function of time, egg shell conductance and the water vapor pressure deficit ($\Delta P_{H2O}$) across the egg shell, Meijerhof and van Beek (1993) demonstrated the eggshell conductance can be calculated by determination of egg weight loss under known $\Delta P_{H2O.}$ The water vapor pressure ($P_{H2O}$) is a function of temperature and relative humidity. To determine $P_{H2O}$, the average temperature and relative humidity between E0 and E7 was determined. The calculation for eggshell conductance was made as follows; egg weight loss / $\Delta P_{H2O}$; where egg weight loss (mg/h) = egg weight loss of fertile eggs between E0 and E7/ (7 x 24) and $\Delta P_{H2O}$ (kPa)= average vapor pressure deficit between E0 to E7. To calculate $\Delta P_{H2O,}$ water vapor pressure inside the eggs was determined as the saturation vapor pressure at 37.8ºC and 100% relative humidity. The water vapor pressure outside of the eggs was calculated based on average temperature (ºC) and humidity (%) of the machine between E0 to E7.

***Fresh Egg Composition and Chick Measurements***

In each batch, 30 fresh eggs of each strain were boiled for 10 min, and thereafter albumen and yolk were separated and weighed. The eggshell excluding shell membranes was dried for 24 h at room temperature and weighed. Albumen and yolk were stored at -20ºC for further analyses.

At 3 h after hatch, chicks were taken out of the chambers, chick weight was measured and blood was collected after decapitation (0.5-1 mL). Chicks were opened and the residual yolk (**RSY**) was removed and weighed. Chick yolk free body mass (YFBM) was determined as chick weight minus RSY weight. Chick YFBM and RSY were stored separately at -20ºC for further analyses.

***Chemical Analysis and Calculations for Energy Utilization***

To have sufficient amount of sample for chemical analyses, albumen and yolk samples of fresh eggs were pooled from 2 eggs and each of RSY and yolk free body (YFB) samples were pooled from 2 chicks.

Proximate analyses were performed for dry matter (**DM**; ISO 6496, 1999) and gross energy (**GE**; ISO 9831, 1998) in albumen and yolk from fresh eggs and RSY and YFB of chicks at 3 h after hatch. Albumen, yolk, and RSY were dried in a freeze dryer before analyses of DM and GE. For YFB analysis, two YFB (without liver) were placed in300 mL of water and autoclaved for 3 h at 120°C. Thereafter, YFB in water was homogenized with an Ultra-Turrax disperser (IKA Werke GmbH & Co. KG, Staufen, Germany) for 10 min, and the suspension was used for DM analysis (ISO 6496, 1999). The remaining suspension was frozen at −20°C and then dried in a freeze dryer before determination of GE (ISO 9831, 1998). The calculations for energy utilization, energy lost, and efficiency of converting energy used to form YFBM ($\mathbf{E_{YFB}}$) were performed as follows;

Energy utilization (kJ) = Albumen (kJ) + Yolk (kJ) – RSY (kJ)
Energy lost (kJ) = [Albumen (kJ) + Yolk (kJ)] – [YFBM (kJ) + RSY (kJ)]

$$E_{YFB} = \frac{\text{YFB (kJ)}}{\text{Albumen (kJ) + yolk (kJ) - RY (kJ)}} \times 100\%$$

***Statistical Analyses***

The setter tray was used as the experimental unit for egg weight loss and eggshell conductance at E7. For other variables, egg or chick were used as the experimental unit in the statistical analyses. Distributions of means and residuals were examined to verify model assumptions. All data were analyzed using PROC MIXED in the statistical software package SAS 9.3 (SAS Institute Inc. 2002–2010, Cary, NC, USA). The model used for the statistical analyses of fresh egg composition, egg weight loss at E7, and eggshell conductance was;

$$Y_{ijk} = \mu + A_i + B_j + AB_{ij} + C(A_i) + e_{ijk}, \quad [1]$$

where $Y_{ijk}$ is the dependent variable, $\mu$ is the overall mean, $A_i$ is the breeder age (i= young or old), $B_j$ is the strain (j= Cobb 500 or Ross 308), $AB_{ij}$ is the interaction between breeder age and strain, $C(A_i)$ is the breeder age nested within batch and this term was used as random effect, and $e_{ijk}$ was the error term. The variables measured after E7, when the EST treatment was applied, were analyzed using PROC MIXED in the statistical software package SAS 9.3 (SAS Institute Inc. 2002–2010, Cary, NC, USA) using model [1] extended with the EST ($D_l$; l= 37.8 or 38.9) and interactions with the other factors (model 2). HP per day from E7 to E18 was analyzed using the MIXED procedure of SAS 9.3 software package (SAS Institute Inc. 2002–2010, Cary, NC, USA) for repeated measurements. Model 2 was extended with the repeated factor day of incubation ($day_m$; m = E7 to E18) and interactions with other factors and an autoregressive covariance structure was used. Least square means were compared using Bonferroni adjustments for multiple comparisons. Values are expressed as LS means. In all cases, a difference was considered significant at $P \leq 0.05$.

# RESULTS

### *Weights and Energy Content of Fresh Eggs and Eggshell Conductance*

Although the eggs were selected on the same egg weight range, egg weight was higher in eggs from old breeder flocks than from young breeder flocks (Δ = 0.5 g; $P = 0.048$) (Table 1). Egg weight of Cobb 500 and Ross 308 did not differ. Albumen wet weight (Δ = 2.95 g; $P = 0.003$), albumen dry weight (Δ = 0.75 g; $P = 0.006$), and amount of energy in albumen (Δ = 17.32 kJ; $P = 0.007$) were higher in eggs of young breeder flocks than that of old breeder flocks. Albumen dry weight (Δ = 0.15 g; $P = 0.013$) and amount of energy in albumen (Δ = 3.7 kJ; $P = 0.009$) in Cobb 500 eggs were higher than in Ross 308 eggs.

A 2-way interaction between breeder age and strain was found for yolk wet weight ($P = 0.043$; Table 1). Wet yolk weight was higher in eggs of old breeder flocks than that of young breeder flocks in both strains, but the difference in Cobb 500 eggs (Δ = 3.20 g) was smaller than in Ross 308 eggs (Δ = 3.81 g). Dry yolk weight (Δ = 1.87 g; $P = 0.004$) and the amount of energy in the yolk (Δ = 64.81 kJ; $P = 0.004$) were higher in eggs of old breeder flocks than that of young breeder flocks, but did not differ between strains. The amount of energy in albumen + yolk of old flock eggs was higher than that of young flock eggs (Δ = 47.49 kJ; $P = 0.005$), but did not differ between strains.

Eggshell conductance at E7 was higher in Ross 308 than in Cobb 500 (Δ = 0.21 mg/h/kPa; $P < 0.001$), but did not differ between breeder ages.

### *Weights and Energy content of RSY and YFB of Chicks*

There was no 3-way interaction for weights and energy content of RSY, YFB, and RSY+YFB of chicks at 3 h after hatch. A 2-way interaction between breeder age and strain was found for wet RSY weight at 3 h after hatch ($P = 0.048$; Table 2). The wet RSY weight was lower in both young and old flocks of Ross 308 chicks than in Cobb 500 chicks, but this difference was more pronounced in the young flocks (Δ = 0.93 g) than in the old flocks (Δ = 0.48 g). The dry RSY weight (Δ = 0.64 g; $P = 0.029$) and RSY energy content (Δ = 28.29 kJ; $P = 0.022$) were

**Table 1.** Egg weight (g), wet and dry weights (g), and energy content (kJ) of albumen and yolk, albumen + yolk energy content (kJ), and eggshell conductance (mg/h/kPa) of Cobb 500 (C) and Ross 308 (R) eggs from young (Y; 29 to 30 wk) and old (O; 54 to 55 wk) flock (LSmeans).[1]

| Effect | Egg wt. | Albumen | | | Yolk | | | Albumen+yolk | Eggshell conductance |
|---|---|---|---|---|---|---|---|---|---|
| | | Wet wt. | Dry | Energy | Wet | Dry | Energy | | |
| | g | g | g | kJ | g | g | kJ | kJ | mg/h/kPa[2] |
| Breeder age x strain | | | | | | | | | |
| Y C | 61.02 | 38.39 | 4.81 | 109.12 | 17.13[b] | 8.20 | 276.25 | 385.37 | 4.04 |
| Y R | 61.19 | 38.61 | 4.67 | 105.9 | 16.89[b] | 8.18 | 273.95 | 379.85 | 4.31 |
| O C | 61.56 | 35.69 | 4.07 | 92.29 | 20.33[a] | 9.97 | 336.95 | 429.24 | 4.41 |
| O R | 61.64 | 35.42 | 3.91 | 88.10 | 20.70[a] | 10.15 | 342.86 | 430.96 | 4.55 |
| SEM | 0.11 | 0.17 | 0.06 | 1.38 | 0.15 | 0.12 | 3.85 | 3.20 | 0.15 |
| Breeder age | | | | | | | | | |
| Y | 61.10[b] | 38.50[a] | 4.74[a] | 107.51[a] | 17.01 | 8.19[b] | 275.10[b] | 382.61[b] | 4.18 |
| O | 61.60[a] | 35.55[b] | 3.99[b] | 90.19[b] | 20.51 | 10.06[a] | 339.91[a] | 430.10[a] | 4.48 |
| SEM | 0.08 | 0.12 | 0.04 | 0.10 | 0.10 | 0.09 | 2.72 | 2.28 | 0.15 |
| Strain | | | | | | | | | |
| C | 61.29 | 37.04 | 4.44[a] | 100.70[a] | 18.73 | 9.08 | 306.60 | 407.30 | 4.22[b] |
| R | 61.41 | 37.02 | 4.29[b] | 97.00[b] | 18.79 | 9.17 | 308.40 | 405.41 | 4.43[a] |
| SEM | 0.08 | 0.13 | 0.04 | 0.97 | 0.10 | 0.09 | 2.72 | 2.26 | 0.11 |
| Source of variation | *P*-values | | | | | | | | |
| Breeder age x strain | 0.724 | 0.152 | 0.886 | 0.719 | 0.043 | 0.423 | 0.293 | 0.259 | 0.137 |
| Breeder age | 0.048 | 0.003 | 0.006 | 0.007 | 0.002 | 0.004 | 0.004 | 0.005 | 0.287 |
| Strain | 0.273 | 0.887 | 0.013 | 0.009 | 0.639 | 0.503 | 0.642 | 0.552 | <.001 |

[1] n = 60 for treatment combination of breeder age x strain. [2] n = 12 for treatment combination of breeder age x strain. [a,b]LSmeans lacking a common superscript within a column and factor differ ($P \leq 0.05$).

**Table 2.** Wet and dry weights (g) and energy content (kJ) of residual yolk (RSY), yolk free body (YFB), and RSY + YFB of Cobb 500 (C) and Ross 308 (R) eggs from young (Y; 29 to 30 wk) and old (O; 54 to 55 wk) flock incubated at an eggshell temperature (EST) of 37.8 or 38.9ºC from E7 until hatching (LSmeans).[1]

| Effect | RSY | | | YFB | | | RSY + YFB |
|---|---|---|---|---|---|---|---|
| | Wet | Dry wt. | Energy | Wet | Dry | Energy | |
| | g | g | kJ | g | g | kJ | kJ |
| Breeder age x strain | | | | | | | |
| Y C | 7.40[ab] | 3.52 | 100.58 | 37.85 | 7.09 | 170.31 | 271.99 |
| Y R | 6.47[c] | 3.06 | 84.67 | 38.52 | 7.21 | 171.61 | 256.36 |
| O C | 7.63[a] | 4.08 | 126.48 | 37.74 | 7.31 | 181.95 | 308.42 |
| O R | 7.15[b] | 3.72 | 115.34 | 38.25 | 7.48 | 185.45 | 300.79 |
| SEM | 0.11 | 0.11 | 3.95 | 0.13 | 0.08 | 2.26 | 3.85 |
| Breeder age x EST | | | | | | | |
| Y 37.8 | 6.46 | 3.10 | 87.92 | 38.71 | 7.30 | 174.16 | 263.25 |
| Y 38.9 | 7.41 | 3.42 | 97.32 | 37.67 | 6.99 | 167.77 | 265.09 |
| O 37.8 | 6.90 | 3.63 | 112.32 | 38.31 | 7.60 | 190.53 | 302.84 |
| O 38.9 | 7.89 | 4.17 | 129.50 | 37.69 | 7.19 | 176.87 | 306.37 |
| SEM | 0.11 | 0.11 | 3.95 | 0.13 | 0.08 | 2.26 | 3.85 |
| Strain x EST | | | | | | | |
| C 37.8 | 7.04 | 3.53 | 104.52 | 38.27 | 7.38 | 181.03 | 286.65 |
| C 38.9 | 7.99 | 4.08 | 122.53 | 37.33 | 7.03 | 171.23 | 293.77 |
| R 37.8 | 6.32 | 3.20 | 95.72 | 38.74 | 7.53 | 183.66 | 279.45 |
| R 38.9 | 7.31 | 3.52 | 104.29 | 38.03 | 7.16 | 173.40 | 277.69 |
| SEM | 0.11 | 0.11 | 3.77 | 0.13 | 0.08 | 2.26 | 3.37 |
| Breeder age | | | | | | | |
| Y | 6.94 | 3.26[b] | 92.62[b] | 38.19 | 7.15 | 170.96[b] | 264.17[b] |
| O | 7.39 | 3.90[a] | 120.91[a] | 37.99 | 7.40 | 183.70[a] | 304.61[a] |
| SEM | 0.08 | 0.08 | 3.02 | 0.09 | 0.06 | 1.60 | 3.30 |
| Strain | | | | | | | |
| C | 7.52 | 3.80[a] | 113.53[a] | 37.80[b] | 7.20 | 176.13 | 290.21[a] |
| R | 6.81 | 3.36[b] | 100.00[b] | 38.39[a] | 7.34 | 178.53 | 278.57[b] |
| SEM | 0.08 | 0.08 | 2.79 | 0.09 | 0.05 | 1.59 | 2.72 |
| EST | | | | | | | |
| 37.8 | 6.68[b] | 3.36[b] | 100.12[b] | 38.51[a] | 7.45[a] | 182.34[a] | 283.98 |
| 38.9 | 7.65[a] | 3.80[a] | 113.41[a] | 37.68[b] | 7.09[b] | 172.32[b] | 285.73 |
| SEM | 0.08 | 0.08 | 2.79 | 0.09 | 0.05 | 1.60 | 2.72 |
| Source of variation | | | | *P*-values | | | |
| Breeder age x strain | 0.048 | 0.491 | 0.507 | 0.535 | 0.790 | 0.627 | 0.158 |
| Breeder age x EST | 0.883 | 0.331 | 0.281 | 0.110 | 0.486 | 0.111 | 0.764 |
| Strain x EST | 0.819 | 0.306 | 0.192 | 0.370 | 0.888 | 0.919 | 0.118 |
| Breeder age | 0.054 | 0.029 | 0.022 | 0.276 | 0.089 | 0.030 | 0.013 |
| Strain | <.001 | <.001 | <.001 | <.001 | 0.069 | 0.290 | <.001 |
| EST | <.001 | <.001 | <.001 | <.001 | <.001 | <.001 | 0.342 |

[1] n = 24 for each treatment combination of breeder age x strain, breeder age x EST, and strain x EST. [2] n for each treatment combination of breeder age x strain, breeder age x EST, and strain x EST for wet weight of RSY and YFB = 120 to 132. [a,b] LSmeans lacking a common superscript within a column and factor differ ($P \leq 0.05$).

higher in chicks of old flock eggs than that of young flock eggs. The dry RSY weight (Δ=0.44 g; $P$<0.001) and RSY energy content (Δ=13.53 kJ; $P$<0.001) were higher in chicks of Cobb 500 eggs than that of Ross 308 eggs. Wet RSY weight (Δ=0.97 g; $P$<0.001), dry RSY weight (Δ=0.44 g; $P$<0.001), and RSY energy content (Δ=13.29 kJ; $P$<0.001) were higher in chicks incubated at an of EST 38.9ºC than at an EST of 37.8ºC.

Chicks of young and old flock eggs did not differ in wet and dry YFB weights, but the amount of energy in YFB of chicks from old flock eggs was higher than that of young flock eggs (Δ=12.74 kJ; $P$=0.030). Ross 308 chicks had a higher wet YFB weight than that of Cobb 500 chicks (Δ=0.59 g; $P$<0.001), but dry YFB weight and energy content did not differ between strains. Chicks of eggs incubated at an EST of 37.8ºC had a higher wet YFB weight (Δ=0.83 g; $P$<0.001), dry YFB weight (Δ=0.36 g; $P$<0.001), and energy content (Δ=10.02 kJ; $P$<0.001) than that of an EST of 38.9ºC.

The amount of energy in RSY + YFB was higher in chicks of old flock eggs than in chicks of young flock eggs (Δ= 40.44 kJ; $P$=0.013). Chicks of Cobb 500 eggs had a higher amount of energy in RSY + YFB than chicks of Ross 308 eggs (Δ=11.64 kJ; $P$<0.001). EST had no effect on the amount of energy in RSY+YFB.



***Nutrient Metabolism***

There were no 3-way and 2-way interactions for nutrient metabolism measured as energy utilization, energy lost, and efficiency of converting energy used to YFB ($E_{YFB}$). Chicks of old flock eggs used a higher amount of energy than that of young flock eggs (Δ=19.98 kJ; $P$=0.046) (Table 3), but the energy lost and $E_{YFB}$ did not differ between flock ages. Chicks of Ross 308 eggs used a higher amount of energy (Δ=12.57 kJ; $P$=0.006), had a higher amount of energy lost (Δ=10.17 kJ; $P$=0.010), and a lower $E_{YFB}$ (Δ=1.91%; $P$=0.024) than that of Cobb 500 chicks. Chicks of eggs incubated at an EST of 37.8ºC used a higher amount of energy than that of an EST at 38.9ºC (Δ=12.52 kJ; $P$=0.006), but the energy lost and the $E_{YFB}$ did not differ between EST.

A significant interaction between strain x incubation day (Figure 1) and EST x incubation day (Figure 2) was found for embryonic heat production (HP).

Ross 308 embryos had higher HP than that of Cobb 500 embryos at E15, E16, E17, and E18 (2.7, 3.8, 4.2, and 3.7 mW/egg, respectively). Embryos of eggs incubated at an EST of 38.9ºC had a higher HP than that of an EST at 37.8ºC at E11, E12, E13, E14, and E15 (3.8, 6.7, 7.9, 7.9, and 5.7 mW/egg, respectively).

**Table 3.** Energy utilization (kJ), energy lost (kJ), and efficiency of converting energy used to yolk free body ($E_{YFB;\ \%}$) of Cobb 500 (C) and Ross 308 (R) chicks from young (Y; 29 to 30 wk) and old (O; 54 to 55 wk) flock incubated at an eggshell temperature (EST) of 37.8 or 38.9ºC from E7 until hatching (LSmeans).[1]

| Effects | Energy utilization | Energy lost | $E_{YFB}$ |
|---|---|---|---|
| Breeder age x strain | | | |
| Y C | 283.07 | 112.76 | 60.40 |
| Y R | 295.35 | 123.74 | 58.13 |
| O C | 302.76 | 120.82 | 60.39 |
| O R | 315.62 | 130.17 | 58.83 |
| SEM | 4.45 | 3.85 | 0.83 |
| Breeder age x EST | | | |
| Y 37.8 | 293.14 | 118.98 | 59.48 |
| Y 38.9 | 285.29 | 117.52 | 59.05 |
| O 37.8 | 317.78 | 127.26 | 60.14 |
| O 38.9 | 300.6 | 123.73 | 59.07 |
| SEM | 4.45 | 3.85 | 0.83 |
| Strain x EST | | | |
| C 37.8 | 301.06 | 120.04 | 60.32 |
| C 38.9 | 284.77 | 113.54 | 60.47 |
| R 37.8 | 309.86 | 126.20 | 59.86 |
| R 38.9 | 301.12 | 127.71 | 57.65 |
| SEM | 4.45 | 3.85 | 0.83 |
| Breeder age | | | |
| Y | 289.21[b] | 118.25 | 59.27 |
| O | 309.19[a] | 125.49 | 59.61 |
| SEM | 3.14 | 2.72 | 0.59 |
| Strain | | | |
| C | 292.92[b] | 116.79[b] | 60.39[a] |
| R | 305.49[a] | 126.96[a] | 58.48[b] |
| SEM | 3.14 | 2.72 | 0.59 |
| EST | | | |
| 37.8 | 305.46[a] | 123.12 | 59.81 |
| 38.9 | 292.94[b] | 120.62 | 59.06 |
| SEM | 3.14 | 2.72 | 0.59 |
| Source of variation | | *P*-values | |
| Breeder age x strain | 0.949 | 0.833 | 0.671 |
| Breeder age x EST | 0.297 | 0.789 | 0.703 |
| Strain x EST | 0.398 | 0.301 | 0.279 |
| Breeder age | 0.046 | 0.201 | 0.723 |
| Strain | 0.006 | 0.010 | 0.024 |
| EST | 0.006 | 0.519 | 0.368 |

[1] n = 24 for each treatment combination of breeder age x strain, breeder age x EST, and strain x EST. [a,b] LSmeans lacking a common superscript within a column and factor differ ($P \leq 0.05$).

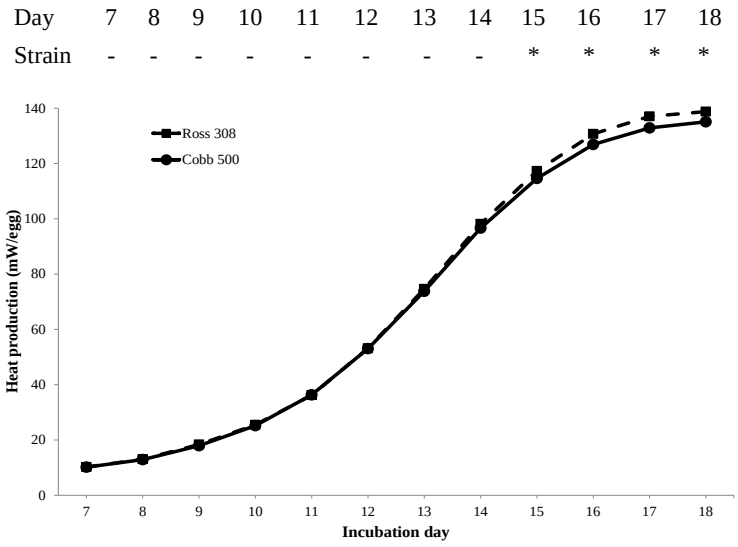


**Figure 1.** The effects of broiler strain Cobb 500 and Ross 308 on embryonic heat production (mW/egg) during E7 to E18. The legend indicates the level of significance (*$P \leq 0.05$).

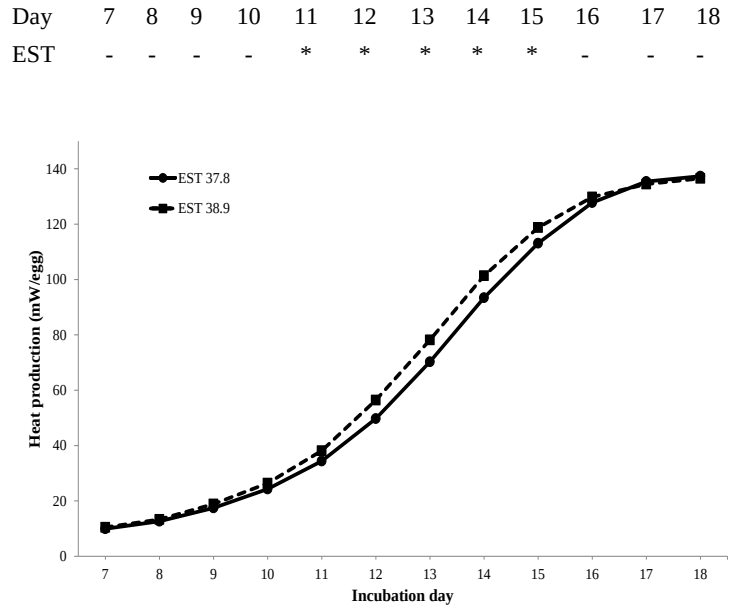


**Figure 2.** The effects of eggshell temperature (EST) of 37.8 and 38.9 ºC from E7 until hatching on embryonic heat production (mW/egg). The legend indicates the level of significance (*$P \leq 0.05$).

# DISCUSSION

Previous studies have shown that embryos of eggs originating from different breeder ages (Nangsuay et al., 2013) and broiler strains (Nangsuay et al., 2015) differ in nutrient availability due to alterations in yolk size. Various studies (Peebles et al., 1998; O'Dea et al., 2004; Nangsuay et al., 2015) suggested that egg origin can also influence eggshell conductance, which can affect $O_2$ availability for the embryos (Ackerman and Rahn, 1981). The availability of both nutrients and $O_2$ is essential for the metabolism and it has been shown that metabolic rate of embryos before E18 is influenced by temperature (Tazawa et al., 1988; Lourens et al., 2007; Molenaar et al., 2010). Although there is information about the role of $O_2$ at different eggshell temperatures (EST; Molenaar et al., 2010), it is still unclear whether $O_2$ availability interacts with the amount of nutrients available in the eggs. The objective of the current study was to investigate effects of breeder age, broiler strain, and EST on nutrient metabolism of the embryos. This study aimed to use eggs from the same egg weight range for both young and old breeder flocks of Cobb 500 and Ross 308. Although the average weights of selected egg in the old flocks was 0.5 g heavier than in the young flocks, it is unlikely that the small difference in egg weight would affect results obtained.

The current results show that breeder age is a determining factor for availability of nutrients in the eggs, whereas the eggshell conductance is influenced by broiler strain. A higher eggshell conductance in Ross 308 than in Cobb 500 eggs confirms previous observations of Nangsuay et al. (2015). In contrast to a previous study (Nangsuay et al., 2015), Ross 308 and Cobb 500 eggs in the current study did not differ in yolk weight and energy content in yolk and albumen + yolk. The inconsistency of results among studies can be due to the distribution of selected eggs compared to the flock average egg weight at each breeder age, which might be different among studies and not fully representative for the normal eggs. This might have influenced yolk: albumen ratio (Nangsuay et al., 2011). The other effect on yolk: albumen ratio might have been energy intake of the breeders, which has been shown to affect yolk size (Peebles et al., 2000). Since the eggs were obtained from different commercial breeder flocks using different diet compositions, an influence of diet and energy intake on the yolk size and yolk

composition and therefore on nutrient availability cannot be totally excluded. Although these both factors might have slightly influenced the results the current study suggests that due to alteration of yolk size the breeder age rather than strain influences nutrient availability in hatching eggs.

The effects of breeder age on nutrient availability were associated with yolk size and thus energy content in the yolk and in the albumen + yolk, which are consistent with a previous study (Nangsuay et al., 2013). However, the current study did not show an influence of breeder age on eggshell conductance, which is in contrast to previous studies of Peebles et al. (1998) and O'Dea et al. (2004). This means that at the start of incubation eggs originating from different breeder ages vary in nutrient availability, whereas eggs derived from different broiler strains vary in eggshell conductance with potential implication for availability of oxygen (Ackerman and Rahn, 1981).

During incubation, embryos take up $O_2$ for nutrient metabolism and produce $CO_2$, metabolic heat (HP), and metabolic water (Etches, 1996). The energy which is generated from metabolism is either lost as heat or being utilized for 1) tissue development for growth; 2) maintenance of existing tissues; and 3) muscular activity especially during the hatching process (Vleck, 1991). This suggests a relationship between the embryonic growth pattern and embryonic HP, in which embryos with a high growth rate would have a high metabolic rate to sustain growth throughout the developmental period. Because embryos before E18 act poikilothermic and thus the nutrient metabolism and growth during this period is temperature dependent (Tazawa et al., 1988), it was expected that the more nutrients available in old flock eggs than in young flock eggs and a higher eggshell conductance in Ross 308 eggs than Cobb 500 eggs would differentially influence nutrient metabolism of embryos, particularly at high EST. Because we did not find a 3-way or 2-way interactions between breeder age, strain, and EST for nutrient metabolism of the embryos, the discussion will focus on the main effects of breeder age, strain, and EST.

Embryos of old flocks had more energy available in the eggs and used more energy during incubation than those of young flocks. In spite of similar dry YFBM between breeder ages, a higher amount of energy used in association with an equal $E_{YFB}$ between breeder ages resulted in a higher amount of energy accumulated into YFBM of old flock chicks than that of young flock chicks. The

current findings for energy utilization, energy content in YFBM, and $E_{YFB}$ are consistent with previous findings (Nangsuay et al., 2013), but the previous study found a higher HP in old flock embryos than young flock embryo from E16 to E18. The more pronounced differences in dry YFBM and energy lost in old flock chicks than in young flock chicks in the previous study compared to the current results might be associated with HP differences in the previous study.

Ross 308 and Cobb 500 embryos did not differ in nutrient availability at the start of incubation, but the energy utilization and embryonic HP during E15 to E18 and YFBM at 3 h after hatch was higher in Ross 308 than in Cobb 500. A higher embryonic HP in Ross 308 than in Cobb 500 can possibly be attributed to a higher oxygen availability due to a higher eggshell conductance. The embryonic HP of Ross 308 and Cobb 500 started to deviate at E15, which was coincided with a higher YFBM at E14 in Ross 308 than in Cobb 500 (Nangsuay et al., unpublished results). It is possible that Cobb 500 embryos experienced oxygen limitation during this phase, which will lead to a lower metabolic rate, less yolk uptake, and lower growth than in Ross 308 embryos. On the other hand, more oxygen availability for Ross 308 embryos permits them to continue a high nutrient metabolism and yolk uptake to sustain their growth rate and this condition might be continued until the moment of external pipping. This can explain the lower wet and dry weights of RSY and a higher wet YFBM of chicks at 3 h after hatch of Ross 308 than Cobb 500. Although Ross 308 embryos were able to use more energy than Cobb 500 embryos, the $E_{YFB}$ was lower in Ross 308. A lower $E_{YFB}$ in Ross 308 was due to a higher amount of energy lost in Ross 308 than in Cobb 500, combined with a similar amount of energy accumulated into YFBM of both strains. The differences in chick YFBM and $E_{YFB}$ between broiler strains are consistent with previous findings by Nangsuay et al. (2015), but the embryonic HP in the previous study was not significantly different. Because numerically the differences in embryonic HP between two strains in the current study and in the previous one were similar, the number of repetitions (current; four vs previous; two) might have influenced the observed differences in statistical significance between the different studies.

An increase in EST from 37.8 to 38.9ºC from E7 onward resulted in an increase in HP during E11 to E15, but this effect disappeared at later stages of incubation. This increased embryonic HP could be related to a high embryonic YFBM at E14 and E16 in the eggs incubated at an EST of 38.9ºC than 37.8ºC

(Nangsuay et al., unpublished results). The effects of EST, which are manifested between E11 and E15, can be explained by the mechanisms suggested by Tazawa et al. (1988) and Whittow and Tazawa (1991). In agreement with these authors, an increase in EST from 37.8 to 38.9ºC in the current study could accelerate metabolic activity and development between E11 and E15 as a result of the temperature dependent Arrhenius-limited stage. As a consequence of high growth rate, energy requirement for maintenance of existing body tissues as well as the energy requirement to sustain growth is increase. To fulfill energy requirements, embryos at an EST at 38.9ºC would require more $O_2$for nutrient metabolism. Since the availability of $O_2$ was comparable due to similar eggshell conductance, the embryos at a high EST at the later stage of development might experience limitation of oxygen availability due to the oxygen-conductance-limited stage (Tazawa et al., 1988; Whittow and Tazawa, 1991). As a consequence, nutrient metabolism and yolk nutrient uptake decreased. This is clearly expressed in a higher wet and dry RSY weight in combination with a lower energy utilization in chicks from an EST of 38.9ºC than that from 37.8ºC. The limitation of oxygen during the later stage of development might explain the disappearance of differences in embryonic HP during E16 to E18. Furthermore, the condition of limited $O_2$ during the last stage of development might be the reason for a lower YFBM and thus lower amount of energy deposited into YFBM of chicks at an EST of 38.9ºC than that in 37.8ºC. However, the total energy lost as well as the $E_{YFB}$ measured at 3 h after hatch did not differ between the EST.



Combining the current findings for the effects of breeder age, strain, and EST, it can be concluded that the availability of oxygen rather than the availability of nutrients influence the metabolism of the embryo. In addition, the current results show that variation in nutrient and oxygen availability due to differences in breeder ages and broiler strains had no influence on responses to changes of EST, especially for YFBM at 3 h after hatch, energy utilization, energy incorporated into YFB, $E_{YFB,}$ and HP.

## ACKNOWLEDGMENTS

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# CHAPTER 8

# General discussion

# GENERAL DISCUSSION

## *I. Introduction*

Commercial hatcheries have to deal with chicken eggs from different origins. The hatching eggs can originate from different breeder ages, breeds, and strains and these eggs may vary in egg size and egg composition. This has initiated questions as 1) whether embryos in eggs from different origins are different in availability of nutrients and $O_2$, 2) whether these embryos are different in nutrient metabolism and development, and 3) what are the mechanisms underlying these possible differences.

On weight basis, the egg consists of 32 to 35% yolk, 52 to 58% albumen, and 9 to 14% shell and shell membranes (Etches, 1996). Although egg macro nutrients are deposited in both albumen and yolk, yolk is the major source for nutrients. Egg yolk composes of approximately 48% water, 33% lipids, 17% protein, and 1% carbohydrates, whereas albumen is a poorer source of nutrients, containing approximately 88 to 90% water, 10% protein, 1% carbohydrates, and no lipids (Romanoff and Romanoff, 1949). The eggshell has an important role in $O_2$ and $CO_2$ exchange. This exchange is determined by eggshell conductance, which is fixed once the eggs are laid (Ackerman and Rahn, 1981). Several studies reported variation in egg composition and eggshell properties due to differences in breeder age, egg size, broiler strain, and breed (O'Sullivan et al., 1991; Vieira and Moran, 1998 a,b; Peebles et al., 2000; Hamidu et al., 2007; Wolanski et al., 2007; Everaert et al., 2008; Ho et al., 2011). This suggests that the origin of eggs, which alters egg composition and eggshell properties, may influence availability of nutrients as well as $O_2$ for the embryo. During incubation, the embryos use these available resources to produce energy to fulfil the requirements for development, growth, and maintenance. Since the embryo develops in the closed environment of an egg that vary in size and composition due to different origin, it can be hypothesized that embryonic development and nutrient metabolism will be altered due to variation in nutrient and $O_2$ availability.

In the biochemical processes of nutrient metabolism, $CO_2$, metabolic water, and heat are produced as by-products. Meijerhof and Van Beek (1993) and

Lourens et al. (2011) showed that embryonic heat production (HP) can have a major influence on the temperature experienced by the embryos, which is reflected in the eggshell temperature (EST). In turn, the EST determines the HP through alterations in embryonic development (Lourens et al., 2011; van den Brand et al., 2015). Several studies have demonstrated that an EST at 37.8°C results in a good hatching success and good chick quality and consequently in good broiler performance (Lourens et al., 2005, 2007; Hulet et al., 2007; Molenaar et al., 2010a,b, Molenaar et al., 2011a). To maintain an optimal EST, heat produced by the embryos needs to be removed. An effective management tool to obtain an optimal EST is a proper machine temperature profile and if the HP is different for eggs of different origins, the required machine temperature profile need to be adjusted accordingly.

Although some studies (O'Dea et al., 2004; Sato et al., 2006; Hamidu et al., 2007; Everaert et al., 2008; Tona et al., 2010) have already investigated effects of egg origin on embryonic development and nutrient metabolism, interpretations of these results are ambiguous. This is because in most of these studies the experimental eggs vary in weight and origin and/or they were incubated in the same machine without an assessment of EST. If the embryos differed in HP as a result of for instance a difference in egg size (Lourens et al., 2006), incubating these eggs at the same machine temperature could lead to alterations in embryo temperature or EST. As a result, effects of egg origin and EST on development and nutrient metabolism could have been confounded in these experiments. Therefore, the approach in this thesis was to select eggs from different origins and to incubate them all at a standardized EST of 37.8ºC (chapter 2, 3, 4, and 5). Further investigations for the mechanisms underlying embryonic development and nutrient metabolism and the interaction between egg origin and EST were made in chapter 6 and 7.



The objective of this general discussion is to discuss the conceptual mechanisms that may cause differences in embryonic development and nutrient metabolism of eggs from different origins. Additionally, practical consequences and implications of the findings in this thesis for incubation management are discussed.

## II. *Embryonic Development and Nutrient Metabolism in Relationship to Requirements and Availability of Nutrients and $O_2$*

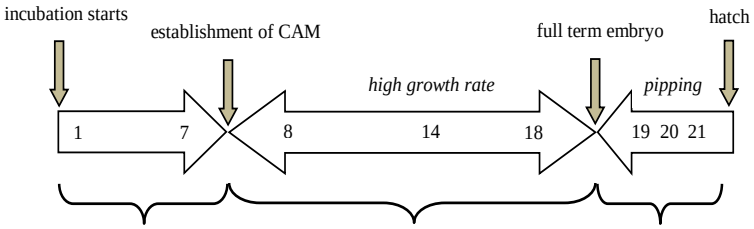
### a. Embryonic development and nutrient metabolism

Development of chicken embryos as well as energy requirements to sustain developmental processes are progressively changing throughout incubation. Figure 1 demonstrates that after the start of incubation, $O_2$ availability at embryonic level changes with the progress of developmental stages and this has an influence on the use of nutrients for energy production. After approximately 48 h of incubation, the yolk sac vascular system starts its function to transport absorbed nutrients from the yolk sac to the embryo (Romanoff, 1952). This vascular system also supports respiration gas exchange until the chorioallantoic membrane (CAM) starts to develop at E8 (Baumann and Meuer, 1992). The CAM is an extremely rich vascular network, which functions as respiratory organ until the onset of lung respiration at internal pipping (Romanoff, 1960; Burton and Tullet, 1985). Compared to the CAM, the vascular system of the yolk sac is limited in its capacity to uptake $O_2$ due to especially the layer of egg albumen on top of the vascularisation area (Baumann and Meuer, 1992). This results in a limited $O_2$ availability at embryonic level during the first week of incubation. Although the CAM is a better system for taking up $O_2$, its function is limited by diffusion through the eggshell and this is determined by the eggshell conductance (Ackerman and Rahn, 1981; Leon-Velarde and Monge, 2004).

As a result of limited access to $O_2$ during the first week of incubation, the embryo uses mainly carbohydrates as energy source via anaerobic glycolysis (Figure 1; Moran, 2007). At the end of the first week almost all carbohydrates are exhausted due to the limited amount available in the eggs (≈1%; Romanoff, 1967). From the second week of incubation onward a sufficient $O_2$ availability, due to CAM development, permits the embryo to use yolk lipids as main energy source (Moran, 2007), providing approximately 90% of total energy requirements (Noble and Cocchi, 1990). The available egg protein is mainly used from the second week of incubation onward, which is the period when major development and growth occur (Romanoff, 1967), and correspondingly energy requirements increase. Although the embryo is structurally completed at E14 (Moran, 2007), the

development towards full body mass and the transition from an embryo to a hatchling during the last week is also an energy demanding process. The amount of $O_2$ consumption as presented in Figure 2, indicates that $O_2$ requirement increases with the progress of the developmental stages. Following the physiological development and the pattern of $O_2$ consumption, Figure 2 also demonstrates that the amount of especially lipids and protein utilization is dynamically and progressively changing throughout the course of incubation. Figure 2 clearly shows that there is an increase in requirements for $O_2$ and nutrients with the progress of incubation.

Because $O_2$ diffusion rate is fixed by the eggshell conductance, a continuous increase in $O_2$ requirements by the embryo will result at a certain stage of incubation in a limitation of $O_2$ availability at embryonic level (Romijn and Roos, 1938; Whittow and Tazawa, 1991). This will result in a reduction of the lipid metabolism and embryos will need to rely more on other nutritional sources for energy. Glucose from glycogenolysis and protein, especially glucogenic amino acids, are alternative sources for energy production (Figure 1). When the hatching process starts at approximately E19, the $O_2$ tension in the air cell is reduced from 21 to 14% (Rahn, 1981; Tazawa et al., 1983). With this limited $O_2$ availability, an aerobic glycolysis of glucose will be the crucial source of energy (Figure 1). Since carbohydrates in the eggs are no longer available after the first week of incubation (Yarnell et al., 1966), a depletion of the glycogen storage is the main supply of glucose to facilitate the completion of hatching.




| $O_2$ availability | Limited | Sufficient | Toward limitation | Limited |
|---|---|---|---|---|
| Main energy source | Carbohydrates | Lipids | Lipids | Lipids |
| Alternative energy source | - | - | Glycogen/ Protein | Glycogen/ Protein |

**Figure 1**. The role of $O_2$ availability at embryonic level on the utilization of nutrients for energy production (after De Oliveira et al., 2008).

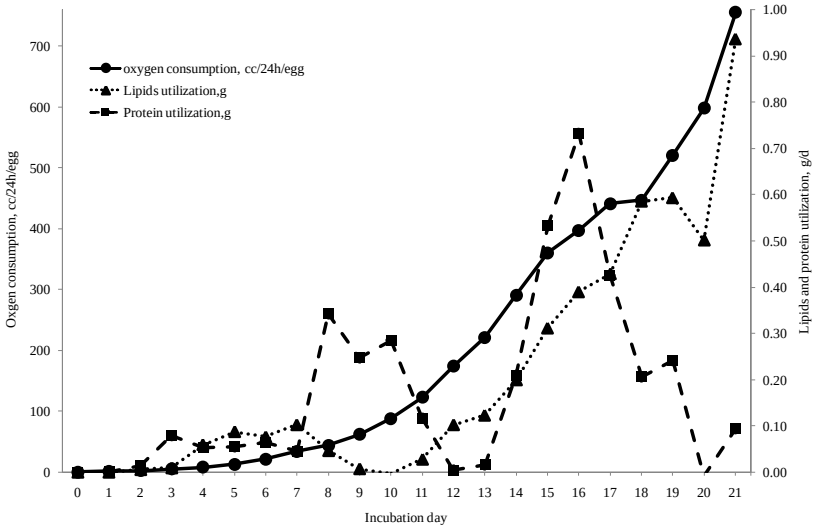



**Figure 2**. $O_2$ consumption of chicken embryos (cc/24h/egg) and amount of lipids and protein utilization throughout incubation (g/d; calculated from the changes in the amount of lipids and protein in the egg).
(after Romanoff, 1967)

Embryos store glucose as glycogen in the yolk sac membrane, heart, muscles, and especially in the liver and this can be detected from approximately E7 of incubation onwards (Beattie, 1964; Garcia et al.,1986; Foye et al., 2007; Yadgary and Uni, 2012). Although lactic acid, glucogenic amino acids, and glycerol are substrates in the gluconeogenesis pathway, Sunny et al. (2007) demonstrated that glycerol is the major substrate being used by chicken embryos. This could explain the storage of hepatic glycogen, which was found to be increased from the second week onward (Garcia et al., 1986), when there is a high intensity of lipid metabolism and as a result a high amount of glycerol available. More glucose availability from gluconeogenesis (Yarnell et al., 1966) might lead to a high glycogen synthesis and it has been shown that glycogen storage reaches its maximum before the start of the hatching process (Garcia et al., 1986; Yadgary and Uni, 2012). By using glycerol as the major substrate for gluconeogenesis, the embryo could spare protein for development and growth. Allocating protein for development and growth is preferable over using it for other purposes as energy production or gluconeogenesis. However, when lipid oxidation is reduced due to insufficient $O_2$ availability at embryonic level and glucose from depletion of glycogen becomes restricted, it might be necessary to allocate especially glucogenic amino acids more toward gluconeogenesis and energy production. The changes of protein allocation for other purposes might affect embryonic development and growth. Molenaar et al. (2010b) demonstrated that embryos at high EST from E7 until E19 had lower yolk absorption and lower efficiency of protein utilization than embryos at normal EST, which resulted in lower yolk free body mass (YFBM) and chick length at hatch. The authors suggested that the effect of high EST could be attributed to a limitation of $O_2$ availability and reported that an increase of $O_2$ to 25% restored the yolk absorption and hatchling development. In later findings, Molenaar et al. (2013) revealed that high incubation temperature increased glucose oxidation and decreased hepatic glycogen storage prior to hatching. The authors proposed that when there is a high glucose oxidation due to high incubation temperature, amino acids obtained from protein catabolism might be used for gluconeogenesis. This will negatively affect embryonic development and hatchling quality as found in other studies (Romanoff 1936; Lourens et al., 2005; Leksrisompong et al., 2007; Molenaar et al., 2010b; Willemsen et al., 2011). In addition, an increase in depletion of hepatic glycogen at high EST might

ultimately lead to insufficient energy for the embryos to complete the hatching process (Willemsen et al., 2010, 2011; Molenaar et al., 2013), resulting in lower hatchability of fertile eggs.

**b. Egg origin and its influence on nutrient and $O_2$ availability**

Development and growth of chicken embryos are continuous processes, but a reduction in growth rate was observed at certain stages of development. Romanoff (1967) reported that embryonic growth rate is generally reduced at E9 and E16 of incubation. Later studies reported differences in embryonic growth patterns at certain embryonic stages, due to factors as breed (Everaert et al., 2008), EST (Lourens et al., 2005), and $O_2$ (Lourens et al., 2007; Molenaar et al., 2011a), but mechanisms underlying these differences remain largely unclear. If the fulfilment of energy requirements is essential to sustain embryonic development and growth, it can be hypothesized that embryos at certain stages of incubation have insufficient available resources (nutrients and/or $O_2$) for energy production. In addition, if there are differences in available resources for energy production, a change in nutrient metabolism to generate energy could have effects on embryonic development and growth pattern. As a result of differences in nutrient metabolism, alterations of embryonic HP could be expected and this could influence embryo temperature or EST. An interrelationship of nutrient metabolism and embryonic development and growth, including nutrient and $O_2$ availability could be explained as in Figure 3.

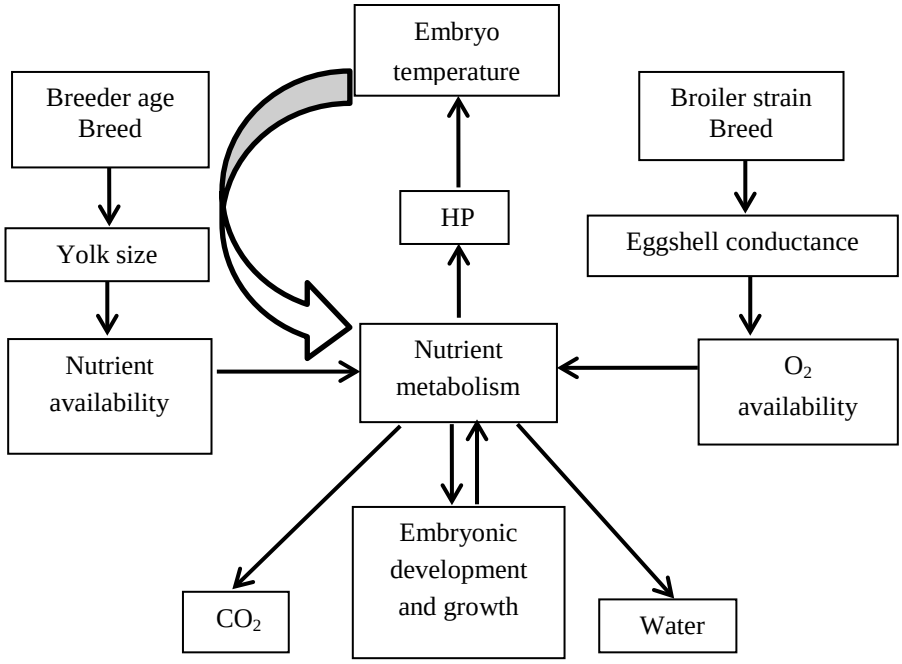



**Figure 3.** Embryonic development and nutrient metabolism of eggs from different origins.

Figure 3 demonstrates that the availability of nutrients and $O_2$ can be influenced by egg origin through alteration of the yolk size and eggshell conductance. As explained earlier, differences in nutrient and $O_2$ availability can lead to differences in nutrient metabolism, which has consequences on embryonic development and growth. In turn, a progressive increase in embryonic growth requires an increase in energy supply for maintenance of the existing body tissues and for the synthesis of new tissues for growth (Vleck, 1991). In the biochemical processes to convert egg nutrients into energy, embryos produce heat, $CO_2$, and water as by-products. This suggests that the availability of nutrients and $O_2$ has also an influence on heat production (HP). If this HP remains in the system in which the eggs are incubated or the removal of this HP varies, the temperature experienced by embryos (EST) will be affected. Since embryos before E18 act poikilothermic (Tazawa et al., 1988), variations in EST could alter metabolic processes as well as embryonic development and growth. This means that the

availability of nutrients and $O_2$ will play a crucial role on how embryos can continue their development and growth at different EST and ultimately this mechanism will affect the quality and hatching success of the embryos.

In the following section the findings of this thesis will be discussed on how this conceptual mechanism could explain differences in nutrient metabolism and embryonic development of eggs from different origins.

**c. Dynamic changes of nutrient metabolism**

To assess nutrient metabolism of embryos during incubation, energy utilization, energy lost during the developmental process, embryonic HP, and the efficiency of converting egg energy into yolk free body ($E_{YFB}$) were determined. Among these measurements, energy utilization, energy lost, and $E_{YFB,}$ which were measured at hatch, provided indications for an overall nutrient metabolism, but could not explain dynamic changes during incubation related to the roles of nutrient and $O_2$ availability. The embryonic HP, which was measured continuously over incubation time, reflected a progressive change of requirements as well as availability of nutrients and $O_2$. Following the pattern of embryonic development and growth, embryonic HP was low during the first week of incubation and progressively increased with incubation time (Chapter 3, 4, 5, and 7). The overall results demonstrated that embryos of eggs from different origins, which varied in nutrient and $O_2$ availability, had similar HP during the first week of incubation (Chapter 3, 4, and 5). This similarity indicates that in normal incubation conditions, the availability of nutrients and $O_2$, which both vary for eggs of different origins, had no influence on nutrient metabolism during the first seven days of incubation. This might be related to the low absolute embryonic weight and thus the low energy requirements during this period.

After E8, the embryonic HP progressively increased, which indicates an increase in utilization of nutrients and $O_2$ to fulfil energy requirements. Since development of the CAM enables embryos to have access to available $O_2$, the diffusion rate of $O_2$ across the eggshell and the availability of nutrients become crucial to optimize nutrient metabolism. The differences in embryonic HP pattern after E8 among egg origins demonstrate an important role of nutrient and $O_2$ availability, which can be explained by Figure 3. However, the interpretations of

embryonic HP could not explicitly explain the dynamic changes of requirements and availability of nutrients and $O_2$. Therefore, the daily HP increments were calculated to investigate the roles of nutrient and $O_2$ availability at the different stages of embryonic development (Figure 4a to 4f).

The daily HP increments were calculated as: HP of each day subtracted by HP of the previous day. The values obtained reflect the change in daily metabolic rate, which is indicative for the dynamic changes of nutrient and $O_2$ availability for energy production. The overall pattern shows that the daily HP increment is low during the first 7 days (Figure 4a and 4b). Thereafter, daily HP increment drastically increased, reaching a plateau stage at approximately E14 and afterward it drastically declined (Figure 4a to 4f). The increase of daily HP increments after E7 and declination after E14 occurred in different magnitude for embryos of eggs from different origins.

The combination of embryonic HP and the daily HP increment after E8 lead to the proposed mechanism underlying differences in nutrient metabolism and embryonic development of eggs from different origins. This mechanism can be attributed to the balance between energy requirements for growth and for maintenance and the availability of nutrients and $O_2$ throughout the different developmental stages. Based on the patterns of changes that occurred in the HP and daily HP increment, embryonic developmental can be divided into three periods 1) E0 to E7, 2) E7 to E14, and 3) E14 until hatching. For further discussion in this thesis, the findings of embryos originating from different egg origins as well as the findings related to incubation factors will be explained based on these 3 developmental periods.

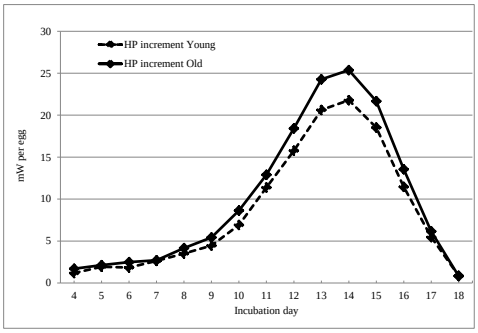



**Figure 4a**. Heat production (HP) increment from E4 until E18 of young and old flock eggs incubated at an EST of 37.8ºC. HP results are presented in Chapter 3.

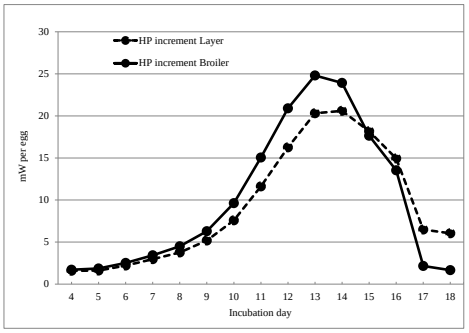



**Figure 4b**. Heat production (HP) increment from E4 until E18 of broiler and layer eggs incubated at an EST of 37.8ºC. HP results are presented in Chapter 4.

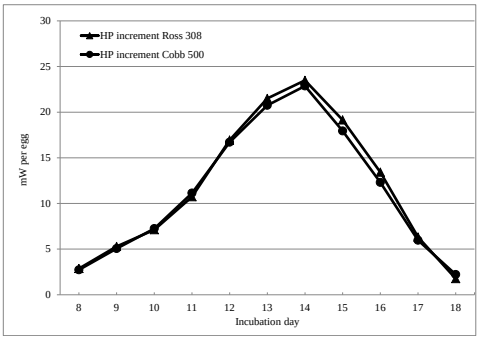



**Figure 4c**. Heat production (HP) increment from E8 until E18 of Ross 308 and Cobb 500 eggs incubated at an EST of 37.8ºC. HP results are presented in Chapter 7.

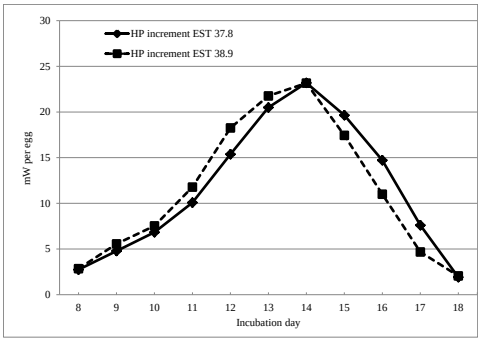



**Figure 4d**. Heat production (HP) increment from E8 until E18 of the broiler eggs incubated at an EST of 37.8 and 38.9ºC. HP results are presented in Chapter 7.

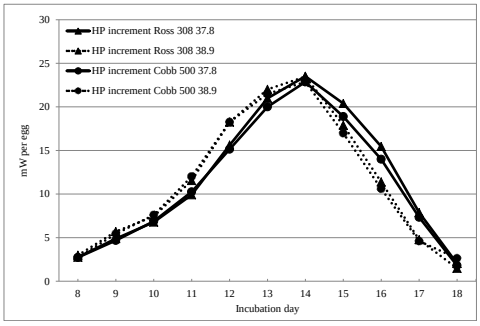



**Figure 4e**. Heat production (HP) increment from E8 until E18 of Ross 308 and Cobb 500 eggs incubated at an EST of 37.8 and 38.9ºC. HP increments were calculated from HP results of Chapter 7.

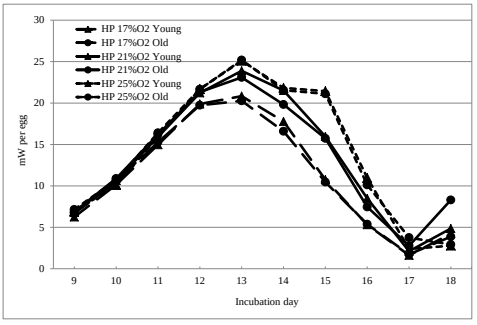



**Figure 4f.** Heat production (HP) increment from E9 until E18 of young and old flock eggs incubated at an EST of 37.8ºC at the oxygen level of 17, 21, and 25% (unpublished data).

***III. Egg and Incubation Related Factors***

**a. Eggs originating from different selection traits: growth and meat yield vs egg production**

It has been proposed and generally thought that selection traits for the grow out period alter development during embryonic stages as well (Tullet and Burton, 1983). In this thesis, the model that best approaches this proposition is the comparison between broilers and layers (**Chapter 4**). Broilers and layers are different in selection traits; broilers are selected for growth and meat yield, whereas layers are selected for egg production. As expected, embryonic development, expressed as YFBM at E16 and at hatch, was higher in broilers than in layers. Consistent with the embryonic growth pattern, embryonic HP was higher in broilers than in layers from E12 until E18. The question arises whether the differences found are due to breed specific growth potential or whether there are other mechanisms, e.g. related to nutrient and $O_2$ availability, involved in these findings.

At a similar egg weight, broiler embryos had a higher nutrient availability, expressed in a larger yolk size and a higher egg energy content, than layer embryos (Chapter 4). Everaert et al. (2008) reported that during the first seven days of incubation the embryonic weight relative to egg weight did not differ between broilers and layers. The same study showed that broiler embryos grew faster than layer embryos from E12 until E16, but there was no difference afterward. In agreement with this study, results in Chapter 4 demonstrate that differences in breed specific growth potential and availability of nutrients and $O_2$ made no differences in embryonic HP and daily HP increment during the first seven days of incubation (Figure 4b).

The daily HP increment of broiler and layer embryos drastically increased from E7 until E14 and decreased afterwards. Numerically, the amount of daily HP increment in broiler embryos started to deviate from layer embryos after E8, the stage when yolk lipid oxidation becomes the main energy supply (Moran, 2007). Based on Figure 1 and 3, broiler embryos after E8 probably benefit from having a higher nutrient and $O_2$ availability than layer embryos due to differences in yolk size and conductance, which gives rise to a high metabolic rate. This might

contribute to a higher growth rate of broiler embryos than of layer embryos until approximately E14. The results during E7 to E14 suggest that differences found between broiler and layer embryos can largely be contributed to differences in availability of nutrients and $O_2$, although a direct influence of breed specific growth potential cannot be completely ruled out for the differences found between E7 to E14.

Broiler embryos had a lower daily HP increment than layer embryos after E14. This daily HP increment pattern occurred coincidentally in the same period as a reduction in embryonic growth rate of broiler embryos takes place (Everaert et al., 2008). Growth reduction of broiler embryos was also found in the results of Chapter 4, where YFBM gain from E16 to hatch was approximately 12% lower in broiler embryos than in layer embryos. Since the daily HP increment is an indication for daily metabolic rate, the patterns suggest that after E14, broiler embryos might experience limitations of resources for nutrient metabolism and this results in a reduction in growth rate. The limitation of resources cannot be explained through nutrients as the RSY at E16 remained approximately 0.8 g bigger in broiler embryos than in layer embryos. Based on Figure 1 and 3, it seems that limitation of $O_2$ availability creates differences in growth rate after E14. Since eggshell conductance was higher in broiler eggs than in layer eggs, the explanations for this proposition cannot be related to only eggshell conductance, but will probably be influenced by the developmental pattern of the embryos and its influence on metabolic rate.

A significantly lower shell weight and numerically higher weight loss at E16 suggests that broiler eggs had a higher eggshell conductance, leading to a higher availability of $O_2$, than layer eggs. This leads to the assumption that limitation of $O_2$ availability in broiler embryos after E14 could have been a result of an earlier high growth rate. A higher embryonic growth rate and a higher absolute weight of broiler embryos than layer embryos during E7 to E14 will lead to a higher requirement for nutrients and $O_2$ to produce energy for growth and for maintenance. Since the capacity of $O_2$ and $CO_2$ exchange is fixed, a progressively higher requirement might ultimately drive broiler embryos after E14 to reach limited $O_2$ availability at embryonic level (Whittow and Tazawa, 1991). This explanation can be attributed to a reduction in daily HP increment (Figure 4b) and

a reduction of embryonic growth in broiler embryos during the last week of incubation.

The findings from the comparison of broilers and layers indicate that the mechanisms underlying differences between breeds could be related to embryonic development as well as metabolic rate of the embryos. The results suggest that the availability of nutrients and $O_2$ plays a crucial role in the metabolic rate and embryonic development. A high metabolic rate and embryonic development during E7 to E14 lead to a high magnitude of limitation in $O_2$ availability after E14. It can be implied that after E14, the availability of $O_2$ is a determinant factor for nutrient metabolism and embryonic growth, whereas a contribution of breed specific growth potential on the results obtained remains unclear.

**b. Eggs originating from similar selection traits: growth**

**i.) Broiler strain**

Since embryos of Ross 308 and Cobb 500 were similar in embryonic HP during E0 to E14 (**Chapter 5 and 7**), the discussion about the effects of broiler strain concentrate on differences found from E14 onward. A crucial role of $O_2$ availability as a determinant factor for nutrient metabolism and embryonic growth after E14 is consistently found for the comparison of Ross 308 and Cobb 500 (Chapter 7). The measurements of eggshell conductance indicates that $O_2$ availability was higher in Ross 308 than in Cobb 500, but yolk size and thus nutrient availability did not differ (Chapter 7). Based on Figure 1 and 3, nutrient metabolism and embryonic growth after E14 was expected to be higher in Ross 308 than in Cobb 500. In accordance with the expectation, Ross 308 embryos had significantly higher HP than Cobb 500 from E15 until E18 (Chapter 7). In fact, a numerically higher HP from E16 to E18 in Ross 308 than in Cobb 500 was observed in Chapter 5. However, a higher nutrient availability due to a 1 g larger in yolk size in Ross 308 than in Cobb 500 hampered the interpretation of the results in Chapter 5. In Chapter 7, it became clear that despite a similar nutrient availability, the daily HP increment during E13 to E16 was slightly higher in Ross 308 than in Cobb 500 (Figure 4c) and as a result, Ross 308 embryos had a higher YFBM than Cobb 500 embryos at E14, E18 and at hatch. The combination of results in Chapter

5 and 7 suggests that during E0 to E14 embryos of eggs with similarity in selection traits were equal in nutrient metabolism and embryonic development, independently of nutrient and $O_2$ availability. After E14, the results confirm that $O_2$ availability is a determining factor for nutrient metabolism and embryonic growth.

**ii.) Breeder age**

The comparisons of young and old breeder age eggs **(Chapter 3, 6 and 7)** provided inconsistent results regarding nutrient metabolism and embryonic development. In terms of nutrient availability, the old flock eggs had consistently 20 to 23% larger yolk size and 13% more nutrient availability, expressed as energy content (Chapter 3 and 7). Other studies have shown that eggshell conductance increased with breeder age (O'Dea et al., 2004). Although it was not significant, the same indications for a higher eggshell conductance in old flock eggs than young flock eggs was found in Chapter 7. By interpreting results based on different developmental periods (E0 to E7, E7 to E14, and E14 to hatch), a similar HP during E0 to E7 (Chapter 3) confirmed that there was no influence of nutrient and $O_2$ availability on nutrient metabolism during this period. There was an indication that breeder age had an influence on embryonic growth at E7 and this was found as well at E14 (Chapter 3). However, the same trend was not observed in Chapter 6. Therefore, it is not clear whether availability of nutrients and $O_2$ can alter embryonic growth during E0 to E7, but nutrient and $O_2$ availability seems to have no effect on nutrient metabolism during the first seven day of incubation. The HP of old flock embryos was numerically higher than that of young flock embryos from E7 until E14 and thereafter significantly higher until E18 (Chapter 3). It was concluded in Chapter 3 that the differences of HP between breeder ages were determined by differences in nutrient availability. However, the patterns of daily HP increment (Figure 4a) suggest that the availability of nutrients as well as $O_2$ might be a reason for this difference.

A reduction of daily HP increment after E14 indicates that the embryos of both breeder ages might reach a limitation in $O_2$ availability at embryonic level (Figure 4a). Nutrient availability will not be limiting during this period as the RSY at E14 is approximately 80 to 85% of the fresh yolk size. A lower HP (Chapter 3)

and daily HP increment (Figure 4a) indicate that the magnitude of $O_2$ limitation after E14 was more critical for embryos of young flock eggs than those of old flock eggs, but they reached the same magnitude of limitation at E17. Although YFBM of young and old flock embryos was not statistically different after E14 (Chapter 3) and for the whole incubation period (Chapter 6 and 7), the dry matter content of the YFBM indicate that embryos of old flock eggs deposited more nutrients into YFBM than those of young flock eggs. This higher nutrient deposition might be due to the fact that embryos cannot gain in weight, due to limitation of space (Wiley, 1950) and because of that will gain in nutrients.

The overall findings for young and old flock embryos fit well within the concept described in Figure 1 and 3; a higher availability of nutrients and $O_2$ leads to higher nutrient metabolism and embryonic growth. However, HP results in Chapter 7 did not show the same pattern as described in Chapter 3. In Chapter 7, embryos of young and old flock eggs had similar HP during E7 to E18. The reason for this inconsistency of HP results among studies is not known. One speculation could be that eggs of different batches and studies differed in eggshell quality and thus eggshell conductance. This might create to some extent differences in $O_2$ availability and this might alter HP results among studies. Although HP results in Chapter 7 were similar between breeder ages, the principle of the mechanism for nutrient metabolism and embryonic development remained applicable for HP results in Chapter 3 as well as for the energy utilization and energy deposition in the YFBM in Chapter 7.



**c. Incubation related factor: EST and $O_2$**

The mechanisms underlying nutrient metabolism and embryonic development were further investigated by applying EST treatments; normal EST at 37.8°C and a high EST at 38.9°C (Chapter 6 and 7). These treatments are based on the principle that chicken embryos before E18 act as poikilothermic (Freeman, 1964; Tazawa et al., 1988) and variations in temperature during this period will alter nutrient metabolism and embryonic development (Figure 3; Lourens et al., 2005, 2007; Joseph et al., 2006; Hulet et al., 2007; Leksrisompong et al., 2007; Willemsen et al., 2010; Molenaar et al., 2010b). In agreement with previous findings (Lourens et al., 2005, 2007, Molenaar et al., 2010b), a high EST initially

accelerated nutrient metabolism, which is shown in higher embryonic HP at E11 to E15 (Chapter 7) as well as higher daily HP increment from E8 until E14 (Figure 4d). The reverse patterns of nutrient metabolism occurred after E14. Embryos at high EST had a lower metabolic rate than embryos at normal EST from E15 to E17 (Figure 4d). The embryonic growth trajectories were altered in line with the pattern of nutrient metabolism. An assessment of YFBM demonstrated that embryos at high EST grew faster than embryos at normal EST at E14 and E16 (Chapter 6), but thereafter a high EST resulted in deceleration of the growth rate. Consequently, incubation at high EST resulted in a lower chick YFBM at hatch.

The results of normal and high EST indicate that nutrient metabolism and embryonic development are stimulated until E16 when eggs are incubated at high EST. Whittow and Tazawa (1991) proposed that an increase in temperature lead to an acceleration of nutrient metabolism as a result of the Arrhenius-limited stage, in which the metabolic rate is directly related to the temperature. A high speed of nutrient metabolism due to high EST, resulting in high embryonic growth (Chapter 7), will lead to a continuously high requirement for energy supply to sustain growth (Figure 3). Since the embryos were equal in nutrient and $O_2$ availability at the start of incubation, an earlier high speed in metabolic rate might drive embryos to reach limitations of available resources for energy production at an earlier moment. Nutrient availability was not limited, as embryos at E14 had a remaining RSY of approximately 85% of fresh yolk available as nutrition source (Chapter 7). This means that embryos of high EST were driven to reach an $O_2$-conductance-limited stage (Whittow and Tazawa, 1991), where diffusion rate of $O_2$ across the eggshell is limited by the eggshell conductance. Embryos at normal EST, which had a lower metabolic rate and embryonic growth rate before E14 were less driven to reach $O_2$ limitation. As a result, the daily HP increment after E14 indicates that embryos at normal EST were able to maintain a higher metabolic rate than embryos at high EST and this resulted in better development as expressed in a higher chick YFBM at hatch.

Effects of EST on embryo metabolism clearly reflect the mechanisms as described in Figure 1 and 3. It has been demonstrated that the metabolic speed is a driving factor leading to limited $O_2$ at embryonic level. It has especially been confirmed that embryonic growth trajectories can be altered through changes in nutrient metabolism. In the case that eggs are incubated in conditions that create a

high nutrient metabolism (e.g. high EST), an acceleration in embryonic growth will create an increase in nutrient and $O_2$ requirements. With a fixed capacity of $O_2$ diffusion across the eggshell, an acceleration of requirement for $O_2$ would force embryos to reach limitations of $O_2$ availability. This will result in low energy production and consequently embryonic growth rate is reduced.

To investigate how the availability of $O_2$ would interact with high EST, Ross 308 and Cobb 500 were incubated at normal and high EST (Chapter 7). Embryos of these broiler strains were equal in availability of nutrients, whereas the availability of $O_2$ was higher in Ross 308 than in Cobb 500, due to differences in eggshell conductance. It was expected that at high EST, embryos of Ross 308 would continue with high nutrient metabolism for a longer period after E14. This, however did not occur as shown in embryonic HP. Embryos of both strains reacted in the same manner to a high EST; the daily HP increment was numerically higher at high EST than at normal EST until E14 and this pattern reversed after E14 (Figure 4e). The lack of an interaction between EST and broiler strain could be due to a pronounced effect of EST, overruling a relative small difference in eggshell conductance between both strains. In previous studies, where the availability of $O_2$ was directly manipulated by changing $O_2$ levels, it was shown that the negative impact of high EST was partly restored by an increase in $O_2$ level to 25% (Molenaar et al., 2010b). The same authors also showed that there was a significant interaction of $O_2$ and EST during E15 to E18. This agrees with the proposition that the availability of $O_2$ is a determinant factor for nutrient metabolism and growth, especially in the condition when there is an acceleration of metabolic speed.

A crucial role of $O_2$ was further investigated as demonstrated in Figure 4f (unpublished results). Figure 4f was obtained from an experiment where eggs were selected from young and old flocks at the same egg weight range and were incubated at $O_2$ levels of 17, 21, or 25%. Eggs were incubated at an EST of 37.8 and $O_2$ treatments were applied from E7 onward. Embryos of old flocks had a higher nutrient availability, expressed as 3.9 g heavier yolk weight than that of the young flocks at the start of incubation. Figure 4f showed that regardless of nutrient availability, daily HP increment after E7 increased with $O_2$ levels. This leads to the conclusion that nutrient metabolism was determined mainly by $O_2$ availability and based on that results, once again it can be suggested that $O_2$ availability is a determinant factor for embryonic development and growth.

***IV. Consequences During Incubation and Practical Implications:***

Incubation temperatures within a range of 37.5 to 38.0ºC have been reported to be optimal for incubation (Wilson, 1991). In recent years, many studies have demonstrated that the temperature experienced by the embryos or EST is critical for an optimal embryonic development. An EST at 37.8 ºC is reported to give good embryonic development, hatching success, and good chick quality (Lourens et al., 2005, Leksrisompong, 2007; Molenaar et al., 2010 a, b). These studies have also shown that one of the major factors influencing the EST is the embryonic heat production (HP; Meijerhof and Van Beek, 1993; Lourens et al., 2011). As EST is the balance of embryonic HP and heat loss, changing embryonic heat production will alter EST, in the case heat is not removed sufficiently throughout the incubation process. The management of heat loss is therefore a critical factor to provide embryos with an optimal EST.

As described in Figure 3, HP is a by-product from nutrient metabolism and is influenced by nutrient and $O_2$ availability. Although the availability of both nutrients and $O_2$ is essential for nutrient metabolism, the findings in this thesis suggest that availability of $O_2$ rather than nutrients determine nutrient metabolism and its effect on HP. It was especially demonstrated that after E14, the embryos with high $O_2$ availability produce more heat. The findings in this thesis also suggest that $O_2$ availability at embryonic level after E14 is determined by both the eggshell conductance and the metabolic rate. Embryos with a high metabolic rate have an increased requirement of $O_2$ and will reach the conductance limiting stage earlier. For the purposes of incubation temperature management, the requirements for $O_2$ in the different stages of incubation should be taken into consideration. Figure 5 provides general suggestions for the factors embryonic HP, eggshell conductance, and nutrient availability and their degree of importance to be considered in developing incubation temperature profiles.

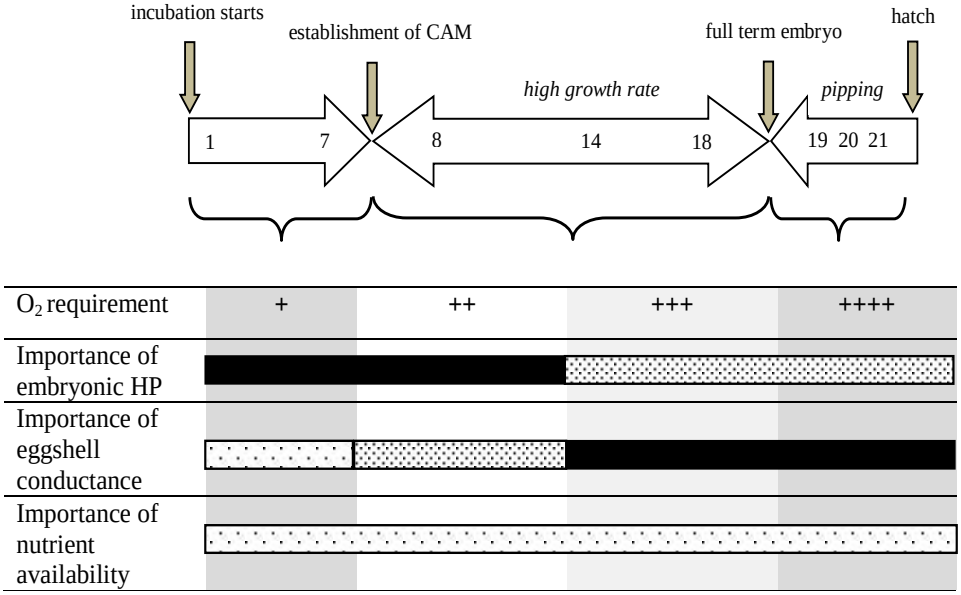



**Figure 5.** Factors and its degree of an importance to be considered in designing incubation temperature throughout the course of incubation ( = low, = medium, and = high).



### i.) Incubation temperature: $O_2$ availability, eggshell conductance and embryonic HP

Nowadays, it is generally recommended in practice to maintain EST at 37.8ºC throughout the incubation period. The general perception is that embryos with high HP should be incubated at a lower machine temperature and vice versa. The findings in this thesis suggest that the embryonic HP is not the only factor to be considered in incubation temperature adjustment. The availability of $O_2$ through eggshell conductance has a crucial role on the fine-tuning of incubation temperature. Combining the available knowledge and the findings in this thesis, practical implications for incubation temperature management should consider the factors as shown in Figure 5. Based on Figure 5, the general concept for

determining incubation temperature profiles should be to consider embryonic HP as a main factor during E0 to E14 and to include the eggshell conductance as a main factor from E14 onward. The findings in this thesis also suggest that the importance of nutrient availability as a factor for adjusting incubation temperature is considered to be low throughout the incubation period. To maintain EST at the same level for embryos of different origins, embryos with high HP should be incubated at a lower machine temperature than embryos with low HP. However, towards E14, the eggshell conductance is increasingly important for the adjustment of incubation temperature. Adjustments made especially during E7 to E14 can have consequences for the rest of the incubation period. This is because the embryos in this period have a high growth rate and at this stage their metabolic rate and growth is temperature dependent. Although the principle of adjusting incubation temperature based on embryonic HP remains applicable, adjustment of temperature during E7 to E14 could result in an early and critical limitation in $O_2$ availability during the rest of the incubation period. If temperature adjustments lead to a high EST, Chapter 6 and 7 suggest that there will be an increase in metabolic rate and $O_2$ requirement, which can result in limited $O_2$ availability at embryonic level after E14 (Figure 4d and 4e). As nutrient metabolism and embryonic development during E7 to E14 are significantly increased, the daily HP increments in Figure 4a to 4f demonstrate that embryos reach a limitation in $O_2$ availability after E14. This is the period when eggshell conductance should be considered as a critical factor for incubation temperature adjustment. Incubation temperature should be lower for eggs with low eggshell conductance than for eggs with high eggshell conductance. Decelerating nutrient metabolism by lower incubation temperature will provide embryos in eggs with low shell conductance more $O_2$ availability at embryonic level to sustain developmental process and complete the hatching process.

The proposed incubation temperature management as described in Figure 5 and especially for the inclusion of eggshell conductance as a main factor after E14 is supported by the findings in this thesis. Chapter 7 demonstrated the negative effects of incubating Cobb 500 eggs, which had lower eggshell conductance, at high EST. Although the interactions between strain and EST were not significant, Cobb 500 embryos at high EST had the lowest heart size at E18 and at hatch, the lowest YFBM at hatch, the shortest incubation duration, the lowest hatch of fertile, and the highest percentage of chicks with bad navel quality. High EST had

negative impact for both strains, but the effects were more pronounced for Cobb 500 than for Ross 308. This was especially exhibited in a lower heart size at E18 (21% for Cobb 500 vs 16% for Ross 308) and a shorter incubation time (13 h for Cobb 500 vs 8 h for Ross 308). When incubating layer eggs, Molenaar et al. (2010a) reported that layer embryos at high EST of 38.9ºC had a lower yolk absorption, lower heart weight relative to YFBM, lower YFBM at hatch, and a 9 h shorter incubation time than layer embryos at normal EST. Similar findings for high EST from E7 until hatching are reported in Chapter 7 and negative effects of high EST for embryos incubated at low $O_2$ were demonstrated by Molenaar et al. (2011b) and Lourens et al. (2007) as well.

It can be concluded that embryonic HP is not the only factor to be considered for determining incubation temperature. Oxygen availability needs to be taken into account to develop incubation temperature settings to obtain an optimal EST. In addition to this point, the observations in the field that Cobb 500 is more sensitive to overheating than Ross 308 might be due to limited $O_2$ availability due to low eggshell conductance. It should be further investigated whether optimal EST differs for embryos varying in $O_2$ availability. The principle behind this question is that eggs should be incubated based on metabolic rate and eggshell conductance to balance requirement and availability of $O_2$.

**ii.) $O_2$ and $CO_2$ level during incubation**



The ambient air during incubation contains approximately 21% $O_2$ and this is generally accepted as an optimal $O_2$ level for incubation. Molenaar et al. (2011b) demonstrated that at a normal EST of 37.8ºC an increase in $O_2$ from 21 to 25% had minimal benefit for the embryos, whereas at high EST of 38.9ºC an increase in $O_2$ to 25% restored the negative impact of high EST. This suggests that optimal $O_2$ during incubation is determined by EST or metabolic rate of embryos. If an EST at 37.8ºC is considered to provide embryos with a normal metabolic rate, then 21% $O_2$ is sufficient to sustain energy production for embryonic development. However, in practical situations, EST can vary and an EST of 38.9ºC is often observed (Elibol and Brake, 2008). This high EST can be found as local hot spots due to non-uniform conditions in the incubator. Additionally, sometimes incubation temperature profiles are incorrect and as a result embryos in the whole machine

experience overheating. In conditions of high temperature, ventilation management of the machines should be considered. Ventilation should be sufficient to remove heat and metabolic water that is produced by the embryos, but also to replenish $O_2$ in the ambient air in the machine.

Furthermore, care should be taken to the $CO_2$ level, which should be kept low especially in high temperature situations. Some hatcheries increase $CO_2$, especially during the last stage of incubation to push the embryos in a condition that stimulates hatching. This is because the process of hatching is triggered by a low $O_2$ or a high $CO_2$ level in the air cell (Visschedijk, 1968). Increasing $CO_2$ in the ambient air will result in an increase of $CO_2$ in the air cell and embryos will be synchronized in hatch time (Tong et al., 2015). However, from an $O_2$ availability perspective, increasing $CO_2$, especially during the last stage of incubation could negatively influence embryonic development and chick quality. Embryos in that stage are limited in $O_2$ availability and a high level of $CO_2$ will further worsen the situation, as it decreases $O_2$ binding to haemoglobin, resulting in reduction in $O_2$ transport to body tissues (Jansen, 2004). These negative synergetic effects could lead to a higher magnitude of reduction in nutrient metabolism and embryonic development and consequently to low chick quality.

**iii.) Selection for better $O_2$ availability**

Since eggshell conductance is a determinant factor for $O_2$ availability, an implication for breeding companies is to emphasize more on eggshell conductance in the selection program, particularly in strains with high metabolic rates. It is generally observed that high eggshell conductance correlates with thin eggshells and low eggshell strength. Therefore, the strategy to select for eggshell conductance should be to remain the eggshell strength and simultaneously improve eggshell conductance. Another potential way is selecting for an improvement of blood circulation by increased vascularization, both in the yolk sac membrane and CAM. A better blood flow will enhance $O_2$ uptake and transportation of $O_2$ to body tissues.

***V. Conclusions of the Discussion:***

The availability of nutrients and $O_2$ through alterations of yolk size and eggshell conductance plays a role on nutrient metabolism and embryonic development at different stages of incubation from E7 onwards. Between E7 and E14, the availability of both nutrients and $O_2$ alter nutrient metabolism and embryonic development. Between E14 and hatching, the availability of $O_2$ at embryonic level, which is determined by diffusion rate through the eggshell, becomes a determinant factor for nutrient metabolism and embryonic development. A high metabolic rate at early stages of incubation is a driving force, accelerating embryos to reach the diffusion limited stage at an earlier moment. As a consequence of limited $O_2$ availability, nutrient metabolism is restricted and embryonic development is depressed. A restricted nutrient metabolism due to limitation of $O_2$ availability has a negative impact on embryonic development and chick quality. The overall findings lead to the proposition that both nutrients and $O_2$ are important for embryonic development and growth, but after E14 the availability of $O_2$ is more limited than that of nutrients. Future implications of this thesis are proposed to maintain the balance between requirements and availability of $O_2$ to sustain embryonic development and growth throughout incubation.

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# Summary

Incubation in practice has to deal with hatching eggs originating from different breeds, strains, and breeder ages and these eggs vary in egg size, egg composition, and shell properties. Because albumen and yolk are the storage of macro nutrients (proteins, lipids, and carbohydrates), variations in egg composition can affect nutrient availability in the eggs. After incubation starts and embryonic development takes place, $O_2$ is being taken up by diffusion through the eggshell. The embryos use $O_2$ in the processes of converting egg nutrients into energy and the by-products $CO_2$, metabolic water and heat are produced. The derived energy is essential to sustain developmental processes for growth and maintenance of embryos and variation in availability of nutrients and $O_2$ may alter nutrient metabolism and embryonic development. As a consequence of differences in nutrient metabolism and embryonic development, embryonic heat production (HP) may vary and this has been shown to influence eggshell temperature (EST), which in turn can affect hatching success and chick quality.

Based on the information above the following questions can be addressed: 1) whether eggs from different origins are different in nutrient and $O_2$ availability, 2) whether embryos in eggs from different origins are different in nutrient metabolism and what are the consequences on embryonic development, and 3) what are the mechanisms underlying these possible differences. Therefore, the aim of this thesis is to investigate effects of egg origin on nutrient and $O_2$ availability and to determine nutrient metabolism of eggs from different origins as well as to examine consequences on embryonic development during incubation.

In Chapter 2 to 5, studies were performed to investigate effects of egg origin on embryonic development and nutrient metabolism. In Chapter 2 and 3, the focus is on breeder age and egg size, in Chapter 4 the focus is on breed, and in Chapter 5 the focus is on broiler strain. The approach in each of these chapters is standardized by selecting eggs from a similar breeder age at the same egg weight range. Before incubation, fresh eggs were examined for nutrients and $O_2$ availability. Egg composition (albumen and yolk) and energy content in the egg were measured as parameters for nutrient availability. Eggshell properties (shell weight or shell conductance) were measured as indicators for $O_2$ availability. Nutrient metabolism, expressed as embryonic HP was determined by incubating the experimental eggs in open circuit climate respiration chambers at an eggshell temperature (EST) of 37.8°C. Embryonic development and changes in residual

yolk were measured during incubation and at hatch. At hatch, the overall nutrient metabolism was assessed in term of energy utilization.

In a normal production cycle, egg size increases with breeder age and studies reported that an increase in egg size influences egg composition and embryonic development. However, in those studies there were confounding effects of breeder age and egg size and the interpretations of results related to egg origins were ambiguous. Therefore, in **Chapter 2** it was investigated whether breeder age or egg size influenced egg composition and embryonic development. The results showed that at similar age, an increase in egg size was mainly the result of an increase in albumen content. At the same egg size, older flock eggs had a higher fresh yolk weight and embryos had a higher yolk absorption rate than those of young flock eggs. However, breeder age had no effect on embryonic development measured as yolk free body mass (YFBM) at hatch. At hatch, egg size was a determinant factor for YFBM, independently of yolk size and yolk absorption rate. Further investigations were made in **Chapter 3** for nutrient availability and nutrient metabolism of eggs from different breeder ages and egg sizes. The results suggest that breeder age rather than egg size alter availability of nutrients via alteration of yolk size and there is a high correlation between yolk size and energy utilization during incubation. Additionally, measurements of embryonic HP showed that embryos of old flock eggs had a higher HP than those of young flock eggs from incubation day (E) E16 onward. These results indicate that nutrient availability is altered through variations in yolk size. An increase in yolk size led to a higher nutrient metabolism, but did not affect chick YFBM at hatch.

In **Chapter 4,** embryonic development and nutrient metabolism were measured for broilers, which are selected for growth and meat yield and for layers, which are selected for egg production. The hypothesis was that the selection for production traits of broilers and layers leads to physiological differences, which may already be present during incubation. The results showed that at the same breeder age and egg size, broiler eggs had more yolk and thus more nutrients available than layer eggs. A lower eggshell weight in broiler eggs than in layer eggs suggested that eggshell conductance and thus $O_2$ availability was higher for broiler embryos than for layer embryos. Embryonic development and nutrient utilization including embryonic HP (from E12 to E18) was higher in broiler embryos than in layer embryos. These results indicate that nutrient and $O_2$

availability may play an important role in embryonic development and nutrient metabolism during incubation.

In **Chapter 5,** comparisons were made between embryos of eggs from similar selection traits, Cobb 500 and Ross 308, which both are selected for growth and meat yield. At similar egg weight and breeder age, eggs of Ross 308 had a higher nutrient availability via a slightly higher yolk size (1 g). Eggshell weight and eggshell thickness was lower in Ross 308 eggs than in Cobb 500 eggs, which suggest a higher eggshell conductance and thus a higher $O_2$ availability. Compared to Cobb 500 embryos, Ross 308 embryos used more nutrients and had a higher YFBM at hatch. Numerically, embryonic HP was slightly higher in Ross 308 embryos than in Cobb 500 embryos from E16 to E18. The results suggest that Cobb 500 and Ross 308 differ in nutrient and $O_2$ availability, which may result in different trajectories for embryonic development and nutrient metabolism, especially after E16.

To investigate possible mechanisms explaining differences in egg origins, in **Chapter 6 and 7,** eggs from young and old breeder flocks of Cobb 500 and Ross 308 strain were used. It was hypothesized that embryos of these eggs, which may vary in nutrient and $O_2$ availability might respond differently to an increase in EST. Hatching eggs of both strains at a given age were selected at the same egg weight range. During E0 to E7, all eggs were incubated at an EST of 37.8°C and thereafter, eggs of each combination of breeder age and broiler strain were incubated at an EST of 37.8 (normal) or 38.9°C (high) from E7 until hatching. The results showed that breeder age rather than broiler strain had an influence on yolk size and thus nutrient availability. Eggshell conductance was higher in Ross 308 than in Cobb 500, whereas eggshell conductance of old flock eggs was numerically higher than that of young flock eggs. During incubation, a high EST accelerated embryonic development and nutrient metabolism expressed as HP from E11 until E15. After E15, embryos incubated at high EST had a lower metabolic rate compared to embryos incubated at normal EST, which was especially shown in nutrient utilization. As a result, chick YFBM at hatch was lower in high EST than in normal EST. Although embryos of old flock eggs had a larger yolk size and a higher nutrient utilization than those of young flock eggs, embryos of young and old flock eggs did not differ in YFBM during incubation and at hatch. Although Cobb 500 and Ross 308 did not differ in nutrient availability, Ross 308 embryos

had a higher nutrient metabolism expressed as embryonic HP from E15 until E18 and a higher nutrient utilization during incubation than Cobb 500 embryos. Consequently YFBM at E14, E18, and at hatch was higher in Ross 308 embryos than in Cobb 500 embryos. A higher EST had a negative impact on embryonic development and chick quality in both strains, but the effects were more pronounced for embryos of Cobb 500 than of Ross 308. The results in Chapter 6 and 7 suggest that a high EST accelerate nutrient metabolism and embryonic development, which result in an acceleration of nutrient and $O_2$ requirements. As a result of high EST, embryos at certain stages might reach a limitation in $O_2$ availability at embryonic level. Embryos with a higher $O_2$ availability, through a better eggshell conductance have less negative impact of higher EST than embryos which have a lower $O_2$ availability.

The overall findings in this thesis suggest that hatching eggs from different origins are not equal in nutrient and $O_2$ availability. The availability of both nutrients and $O_2$ plays a role on nutrient metabolism and consequently on embryonic development. During the first 7 days of incubation, nutrient and $O_2$ availability has no influence on nutrient metabolism and embryonic development. Between E7 and E14, nutrient and $O_2$ availability might have played a role as shown in the results of the broiler and layer comparison. Between E14 and hatching, the availability of $O_2$ becomes a determinant factor for nutrient metabolism and consequently for embryonic development. An increase in EST from 37.8 to 38.9°C from E7 onward resulted in an acceleration of nutrient metabolism and embryonic development until E16, but thereafter embryonic development reduced. Due to a high EST, the embryos with an accelerated metabolic speed at an early stage of incubation might reach limited $O_2$ availability at a higher magnitude than the embryos at a normal EST. As a result, nutrient metabolism is restricted and embryonic development is depressed. A restricted nutrient metabolism due to limitation of $O_2$ might be the explanation for poor embryonic development and a high percentage of low quality chicks. It can be concluded that HP should not be the only factor for developing machine temperature to obtain an optimal EST. The availability of $O_2$ is crucial as well and should be taken into account. The principle to develop machine temperature profiles for optimal EST is to maintain the balance between requirement and availability of $O_2$. If these are balanced, a continuing optimal nutrient metabolism

would generate sufficient energy to sustain development and growth throughout incubation.

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# About the Author

## CURRICULUM VITAE

Ampai Nangsuay was born on January 27, 1975 in Trang, Thailand. After completing a Vocational Certification (Fishery) at Trang College of Agriculture and Technology, Thailand, Ampai obtained her BSc in Animal Science from the Faculty of Agriculture, KamphaengSaen Campus, Kasetsart University, Thailand. During her BSc, Ampai took the opportunity to join the internship program for six months to learn about dairy farm management in Iowa, the United States. In 1998, Ampai started to work in an integrated broiler company, Sun Food Co., Ltd., Thailand, being responsible for technical issues in broiler breeder farms and hatchery, including the production planning for breeder farms, hatchery and broiler farms. In 2005, Ampai joined the broiler breeding company, Hybro B.V., the Netherlands, where she provided technical services for clients in Thailand, Bangladesh and Sri Lanka. In 2008, Ampai continued her education to obtain a M.Sc. in Animal Nutrition and Feed Technology, Animal Science Department, Faculty of Agriculture, KamphaengSaen Campus, Kasetsart University, Thailand. In 2011, Ampai spent six months as a guest researcher at the Adaptation Physiology (ADP) group, Wageningen University, the Netherlands. Ampai started her PhD at ADP group in 2013 and the outcomes of her research are described in this thesis.

## PUBLICATIONS

### Refereed Scientific Journals

**Nangsuay, A**., Y. Ruangpanit, R. Meijerhof, and S. Attamangkune. 2011. Yolk absorption and embryo development of small and large eggs originating from young and old breeder hens. Poult. Sci. 90:2648-2655.

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## WIAS Training and Supervision Plan[1]

| Description | Year |
|---|---|
| **The Basic Package (3.0 ECTS)** | |
| WIAS Introduction Course | 2013 |
| WGS course Ethics and Philosophy in Life Sciences | 2013 |
| | |
| **International Conferences (3.5 ECTS)** | |
| XIVth European Poultry Conference (EPC), Stavanger, Norway | 2014 |
| Poultry Science Annual (PSA) Meeting, Texas, USA | 2014 |
| IFRG Meeting and PDP Workshop, Berlin, Germany | 2015 |
| | |
| **Seminars and Workshops (0.8 ECTS)** | |
| WIAS Science Day, Wageningen, the Netherlands | 2014 |
| IFRG Meeting, Lunteren, the Netherlands | 2014 |
| | |
| **Presentations (5 ECTS)** | |
| Oral presentation at XIVth European Poultry Conference (EPC), Stavanger, Norway | 2014 |
| Oral presentation at PSA Meeting, Texas, USA | 2014 |
| Poster presentation at WIAS Science day, Wageningen, The Netherlands | 2015 |
| Oral presentation at IFRG Meeting and PDP Workshop, Berlin, Germany | 2015 |
| Oral presentation at Cobb Technical Meeting, Verona, Italy | 2015 |
| | |
| **In-Depth Studies (7.6 ECTS)** | |
| Epigenesis and Epigenetics | 2014 |
| Incubation Biology and Management | 2014 |
| Nutrition and (hot) Climate in Poultry | 2014 |
| Gut Health in Pigs and Poultry | 2014 |
| Quality of Protein in Animal Diets | 2015 |
| Early Programming: How early life shapes human development | 2015 |

| | |
|---|---|
| Advanced Statistics Course: Design of Experiments | 2014 |
| Statistics for the Life Sciences | 2015 |
| | |
| **Statutory Courses (3 ECTS)** | |
| Laboratory Animal Science | 2014 |
| | |
| **Professional Skills Support Courses (6.8 ECTS)** | |
| Reviewing a Scientific Paper | 2013 |
| Scientific Publishing | 2013 |
| Techniques for Writing and Presenting a Scientific paper | 2013 |
| Project and Time Management | 2014 |
| Data management | 2014 |
| PhD Competence Assessment | 2013 |
| Scientific Writing | 2014 |
| Efficient Writing Strategies | 2015 |
| | |
| **Research Skills Training (6 ECTS)** | |
| Preparing own PhD research proposal | 2013 |
| | |
| **Didactic Skills Training (4.5 ECTS)** | |
| Supervising 1 BSc and 2 MSc thesis students | |
| | |
| **Education and Training Total** | **40.2** |

[1]1 ECTS credit equals a study load of approximately 28 hours

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| | |
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