

Theoretical and empirical studies on temperature and moisture loss  
of hatching eggs during the pre-incubation period

Theoretisch en empirisch onderzoek naar de temperatuur en het  
vochtverlies van broedeieren tijdens de periode voor het broeden

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of hatching eggs during the pre-incubation period

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## STELLINGEN

### I

In de vermeerderingssector verdient het gebruik van wegrolnesten de voorkeur boven het gebruik van strooiselnesten.

*Dit proefschrift*

### II

Het belang van het handhaven van een hoge bewaartemperatuur voor broedeieren wordt onder Nederlandse omstandigheden overschat.

*Dit proefschrift*

### III

Door het vermogen van eieren om tenminste een gedeelte van het vochtverlies tijdens de bewaring te compenseren tijdens het broedproces is het handhaven van een hoge relatieve luchtvochtigheid tijdens het bewaren alleen onder extreme omstandigheden noodzakelijk.

*Dit proefschrift*

### IV

Voor een juiste opzet en interpretatie van broedproeven, evenals voor een juiste behandeling van broedeieren onder praktijkomstandigheden, dient rekening gehouden te worden met de leeftijd van de moederdieren.

*Dit proefschrift*

### V

De bewaring van broedeieren op voorbroedladen verdient de voorkeur boven bewaring op kartonnen tray's of plastic tray's.

*Dit proefschrift*

### VI

Hoewel in het algemeen wordt ontraden om appels en peren met elkaar te vergelijken, kan een vergelijking van appels en eieren veel nuttige informatie opleveren.

### VII

Indien de mening van de kiezer evenveel aandacht zou krijgen in een lopende kabinetsperiode als tijdens de verkiezingen zou politieke besluitvorming op een breder maatschappelijk draagvlak kunnen rekenen.

### VIII

Voor een verantwoord en doeltreffend gebruik van statistiek in landbouwkundig onderzoek is gezond verstand en biologische interpretatie belangrijker dan wiskundig inzicht

### IX

De tendens binnen de overheid om een niet naar tevredenheid functionerende organisatie ingrijpend te reorganiseren in plaats van tijdig bij te sturen resulteert in een ondoelmatig gebruik van mensen en middelen.

### X

De algemeen heersende tendens om toegepast gedragsonderzoek als synoniem voor welzijnsonderzoek te beschouwen leidt tot een onderbenutting van de mogelijkheden van dit vakgebied.

### XI

Zowel vanuit het oogpunt van onderzoek als van onderwijs is een toename van het aantal "dringers" dringend gewenst.

R. Meijerhof

Theoretical and empirical studies on temperature and moisture loss of hatching eggs during the pre-incubation period. Wageningen, 17 mei 1994.

## Voorwoord

De weergave van de resultaten van een wetenschappelijk onderzoek zoals dit voor u ligt laat weinig tot geen ruimte voor het verwoorden van de inspanning en de problemen waarmee een dergelijk onderzoek gepaard gaat, evenmin als het de mogelijkheid biedt om de voldoening en de plezierige kanten van het onderzoek te illustreren. Zo is alleen voor de direct betrokkenen de inspanning te schatten die het kost om duizenden eieren te openen voor het beoordelen van de werkelijke bevruchting. Ook met de radio aan en een koffiepauze op zijn tijd is het een kwestie van vele uren emmers met struif vullen. Ook het plezier van samenwerking met collega's bij het 's nachts verzamelen van de eieren direct onder de kip vandaan of het bepalen van de afkoeling van eieren in de nesten laat zich in een dergelijk verslag niet vatten, evenmin als de voldoening die het geeft als het uitwisselen van gegevens en gedachten met andere onderzoekers leidt tot een doorbraak voor beide kanten in de gedachtenvorming.

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## GENERAL INTRODUCTION

In the Netherlands, approximately 800 million hatching eggs are produced per year for the production of broiler chicks (Jaarverslag PPE, 1993). A certain percentage of these eggs is unfertile, dependent on e.g. method of housing and feeding, rearing, strain, age, ratio males/females. During incubation, an embryo is formed out of the egg content. In modern artificial incubators, this complex biological process is performed with a high rate of success. Although the success of this performance has increased over the years (Tullett, 1990), at this moment approximately 10% of the fertile eggs do not hatch. This is caused by death of the embryo somewhere in the period between fertilization and hatching. Also some embryos are not able to complete the pipping process at the end of the incubation period, resulting in a reduced hatching record. Some of the causes of this reduced hatchability of fertile eggs are situated in the period before the actual incubation process has started.

In commercial breeding operations, hatching eggs are produced in nest boxes, collected, stored for several days, transported to the hatchery and set, after which the incubation process is started. The treatment of hatching eggs in the period between oviposition and setting varies in commercial operations. These variations are mainly characterised by differences in temperature, storage time and moisture loss. It is recognised (Mayes and Takeballi, 1984) that the storage time and the temperature and moisture loss during storage are important for maintaining hatchability of fertile eggs. For several influencing factors, the mechanism responsible for the decrease in hatchability is not known. In commercial operations, it is not always possible to set the pre-incubation treatments according to the reported optimal conditions. To evaluate conditions that occur in commercial operations, the influence of non-optimal pre-incubation treatments on hatching results must be estimated. More knowledge about the mechanisms responsible for the influence of pre-incubation treatments on hatchability is necessary to evaluate these conditions and estimate their influence.

The aim of this study was (1) to formulate analytical equations to calculate the temperature development and moisture loss of hatching eggs, (2) to determine the actual conditions in the pre-incubation period that occur in field situations and (3) to determine the influence of these conditions on hatchability. The conditions studied were set at levels that represent field conditions. To be able to evaluate the influence of management decisions in the period before incubation on hatching results, some hypothesis about the involved mechanisms are formulated.

In chapter 1, the pre-incubation conditions and their influence on hatchability are reviewed. In

chapter 2, mathematical methods based on analytical equations are presented which can be used to calculate the temperature development and moisture loss of eggs. With these thermo-dynamic equations the temperature development and moisture loss of eggs can be predicted and results of experiments can be evaluated.

In chapter 3, the temperature development and moisture loss of eggs produced in different types of laying nests is determined and the results are compared with analytical equations. In chapter 4, temperature development and moisture loss of eggs stored in different types of egg containers and in various storage situations are determined and results are compared with analytical equations.

In chapter 5, the influence of temperature in the nest period, storage time and temperature during storage and temperature during the period just prior to setting on hatchability is determined. This is done with eggs produced by birds of two different ages. The level of the conditions used in this experiment are partly based on the results of chapter 3 and 4 and partly on the situations that occur in the field.

It is assumed that moisture loss during storage must be prevented to maintain maximum hatchability, because an optimum amount of moisture loss at the end of the incubation period is necessary to obtain maximum hatchability. However, it can be questioned if moisture loss during storage is detrimental if correction for this moisture loss during incubation is provided and therefore the reported optimum total moisture loss at the end of the incubation period is obtained. In chapter 6, the influence of moisture loss during storage in combination with moisture loss during incubation on hatchability is determined.

In the general discussion (final chapter) the practical implications of the results obtained in the experiments are discussed and hypothesis for underlying mechanisms are given.

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CHAPTER 1

PRE-INCUBATION HOLDING OF HATCHING EGGS

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**ABSTRACT**

The time between a hatching egg being produced and set in an incubator can be divided into a laying house period and a storage period. The period in the laying house affects hatching results by the stage of embryonic development at oviposition as well as by the subsequent environmental conditions. Advanced embryonic development at oviposition or prior to storage seems to improve hatchability, especially when storage is prolonged. Stage of embryonic development at oviposition is influenced by parental age, strain, individual bird variation and clutch length. After oviposition and before storage embryonic development can be influenced by the type of nest, egg collection pattern and temperature.

During storage, hatchability is influenced by the length of the storage period, temperature, humidity, gaseous environment and the orientation of the eggs. A decrease in hatchability can be detected in eggs stored for two to three days or more. Storage temperature should be decreased with extended length of storage. Temporary heating before incubation and enclosing eggs in plastic bags during storage improves hatchability, especially when storage is prolonged. A high humidity during storage improves hatchability as well, probably due to a reduction in water loss.

The changes in albumen pH during storage are discussed in so far as they provide a possible explanation for relationships between environmental conditions during storage and hatching results.

**INTRODUCTION**

Ideally every fertile egg would produce a healthy hatchling. In a commercial hatchery, however, this optimal situation is never achieved. This is due to a number of factors including lethal chromosomal abnormalities which cannot be influenced once the hatching egg is produced, insufficient availability of nutrients in the egg and the fact that eggs may be exposed to conditions not matching the demands of the developing embryo.

The process from egg formation to hatching is very complex and many factors may influence hatching results. Some of these can be influenced by management. To achieve optimum results it is necessary to determine the influence of management decisions on hatchability at different stages of the process. Knowledge of the demands of the embryo and properties of the hatching egg is therefore essential.

The reproduction process can be divided into a pre-incubation period and an incubation period. The pre-incubation period can be divided into a laying house period and a storage period. In this paper, the influence of climatic conditions during the pre-incubation period on hatchability and possible relationships with management decisions will be discussed, with a distinction between the period in the laying house and the storage period. The aim of this article is to give an overview of the relevant research and to indicate possible avenues of further research.

#### LAYING HOUSE PERIOD

The laying house period is defined as the interval between ovulation and egg collection. In this period ovulation, fertilization, egg formation, oviposition and egg collection occur. The length of this period will vary between approximately 24 and 32 hours, depending mainly on time between oviposition and collection of the individual eggs.

Breeder flocks are commonly housed on litter floors. When housed in this way, nest boxes are provided, which may be either litter or roll-away type. Timing and frequency of egg collection depends on the availability of labour, type of nest and number of birds per nest. In the Netherlands the first egg collection is usually done when 60-70% of the day's eggs have been produced, approximately 6-7 hours after the start of the photoperiod.

#### *Embryo development*

As fertilization occurs within 15 to 20 minutes of ovulation (Howarth, 1970; Bakst and Howarth, 1977), the embryo has a chronological age of 24 to 26 hours at oviposition. The first cleavage division in the germinal disc is observed some 4 h after ovulation (Perry, 1987). The stage of embryonic development at oviposition influences hatching results. Hays and Nicolaidis (1934) had already observed differences in stages of embryonic development at oviposition and reported that pre-gastrula and early gastrula stages were common in eggs from birds with poor hatching results, while eggs from birds with good hatching results contained embryos at an advanced gastrula stage.

Mather and Laughlin (1979) reported that the blastoderm area in fresh unincubated eggs increased with parental age. When incubated, embryonic development of embryos in eggs laid by older birds was advanced. This is in agreement with results of Crittenden and Bohren (1962) and

Smith and Bohren (1975) who reported that eggs from older birds hatch earlier than those from younger birds.

Several researchers have reported an advanced embryonic development in populations selected for low body weight, in both domestic fowl (McNary et al., 1960; Coleman et al., 1964) and turkey (Weisbroth, 1960; Kosin and Mun, 1965; Arora and Kosin, 1964; Arora and Kosin, 1966b). Turkeys selected for high mature body weight produced eggs which more frequently contained embryos at an early gastrula stage than when selected for low body weight (Arora and Kosin, 1964; Kosin and Arora, 1966). The blastoderm in eggs from turkeys selected for low body weight, however, was smaller (Arora and Kosin, 1966b). When incubated, groups with an advanced developmental stage at oviposition showed a higher rate of development in the first few days. In a similar study, Coleman and Siegel (1966) reported that hens selected for low body weight at 8 weeks of age produced eggs with greater embryonic development at oviposition and an increased hatchability when compared with eggs from hens selected for high body weight. Heating eggs from high body weight selected hens for four hours at  $37.5^{\circ}\text{C}$  to obtain a comparable stage of embryo development to the eggs from low body weight selected hens increased hatchability of these eggs to a comparable level. In another study with the same selection lines, Coleman et al. (1964) reported a higher rate of embryonic development in the first days of incubation for the low body weight selected hens. It was suggested that advanced embryonic development at oviposition is beneficial in helping the embryo to withstand storage (Coleman and Siegel, 1966; Arora and Kosin, 1966a). This was confirmed by Kosin (1956) and Becker and Bearnse (1958) who reported that heating of turkey and chicken eggs prior to storage resulted in an increased hatchability.

Several explanations for the reported differences in embryonic developmental stage might be valid. Changes in inherent rate of growth or development may already appear at an early embryonic stage. This provides a suitable explanation for differences between selected lines, but it does not explain the parental age effect.

McNally and Byerly (1936) suggested that egg weight might be positively correlated with early development of embryos, which may explain the differences between birds of different ages. Coleman et al. (1964) reported a positive correlation within lines of egg weight and embryo development after 42 hours of incubation. However, Arora and Matsumoto (1968) and Mather and Laughlin (1979) reported no significant relationship between egg weight and developmental stage

after 2 days of incubation. Egg weight as an explanation of differences between body weights of selected lines is not likely to be valid, because birds from low body weight selected stocks are reported to produce eggs with embryos in advanced stages and probably produce smaller eggs.

The time spent in the oviduct, determined by length of the oviduct and/or the passage rate of the egg, will influence developmental stage. Bernier et al. (1951) reported that first and last eggs of clutch sequences contained more advanced embryos at oviposition and after a short period of incubation, than intermediate eggs. Sturkie (1986) reported that eggs in the terminal position in the sequence remain in the oviduct for a longer period, which might cause differences in embryonic development at oviposition. Low production rate, arising from bird age, bird condition or selection is characterized by shorter clutches resulting in relatively more terminal and fewer intermediate eggs. On average, embryonic development at oviposition would be expected to be more advanced. Coleman et al. (1964) however, reported that correlation between embryonic development after 48 hours of incubation and position in the clutch sequence is low.

Body temperature of the bird will influence the rate of development and therefore change the developmental stage at oviposition, even if time spent in the oviduct is not influenced. The author is not aware of experiments involving developmental responses in relation to body temperature.

#### *Possible influences of management decisions*

In the housing period, management decisions may influence the hatching results. The temperature history of the egg depends on type of nest, time of oviposition, time and frequency of egg collection and environmental conditions in the house. Fassenko et al. (1991) reported an advanced embryonic development at the moment of egg collection when eggs remained in litter nests for 6 to 7 h compared with egg collection within 1 h after oviposition. Kirk et al. (1980) reported a slightly reduced hatchability when eggs were collected hourly as compared to collection of eggs 5 h after lay and set within 5 days. This might be explained by differences in egg cooling rate and, therefore, embryo development, which is reported to influence hatchability (Coleman and Siegel, 1966). When eggs were stored for 8 days, no differences could be observed (Kirk et al., 1980; Skoglund and Brown, 1956). This was not confirmed by Kosin (1956) and Becker and Barse (1958) who suggested that an advanced developmental stage of the embryo is beneficial when eggs are stored.

The type of nest that is used for egg collection will influence the cooling rate of the egg. Eggs laid in a litter nest are more protected against heat loss than those laid in roll-away nests and, in addition, tend to be kept warm by hens sitting on them. This seems likely to result in a more advanced stage of development being reached by embryos in eggs laid in litter nests compared with roll-away nests. Considering the variation in time of oviposition, it can also be assumed that there will be more variation in embryonic development of eggs laid in litter nests compared with eggs laid in roll-away nests.

Factors during the production period reported to influence hatching results, the possible sources of reported differences and related management factors

factor influencing results	possible source of differences	possible mechanism	related management factor
development at oviposition	embryonic growth rate	genetic difference body temperature	breeding program
	time in oviduct	passage rate oviduct length clutch length	breeder age production level
pre-storage development	embryonic growth	cooling rate	nest type collection rate

## EGG STORAGE

Normally eggs are stored at the farm, transported to the hatchery, stored at the hatchery and then set. Orientation of the egg during storage is normally pointed-end-down, although it is suggested that storage pointed-end-up may be beneficial. A review of the research on this subject is given by Mayes and Takeballi (1984). Duration of the storage period may vary from one day to over a week, depending on distance between farm and hatchery, capacity of the hatchery and market situation. After collection eggs are placed on pulp or plastic trays in containers with open

sides. Eggs used for export are often stored in cardboard boxes. At most farms a separate storage room is used in which temperature and humidity can be more or less controlled.

#### *The embryo during storage*

Pre-incubation storage leads to morphological changes in the blastoderm and malformations in the embryo (Weisbroth and Kosin, 1966; Arora and Kosin, 1966a; Mather and Laughlin, 1979) with increased cell necrosis (Arora and Kosin, 1966b) and to a lower growth rate of the embryo (Singal and Kosin, 1969). Mather and Laughlin (1979) reported shrinkage of the blastoderm during storage and a delay in initiation of development of the embryo after storage.

Hatchability decreases when eggs are stored, dependent on length of the storage period and environmental conditions during storage. The decrease in viability of the embryo may be caused by changes in the embryo and/or by changes in the other contents of the egg.

#### *Length of the storage period*

Numerous studies have shown that fertile eggs can be stored for several days without a major loss in hatchability, when appropriate conditions are maintained. An extensive review of the early research on this subject is given by Kosin (1964), Landauer (1967) and Mayes and Takeballi (1984).

When storage time is extended, hatchability may be reduced by 0.5% per day (Hodgetts, 1981 - cited by Mayes and Takeballi, 1984). It is often suggested that hatchability starts to decline after 7 days of storage (Waite, 1919; Scott, 1933; Funk, 1934; Funk et al., 1950). Tandron et al. (1983), however, reported a decrease in hatchability starting 2-3 days after lay. Hatchability of eggs from older birds decreased more with increasing storage time (Kirk et al., 1980). Furthermore, eggs laid by birds having a low rate of hatch were affected more by increased storage time than eggs from birds in the same flock with a higher rate of hatch (Bohren et al., 1961). This might be explained by differences in embryonic development at oviposition (Hays and Nicolaidis, 1934) which might influence the capability of the embryo to withstand storage (Coleman and Siegel, 1966; Arora and Kosin, 1966a).

Storage causes a delay in the initiation of development (Kaufman, 1939; Arora, 1965; Becker et al., 1968, MacLaury and Insko, 1968; Mather and Laughlin, 1976). Mather and Laughlin (1979)

also reported a decrease in rate of development after storage. These effects explain the increase in hatching time after storage as reported by Bohren et al. (1961). Mather and Laughlin (1976), Becker et al. (1968) and Kirk et al. (1980) indicated that every day of storage added about one hour to the average hatching time.

Length of storage has an influence on embryo quality and chick quality. Mather and Laughlin (1977; 1979) reported an increase in malformed embryos with egg storage time. Byng and Nash (1962) reported that quality of hatching chicks (characterised by percentage of culls) decreased with increased storage time. Becker (1960) and Merritt (1964) reported a reduced growth rate for chickens from stored hatching eggs.

#### *Temperature during storage*

Fertile eggs should be stored at a temperature below "physiological zero" to maintain dormancy of the embryo (Proudfoot and Hulan, 1983). These researchers suggested that for chicken embryos this temperature is 20-21°C, which is in agreement with the earlier results of Edwards (1902). A higher physiological zero for chicken embryos is suggested by Funk and Biellier (1944) and by Lundy (1969) who reported temperatures of approximately 27°C and 25-27°C, respectively. Although dormancy of the embryo is maintained below the physiological zero, morphology of the embryo is not static. Arora and Kosin (1966b) reported a series of recognizable regressive changes in the structure of the blastoderm when eggs were stored at 13°C. Funk and Biellier (1944) reported shrinkage of the blastoderm when eggs were stored at 10°C.

Many authors, as reviewed by Mayes and Takeballi (1984) and Wilson (1991) reported that optimum storage temperature should be decreased with increasing storage time. In general, suggested temperatures are 20-25°C when storing eggs of the domestic fowl for less than 4 days, 16-17°C when storing for 4 to 7 days, and 10-12°C for storage of more than 7 days.

Heating the eggs before incubation affects hatchability. It is concluded that pre-incubation heating of eggs is generally beneficial when the storage period is in excess of 7 days (Mayes and Takeballi, 1984). Heating can be done prior to storage, during storage or prior to setting. Becker and Bearse (1958) showed an improvement in hatchability when eggs were warmed for 1 to 5 h at 37°C following oviposition. Kosin (1956) reported similar results. Lancaster and Jones (1986) reported a decrease in hatchability when heating was done for more than 5 h prior to storage.

Temporary heating of the stored eggs to incubation temperature for 1 h (Kosin, 1956) or for 2-4 h (Nikolaeva, 1958) on a daily basis reduces the decline in hatchability, especially when the storage period is extended. Beneficial effects of heating eggs prior to storage or during storage may be explained by the assumption that an advanced stage of development of the blastoderm is beneficial in helping the embryo to withstand storage (Kosin, 1956).

Heating stored eggs just prior to setting is reported to improve hatchability. Becker and Bearse (1958) and Proudfoot (1966) reported a positive influence on hatchability when eggs were warmed for 18 hours at 23°C or for 1-5 hours at 37.8°C before setting. These authors reported that eggs stored for longer periods benefitted most from the treatment. This is not confirmed in the experiments of Funk and Forward (1960) and McConachie et al. (1960) who reported no beneficial effects after storage for more than 5 days.

#### *Humidity during storage*

During storage, water is lost through evaporation, being influenced by relative humidity (R.H.), temperature and shell porosity. Air movement has a minor effect on moisture loss (Kaltofen, 1969; Spotila et al., 1981).

Many investigators reported a better hatchability when R.H. during storage was maintained at a high level (90% vs. 60-80%) (Cooney, 1943; Funk and Forward, 1951; Proudfoot, 1976). Becker et al. (1968), reported an average weight loss of 0.04 g per 24 h when eggs were stored in the temperature range 11.7 - 13.9°C and R.H. 75%.

Kosin and Konishi (1973) concluded that beneficial effects of enclosing eggs in plastic during extended storage were largely due to a reduction in water vapour loss. Kaufman (1939) concluded from experiments with extended moisture loss by artificial reduction of air pressure that dehydration of eggs is not the main cause for the increase of embryo mortality after prolonged storage. Mayes and Takeballi (1984) concluded that attempts should be made to prevent any weight loss during storage because it increases the total moisture loss between laying and end of incubation and, therefore, affects hatchability. In practise storage at high R.H. is not recommended because condensation on the eggs might occur, thus stimulating bacteria and mould to grow (Sharp and Stewart, 1936).

Hulet et al. (1987) showed that a relative small reduction in total moisture loss (approximately

9.9% compared with 11.0%) significantly reduced hatchability of turkey eggs. Although moisture loss during storage is small compared with total moisture loss during incubation, it might be assumed that moisture loss during incubation should be corrected for the amount of loss during storage to obtain maximal hatchability. The author is not aware of any research on this subject.

#### *Carbon dioxide concentration during storage*

After oviposition carbon dioxide is released from the egg, ultimately resulting in an increase in albumen pH from 7.6 to 9.5 (Kosin and Konishi, 1973; Dawes, 1975). Goodrum et al. (1989) reported that albumen pH of table eggs is increasing more rapidly when eggs are stored at high temperature (38°C versus 4°C). After approximately 6 days, all groups showed a stabilized pH with values of 9.0 (4°C) - 9.5 (38°C). Proudfoot (1965) reported a pH of albumen of approximately 9.4 when eggs were stored for 8 days at 10-12°C. The pH of the yolk remains rather constant at 6.15, with a small increase to 6.25 after 20 days of storage (Proudfoot, 1965). The blastoderm is situated between albumen and yolk and is separated from the albumen by the vitelline membrane (Romanoff and Romanoff, 1949). Therefore, a 1000-fold  $[H^+]$  gradient (3 pH units) may occur across the blastoderm (Stern, 1991). This author discusses the developmental functions of this gradient and estimates values for intracellular pH of 6.9 and sub-embryonic fluid pH of 7.4 after oviposition. Embryonic pH value changes rapidly during the start of the incubation process according to Gillespie and McHanwell (1987). They reported an intra-embryonic pH of 8.3 after 24-60 h of incubation. Sauveur et al. (1967) reported an albumen pH during storage of 8.2 as optimal to maintain hatchability. This suggests an interaction between storage pH and hatchability, which may be related with the developmental responses of the embryo at the beginning of the incubation process.

When eggs are enclosed in plastic bags, loss of carbon dioxide is retarded and albumen pH will stay at a low level (Fletcher et al., 1959; Proudfoot, 1965). Storing eggs in plastic bags improved hatchability, especially when storage time was prolonged (Becker, 1964; Proudfoot, 1964a,b; Warren et al., 1965). Storing eggs with high levels of carbon dioxide did not improve hatchability and when combined with longer storing periods resulted in reduced hatchability (Becker et al. 1964, 1968; Proudfoot 1964b). Kosin and Konishi (1973) replaced the lost carbon dioxide to maintain its concentration in the albumen at the level of newly laid eggs and obtained no improvement in

hatchability. They concluded therefore that the advantage of packing the eggs is more related to differences in water loss. This is in agreement with the results of Reinhart and Hurnik (1982), who reported hatchability after long-term storage at high humidity similar to that of eggs stored in Cryovac (polyvinylidene chloride copolymer) bags.

Becker et al. (1968) showed that lowering the pH-level of the albumen to the level of a fresh laid egg by placing eggs for one hour in CO<sub>2</sub>-enriched air prior to setting did not improve hatchability.

#### *Possible influences of environmental conditions*

Although periods of less than 4 days are preferable for storage of hatching eggs, the market situation and hatchery organisation can demand longer storage periods. The rate of decline in hatchability after storage depends on storage conditions. The functional effect of different storage conditions on embryo and hatchability is not fully understood, but pH levels of albumen and embryo can be relevant factors. It might be suggested that albumen pH should be similar to embryo pH at the start of incubation so as to supply an optimal medium for development. This is supported by the results of Sauveur et al. (1967), who suggested an optimal storage pH of 8.2. This is within the range of the intra-embryonic pH at the beginning of the incubation period as reported by Gillespie and McHanwell (1987).

Albumen pH at the start of the incubation process can be influenced in different ways. Storage temperature influences the loss of carbon dioxide and, therefore, the increase of pH. Goodrum et al. (1989) reported that a positive correlation between albumen pH after 6 days of storage and storage temperature. This might explain the need for low temperature when the storage period is extended, as also the required high temperature during storage for a limited period, as reported by Kaltofen and El-Jack (1972).

After prolonged storage, albumen pH is 9.0 - 9.5, depending on storage temperature (Goodrum et al., 1989). Metabolic activity of cells of the early embryo produces carbon dioxide (Kucera and Raddatz, 1980) which influences the pH of the tissues, thus creating a more suitable environment. Temporary heating of the eggs to incubation temperature during storage or heating the eggs before incubation will induce metabolic activity of the embryo and, therefore, lower the pH in the tissues. This might be a suitable explanation for the beneficial effects of this treatment.

Packing the eggs in plastic will prevent the escape of carbon dioxide, but allows a limited increase in albumen pH in the period between oviposition and packing and after packing until the diffusive balance with the surrounding air is reached. Experiments with supplemental carbon dioxide during storage have aimed at maintaining an albumen pH level close to that of freshly laid eggs. According to the results of Gillespie & McHanwell (1987) and Sauveur et al., (1967) this level is too low to obtain maximum hatching results.

Factors during storage reported to influence hatching results, possible sources of reported differences and relevant management factors during storage

factor influencing results	possible source of differences	possible mechanism	relevant management factor
developmental stage	developmental stage at oviposition		
	temperature treatment	biological activity	pre-storage heating temporally heating
dormancy of the embryo	temperature	biological activity	temperature
pH albumen	temperature	CO <sub>2</sub> solubility	time-temperature
		embryo metabolism	temporally heating pre-setting heating
	carbon dioxide	chemical balance	packaging adding carbon dioxide
obsolescence	temperature	chemical/physical reactions	time-temperature

## CONCLUDING REMARKS

In the period between egg formation and incubation, environmental conditions and storage time can have an influence on hatching results. Much research has been done on the influence of different factors during storage on hatching results. However, the mechanisms that are responsible for the results obtained in the experiments are not clear for every influencing factor. To determine the consequences of management decisions, it is necessary to obtain more knowledge of the mechanisms involved. In particular the relationship between albumen pH during storage and embryo development seems an area that needs further research.

Another interesting research area is the relationship between the time-temperature history of the hatching egg in the nest, as a result of nest type and collection pattern, together with embryo development in this period and capability of the embryo to withstand storage. It might be feasible to adapt storage treatment to the time-temperature history of the eggs.

A third relevant research area is the moisture loss pattern during storage and incubation. If the moisture losses during storage and incubation are interchangeable, an optimum total moisture loss can be obtained by correcting for storage moisture loss during incubation.

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## CHAPTER 2

# MATHEMATICAL MODELLING OF TEMPERATURE AND MOISTURE LOSS OF HATCHING EGGS

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**ABSTRACT**

Analytical equations are formulated to describe the influence of climatic conditions on moisture loss and temperature of hatching eggs. The equations are based on physical properties and are used to calculate moisture loss and temperature of eggs in different situations. Temperature development during cooling of eggs, moisture loss of eggs during cooling or heating and at constant temperature and temperature in the egg during development of the embryo is described. For every characteristic, analytical equations are formulated and practical impacts are discussed.

**INTRODUCTION**

Hatching eggs are exposed to various climatic conditions during production, storage, incubation and hatching. The climatic condition in the respective stages affect embryo development and hatching results. Little is known about the influence of the climate in the nest on hatchability. Kirk et al. (1980) reported a slightly reduced hatchability when collecting eggs hourly instead of every five hours. North (1984: 72) recommended that eggs should be collected at least four times daily to obtain maximum embryo viability and hatchability. Environmental temperature after oviposition influences the rate of embryonic development (Kaplan et al., 1978), especially during early stages (Romanoff, 1939). This early embryonic development will depend on collection pattern, which may affect viability and hatchability of the embryo.

After collection, eggs are often stored for several days prior to setting. Hatchability decreases when eggs are stored for 7 days or more (Funk et al., 1950). Tandron et al. (1983) reported a decrease in hatchability after 2-3 days of storage. To obtain maximum hatchability, optimum storage temperature is reported to decrease with increasing storage time (Kaltofen and El-Jack, 1972; Kirk et al., 1980). Proudfoot (1976) reported that relative humidity during storage should be high to prevent moisture loss. Kaufmann (1939) concluded that dehydration caused by extended moisture loss during storage is not the main cause for decrease in hatchability after prolonged storage.

Temperature and moisture loss during incubation have a major impact on hatching results. In practise, incubator temperature is fixed at 37.5 - 37.8°C. Hatchability and chick quality decreases, hatching time changes and more anomalies occur when incubator temperature is too high or too low (Romanoff, 1960). During incubation, egg temperature increases to a level above the temperature of the surrounding air, due to metabolic activity of the embryo (Tazawa and Rahn, 1987).

A certain amount of moisture evaporates through the shell during incubation. This amount can vary within a batch of eggs. The coefficient of variation of moisture loss during incubation is reported to be as high as 22% for chicken eggs (Visschedijk et al., 1985). Although several experiments (Hoyt, 1979; Simkiss, 1980) have shown that chicken embryos are able to compensate at least partly for suboptimal moisture loss, it is well accepted that there is an optimal range. Ar and Rahn (1980) suggested that 15% of the initial egg weight should be lost as metabolic water. Others (Tullett, 1981; Meir et al., 1984; Hulet et al., 1987) reported an optimum moisture loss of 12%. Meir and Ar (1987) and Hulet et al. (1987) showed that regulating incubator humidity to obtain a specified moisture loss can improve hatchability of turkey eggs.

In the period from oviposition to hatching, eggs are exposed to various environmental conditions. To determine the effects of climatic conditions on the development of the embryo, it is necessary to estimate the effects of macro-climate on micro-climate and therefore on egg content and embryo. In order to estimate these effects, it is necessary to formulate the physical properties of eggs and the physical laws involved with temperature changes and moisture loss of eggs by means of analytical modelling.

In this article, physical aspects of temperature and moisture loss are described. Analytical equations are formulated to calculate temperature development and moisture loss of eggs, as well as temperature in the egg during development of the embryo.

## COOLING AND HEATING OF EGGS

### *Theoretical aspects*

Basically three different factors influence the cooling process of eggs, namely the temperature difference between egg and surrounding air, heatflow through the egg content and heat transfer between egg shell and surrounding air.

The basic shape (plate, cylinder, sphere) that approximates the shape of a hatching egg is the sphere. The governing equations for conducting heat transfer through a homogeneous sphere, initially at a uniform temperature  $T_i$  and surrounded by air of constant temperature  $T_a$ , are (Luikov, 1968: 247):

$$\frac{\partial T}{\partial t} = a \left( \frac{\partial^2 T}{\partial r^2} + \frac{2}{r} \frac{\partial T}{\partial r} \right) \quad (1)$$

with boundary condition:

$$\lambda \left( \frac{\partial T}{\partial t} \right)_{r=R} = \alpha (T_{surrounding} - T_{ambient}) \quad (2)$$

and starting condition (initial temperature of the egg constant):

$$T(r=0) = T_{initial} \quad (3)$$

This set of equations results in the following solution:

$$\theta = \sum_{n=1}^{\infty} \frac{A_n \cdot R \cdot \sin(\mu_n \cdot r/R)}{r \cdot \mu_n} \exp(-\mu_n^2 Fo) \quad (4)$$

The constant  $A_n$  in equation 4 is defined as:

$$A_n = \frac{2(\sin \mu_n - \mu_n \cos \mu_n)}{\mu_n - \sin \mu_n \cos \mu_n} \quad (5)$$

while  $\mu_n$  are the roots of the transcendental equation:

$$\tan(\mu_n) = \frac{-\mu_n}{Bi - i} \quad (6)$$

The dimensionless numbers used in these equations are defined as:

$$Fo = \text{Fourier} = a \cdot t/R^2$$

$$Bi = \text{Biot} = \alpha \cdot R/\lambda$$

$$\theta = \text{theta} = (T - T_{ambien})/(T_{initial} - T_{ambien})$$

With equation 4 it is possible to calculate the temperature as a function of time for every spot in a spherical egg. However, the shape of an egg is more elliptic than spheric. Analytical solutions

to estimate the temperature in an ellipsoid are not known by the authors. Preliminary research (unpublished) indicated that cooling time at the germ position is about 10% longer for a sphere when compared with an egg of identical volume. The difference is about 15% when surface area is identical.

### *Thermal properties*

In order to compare the calculation of the temperature of hatching eggs during cooling with experimental data, the thermal properties of the egg and the convective heat transfer must be estimated. The thermal properties of eggs can be calculated from the chemical composition (Miles et al., 1983). The calculated thermal properties of yolk and albumen as a function of temperature, based on the chemical composition of the egg (Romanoff and Romanoff, 1949: 311) are given in Table 1.

Table 1: Calculated thermal properties of yolk (Y) and albumen (A) based on the chemical composition

temp. °C	conductivity W/(m/K)		spec. heat J/kg/K		density kg/m		diffusivity m <sup>2</sup> /sec	
	Y	A	Y	A	Y	A	Y	A
0	0.37	0.52	3048	3929	1026	1037	1.20 <sup>-7</sup>	1.28 <sup>-7</sup>
10	0.38	0.54	3048	3929	1026	1037	1.23 <sup>-7</sup>	1.32 <sup>-7</sup>
20	0.39	0.55	3048	3929	1024	1035	1.26 <sup>-7</sup>	1.36 <sup>-7</sup>
30	0.40	0.57	3048	3929	1023	1032	1.28 <sup>-7</sup>	1.40 <sup>-7</sup>
40	0.41	0.58	3048	3929	1021	1031	1.31 <sup>-7</sup>	1.44 <sup>-7</sup>

A method to calculate the cooling rate of an egg is given by Luikov (1968: 417), using the analytical solution for cooling of a system of two spherical bodies with different thermal properties. However, the drawback of this method is that the Biot number (the ratio between convection and conduction) is infinite. While the difference in thermal diffusivity of yolk and albumen is relatively small, a more simplified method would be to use the mean value of yolk and albumen and calculate the cooling rate with equation 4. To compare both methods, the dimensionless temperature  $\theta$  of a cooling egg with mean and real thermal properties of yolk and albumen is calculated as a function

of time. To be able to compare the results of both methods, convective heat transfer  $\alpha$  in the calculations with mean thermal properties is set at infinite level. The results of this comparison are shown in Table 2.

Table 2: Dimensionless temperature of a cooling egg with mean and real thermal properties of yolk and albumen

time (min)	dimensionless temperature	
	mean	real
15	0.537	0.561
30	0.224	0.267
31		0.258
32		0.234
33		0.231
34		0.220

This table shows that the difference in time required to reach the dimensionless temperature 0.22 between both analytical solutions is about 10%. Because of this relative small difference and the mentioned drawback, we prefer to calculate cooling rates with equation 4.

#### *Heat transfer coefficient*

The heat transfer coefficient is the most difficult variable to estimate. The relation between the Nusselt number (dimensionless heat transfer) and the Reynolds-number (dimensionless air velocity) is:

$$Nu = 2 + 1.3 Pr^{0.15} + 0.66 Re^{0.5} Pr^{0.33} \quad (7)$$

The dimensionless numbers are defined as:

$$Pr = \frac{\nu}{\alpha_{air}} \quad (8)$$

$$Nu = \frac{\alpha \cdot 2R}{\lambda_{air}} \quad (9)$$

$$Re = \frac{v \cdot 2R}{\nu} \quad (10)$$

Fig. 1 shows the increase of the heat transfer coefficient of different sized spheres (comparable with large, medium and small sized eggs) with increasing air velocity. Although equation 7 is often used to predict the heat transfer coefficient, the measured value can be higher because of buoyancy motion of air in the stagnant air layer around the sphere. The result of the calculations depends largely on the thermal conductivity of air, as is shown in fig. 1.

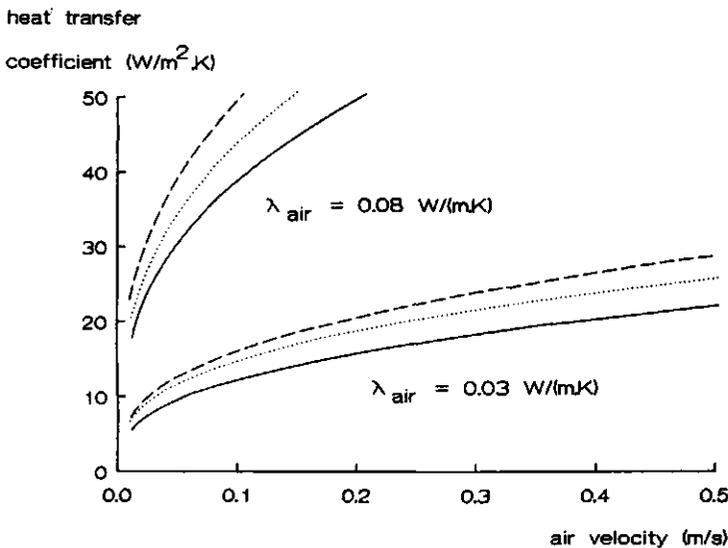


Figure 1: Relation between heat transfer coefficient of different-sized spheres and air velocity at two levels of thermal conductivity of air: (—),  $R = 3.0$  cm; (···),  $R = 2.5$  cm; (---),  $R = 2.0$  cm.

#### Half-cooling time

The half-cooling time, defined as the time needed to cool a body through half of the possible temperature range, is a practical property to compare cooling rates. The first half-cooling time of a spherical body can be calculated with equation 4. The first half-cooling time is longer for the centre of the sphere than for the surface. The difference depends on the thermal characteristics of the

sphere. Fig. 2 shows the calculated temperature development of the centre, surface and germ-position of a spherical egg with a radius of 2.5 cm, being a characteristic dimension for an egg of 55 g. The contribution of the trailing roots in equation 4 is neglectable after the first

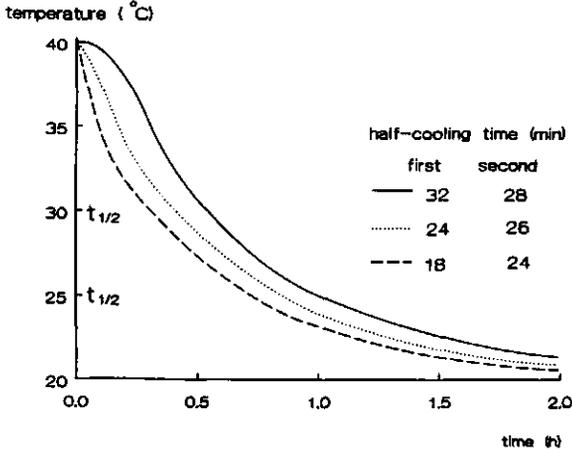


Figure 2: Calculated temperature development at three places in a spherical egg (radius 2.5 cm) with initial temperature of 40°C and ambient temperature of 20°C. (—), in centre  $r = 0$  cm). (...), at germ ( $r = 2$  cm); (- -) at surface ( $r = 2.5$  cm).

half-cooling time, as can be shown with the solution of equation 6. This means that the second and following half-cooling times are equal for all coordinates in the sphere because equation 4 can be transformed to:

$$\ln t = \ln \left( \frac{A_1 \cdot R \cdot \sin(\mu_1 \cdot r/R)}{r \cdot \mu_1} \right) - \mu_1^2 \cdot Fo \quad (11)$$

Equation 11 shows a linear relation between logarithmic temperature and time. The half-cooling time, after the lag period, can be calculated with:

$$t_{1/2} = \frac{R^2 \cdot \ln \cdot 2}{\alpha \cdot \mu_1^2} \quad (12)$$

The intercept values in equation 11 for centre, surface and germ-position of an egg are  $\ln t = 1.29, 0.81$  and  $1$ , respectively, as Fig. 3 shows. To be able to calculate cooling rate with equation

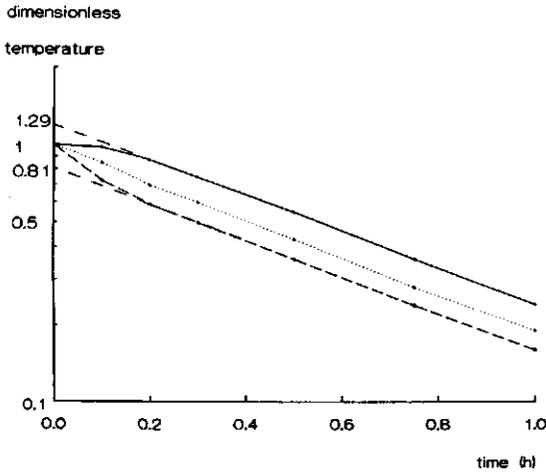


Figure 3: Calculated logarithmic temperature development at three places in a spherical egg during cooling. (—), in centre; (…), at germ; (---), at surface.

11 or 12, the first roots  $\mu_1$  and  $A_1$  as a function of the Biot-number are given in Fig. 4. The Biot-number of a single egg is about  $Bi = \alpha.R/\lambda = 15 \cdot 0.25/0.4 = 1$ , indicating a half-cooling time of 30 minutes at the germ position.

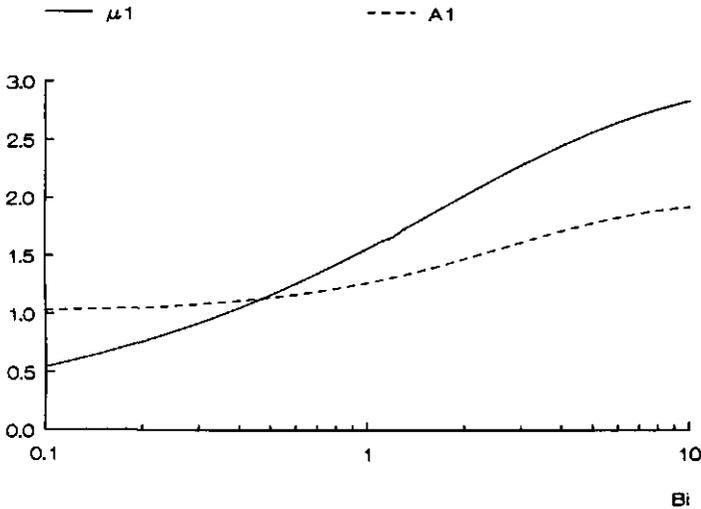


Figure 4: Relation between the Biot-number and the first roots of  $u_1$  and  $A_1$  (based on Luikov, 1968).

### Cooling through evaporation

Moisture loss accelerates the cooling of an egg and retards the heating process while the heat loss through evaporation acts as a cooling source (van Beek and Meffert, 1981). This means that the actual cooling rate of an egg will be higher than the cooling rate calculated with equation 4, if moisture loss appears. To be able to calculate the cooling rate of a sphere with internal heat production, equation 4 can be transformed to:

$$\theta = \frac{Po}{6} \left( 1 - \frac{r^2}{R^2} + \frac{2}{Bi} \right) + \sum_{n=1}^{\infty} \left( 1 - \frac{Po}{\mu_n^2} \right) \cdot \frac{A_n \cdot R \cdot \sin(\mu_n \cdot r/R)}{r \cdot \mu_n} \cdot \exp(-\mu_n^2 \cdot Fo)$$

(13)

The Pomerantsev-number (dimensionless heat production) used in equation 13 is defined as:

$$Po = \frac{q \cdot R^2}{\lambda \cdot (T_{initial} - T_{ambient})}$$

(14)

Moisture loss of eggs is accompanied by heat loss. To estimate the effect of moisture loss on temperature with equation 11, a homogeneous heat generation in the egg must be introduced. This is allowed if the Biot-number is less than 1, which means that temperature distribution in the egg is rather homogeneous.

In Table 3 calculated temperatures after 20 minutes and at infinitive time (5 halftimes or more) in the centre and at the surface are given for a cooling egg (40°C - 20°C) of 60 g with moisture loss of 0 %/hr, 0.01 %/hr and 0.1 %/hr and therefore a heat production of 0 W/m<sup>3</sup>, -70 W/m<sup>3</sup> and -700 W/m<sup>3</sup>, respectively. This table shows that moisture loss of the egg during cooling has a relative small influence on temperature at the surface and in the centre of the egg. This means that moisture loss during cooling and heating can be calculated rather accurate from surface temperature without correction for heat production caused by moisture loss.

Table 3: Temperature of a cooling egg (from 40°C to 20°C) of 60 g with heat production of 0 W/m<sup>3</sup>, -70 W/m<sup>3</sup> and -700 W/m<sup>3</sup> after 20 minutes and after infinite time

time (min)	surface		centre	
	20	∞	20	∞
heat production (W/m <sup>3</sup> )				
0	29.74	20.00	35.17	20.00
-70	29.73	19.97	35.16	19.96
-700	29.62	19.71	35.01	19.56

### MOISTURE LOSS OF EGGS DURING COOLING, HEATING AND AT CONSTANT TEMPERATURE

#### *Theoretical aspects*

Moisture loss of an egg is related to the driving force for moisture loss and the porosity or conductance of the shell. Several properties can be used as driving force: water potential (Pa), water vapour concentration (kg/m<sup>3</sup>), water vapour pressure (Pa) and mol fraction (mol/mol). Theoretically, the best property to use is the mol fraction because the related coefficient of diffusion of water vapour only depends on temperature to the power 0.8 and is independent of pressure (Nobel, 1983). However, using water vapour pressure is more commonly used. Moisture loss of eggs can be described based on surface area, which is theoretically the best, or based on mass, which is more practical, with:

$$J = k_a \cdot A \cdot dp \quad \text{or} \quad J = k_m \cdot m \cdot dp \quad (15)$$

#### *Water vapour pressure*

Evaporation of water is forced by a difference in water vapour pressure between egg and surrounding air. The water vapour pressure of air can be determined using the Mollier diagram. Because of the high water content the water vapour pressure in the egg is nearly saturated. Absolute humidity is related to water vapour concentration by:

$$x = 0.622 \cdot p / (p_{atm} - p) \quad (16)$$

The saturated water vapour pressure only depends on temperature and can be predicted with the equation of Magnus:

$$p_s = \exp \left( 6.414 + \frac{17.26 \cdot T}{(237.2 + T)} \right) \quad (17)$$

#### *Transpiration coefficient*

The transpiration coefficient of eggs can be calculated by determining the weight loss of eggs as a function of time, temperature and relative humidity when sufficient ventilation around the eggs is allowed. When the climatic conditions are known, water vapour pressure deficit can be calculated and transpiration coefficient based on mass can be calculated with:

$$k_m = \frac{dm}{m \cdot dp \cdot dt} \quad (18)$$

The influence of the mass of the egg on the measured transpiration coefficient is theoretically to the power -0.33, if thickness of the shell is constant for every mass of the egg, or +0.33, if mass of the shell is constant for every mass of the egg. The results of Scriba (1987) indicate that shell thickness is relatively independent of egg mass, which means that the relationship should be to the power -0.33. This is in agreement with results obtained in our experiments (unpublished).

To be able to calculate  $k_a$  from  $k_m$ , or reversed, we use the equation of Bonnet and Mongin (1965) to calculate the surface area of an egg from its mass:

$$A = c * (m * 1000)^{0.66} \quad (19)$$

$c = 4.68$ -constant for eggs of 60-70 gram

$c = 4.69$ -constant for eggs > 70 gram

$c = 4.67$ -constant for eggs < 60 gram

#### *Moisture loss of eggs during cooling*

Fig. 5 shows the development of water vapour pressure deficit during cooling for an egg with initial temperature of 30°C in air of 10°C and 60% relative humidity. According to the Magnus-equation (17) the water vapour pressure in the egg is 4244 Pa. The water vapour pressure

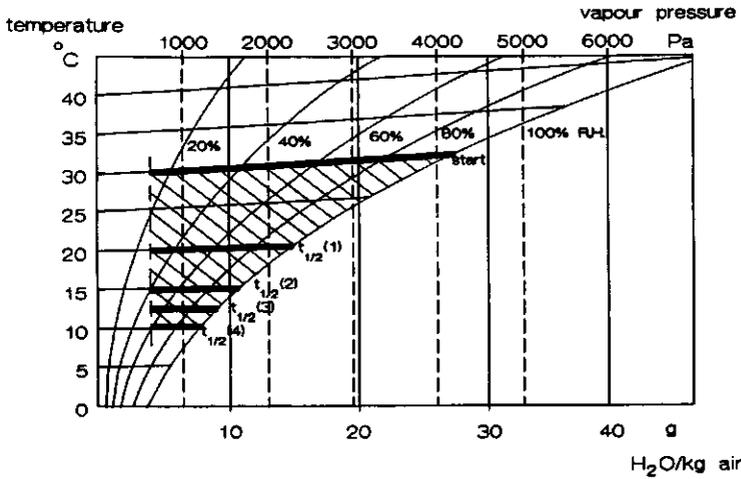


Figure 5: Development of water vapour pressure deficit of an egg during colling from 30°C to 10°C in air with 60% relative humidity.

of the cold air is a fraction, defined by the relative humidity, of the saturated water vapour pressure at 10°C:  $0.6 \cdot 1228 = 736$  Pa. At the start of the cooling process, the water vapour pressure deficit is  $4244 - 736 = 3508$  Pa. After 1 half-cooling time, the temperature of the egg is 20°C and the deficit is  $2338 - 736 = 1602$  Pa. The water vapour pressure deficit reduces during the cooling process to 492 Pa at 10°C after 5 or more halftimes.

*Moisture loss of eggs during heating*

When an egg is heated from 10°C to 30°C, with air of 60% R.H. and 30°C, the total moisture loss is considerably less compared to cooling over the same temperature interval. The water vapour pressure deficit as a function of temperature during heating is shown in Fig. 6. In the beginning water will condensate on the cold surface of the egg, because the water vapour pressure of the warm air,  $0.6 \cdot 4244 = 2546$  Pa, is more then the saturated water vapour pressure of the cold air layer around the egg (at 10°C the saturated water vapour pressure is 1228 Pa). If the temperature of the egg exceeds the dewpoint (21°C) of the surrounding air the condensated water will start to evaporate and moisture loss of the egg will begin. The water vapour pressure deficit will increase during the heating process. At the end of the process, after 5 halftimes, the resulting deficit is  $4244 - 2546 = 1698$  Pa.

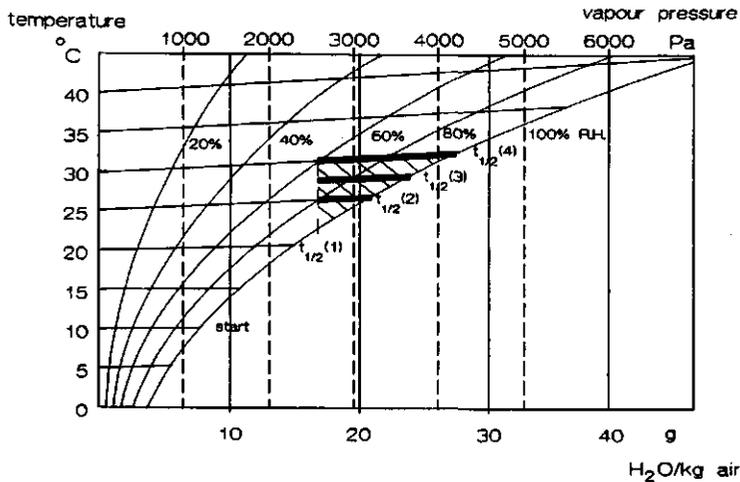


Figure 6: Development of water vapour pressure deficit of an egg during heating from 10°C to 30°C in air with 60% relative humidity.

#### Moisture loss of eggs at constant temperature

Moisture loss of eggs at constant temperature can be calculated directly with eqn (15). Results of our experiments (unpublished data) show good relation with calculations if the transpiration coefficients of the eggs are known.

Knowing by calculation the temperature just under the shell of a hatching egg as a function of time and knowing the development of the water vapour pressure of the air, it is possible to calculate the water vapour pressure deficit as a function of time. Using eqn (15), moisture loss can be calculated as a function of time if the transpiration coefficient or conductance is known.

#### TEMPERATURE IN THE EGG DURING DEVELOPMENT OF THE EMBRYO

During incubation, mean temperature of the embryo is a function of ambient temperature, metabolic heat production and heat loss. Under stationary conditions with high metabolic heat production compared to the latent heat loss, temperature difference between a certain spot in the egg and the surrounding air is described by the first part of eqn (13):

$$\theta = \frac{Po}{6} \left( 1 - \frac{r^2}{R^2} + \frac{2}{Bi} \right) \quad (20)$$

or for the centre of the egg:

$$T_{centre} - T_{air} = \frac{q R^2}{6 \lambda} \cdot \left(1 + \frac{2 \lambda}{\alpha R}\right) \tag{21}$$

The latent heat loss caused by moisture loss can not be incorporated in equation 21. The influence of the evaporation on the centre temperature of the egg can be described using the heat balance: latent heat=heat of convection by equation 21. This will be a negative difference.

$$T_{centre} - T_{air} = \frac{-ka * dp * h}{\alpha} \tag{22}$$

The theoretical relationship between size of the egg, heat production and temperature difference between egg and surrounding air at two different levels of air velocity is shown in Fig. 7 and 8. The calculations are based on the situation in an incubator, with air temperature of 37.5°C. In the calculations it is assumed that thermal conductivity of the egg increases from 0.5 W/(m.K) to 50 W/(m.K) with increasing heat production, because of the increasing blood flow in a developing embryo. The figures show that the relationship between egg size and temperature difference

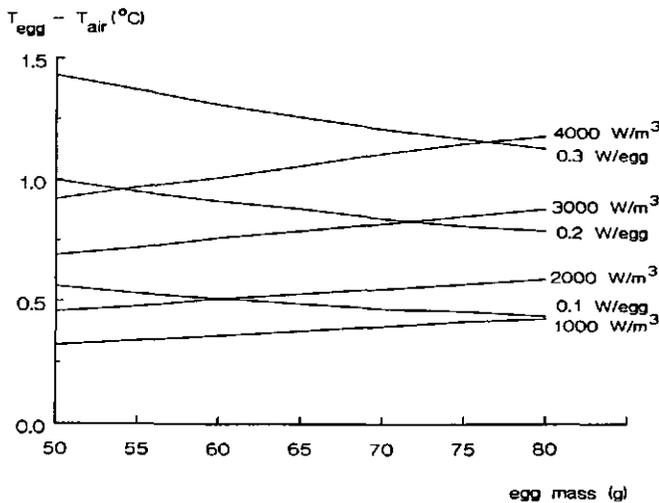


Figure 7: Calculated temperature difference between egg (germ position) and surrounding air at different levels of heat production and air velocity of 2 m sec<sup>-1</sup>.

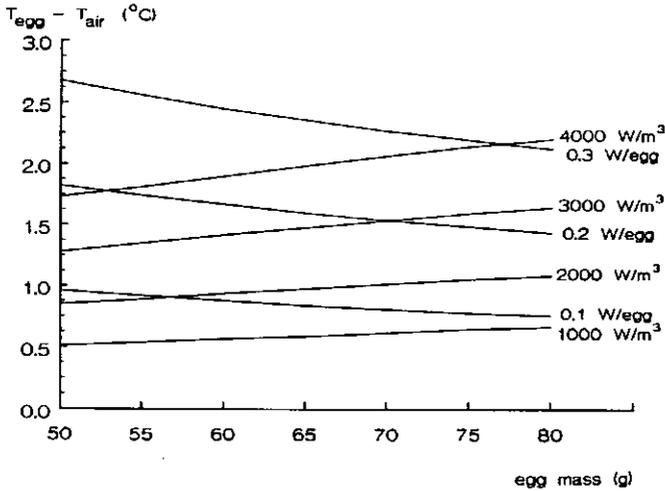


Figure 8: Calculated temperature difference between egg (germ position) and surrounding air at different levels of heat production and air velocity of  $0.5 \text{ m sec}^{-1}$ .

between developing embryo and surrounding air depends on the property of the heat production  $q$ . If heat production per gram egg is independent of egg size, as is suggested by Ar and Rahn (1978), temperature difference will increase with increasing egg mass. If heat production per egg is independent of egg size, temperature difference will decrease with increasing egg mass.

Because of the relationship between heat transfer coefficient and air velocity, as described earlier, the influence of air velocity on temperature differences is relatively high. This means that differences in air velocity in the incubator can cause differences in embryonic temperature and therefore in embryonic development within batches of eggs.

## DISCUSSION

### *Temperature*

Sotherland et al. (1987) showed that the temperature of an egg can be calculated rather accurately based on its thermal properties. These authors also showed that the heat transfer calculated for spheres and measured at eggs match well. However, the method presented by Sotherland et al. (1987) does not account for heat convection in the egg. With the method presented here, the temperature at every spot in the egg can be calculated as a function of time during cooling and heating. The results of this method showed that the influence of the heat convection through the egg on the cooling process is rather small, because the radius of hen's eggs is rather small and

the biot number of eggs is close to 1. Only at the beginning of the cooling process, a difference in cooling rate can be expected at different spots in the egg. After a small time lag, the cooling rate at every spot will be identical.

The influence of the air velocity on the cooling rate of eggs is high because the heat transfer coefficient depends on air velocity. The influence of the air velocity depends on the size of the egg. This is in agreement with the calculations and measurements of Sotherland et al. (1987).

The influence of moisture loss on the cooling rate is small. Even a high rate of moisture loss of 0.1% per h will cause a temperature difference of less than 0.5°C when compared with a situation without moisture loss.

#### *Moisture loss*

Moisture loss of eggs is often predicted with Ficks's first law of diffusion (Ar et al., 1974). This method is based on egg shell conductance and water vapour pressure deficit across the shell. The method presented here is based on the same principle but calculations are made with different properties. Instead of egg shell conductance, expressed as milligrams of water per torr per day, we prefer to use a transpiration coefficient, based on SI units, which allows to use the Mollier diagram to determine water vapour pressure deficits under different circumstances. This facilitates calculation of moisture loss during cooling and heating.

#### *Internal heat production*

It is well known that embryo development causes heat production in the latter part of the incubation process (Tullett, 1990). Due to this metabolic heat production, embryo temperature rises above air temperature (Sotherland et al., 1987). Because of the susceptibility of the embryo development for temperature, it can be questioned if the setter temperature should be lowered during the latter part of the incubation process to retain the embryo temperature at a constant level (Tullett, 1990).

However, because of the dependency of the cooling rate of eggs on air velocity, it can be questioned if embryo temperature is relatively constant between eggs at different places in an incubator. Experiments concerning air velocity in incubators in relation to embryo temperature are not at the authors knowledge. The design of modern incubators, however, will probably cause a great variety in air velocity over eggs at different spots in the incubator, as is confirmed by our own

measurements (unpublished) and by Owen (1991). Based on the calculations presented, it can be assumed that uni-directional flow of air through the incubator is beneficial for uniformity in development and hatching.

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Symbol	Unit	Property
T	°C	temperature
t	s	time
$a$	$m^2/s$	thermal diffusivity
$\lambda$	W/(m.K)	thermal conductivity
$\rho$	$kg/m^3$	density
c	J/(kg.K)	specific heat
$\alpha$	W/( $m^2$ .K)	convective heat transfer coefficient
v	m/s	air velocity
$\nu$	$m^2/s$	kinematic viscosity of air
x	kg/kg	absolute humidity of air
p	Pa	partial water vapour pressure
dp	Pa	vapour pressure deficit
$p_{atm}$	Pa	total pressure of air
$p_s$	Pa	saturation water vapour pressure
$k_m$	Kg/(kg.Pa.s)	transpiration coefficient based on mass
$k_a$	kg/kg( $m^2$ .Pa.s)	transpiration coefficient based on surface
m	kg	mass of egg
A	$cm^2$	surface of egg
R	m	radius of sphere
r	m	radial distance from center
q	W/ $m^3$	heat production
h	J/kg	latent heat of evaporation
J	kg/s	moisture loss

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CHAPTER 3

TEMPERATURE AND MOISTURE LOSS OF HATCHING EGGS PRODUCED IN  
TWO TYPES OF LAYING NESTS

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**ABSTRACT**

Two experiments were conducted to investigate the temperature and moisture loss of eggs in different types of laying nests. The objectives of this study were to determine the influence of type of nest on egg temperature and moisture loss of eggs and to compare the experimental results of the measurements with theoretical calculations.

The cooling of eggs imbedded in litter took about twice as long as that of eggs produced in roll-away nests or of eggs lying on litter. If birds had access to the nests, the average egg surface temperature in the litter nests was constantly above the temperature at which dormancy of the embryo occurs.

Moisture loss was higher in litter nests than in roll-away nests because of the average high egg surface temperature. The amount of moisture lost during the period in the laying nests was relatively small compared with the total amount of moisture lost during incubation.

**INTRODUCTION**

For broiler breeders traditionally litter nests are used with wood shavings, oat hulls or chopped straw as nesting material. In these nests eggs are more or less protected against heat loss and therefore a relatively slow cooling process of the produced eggs can be expected. More recently roll-away nests were introduced, in which eggs are not protected against heat loss by the nesting material and will experience a higher cooling rate. Also differences in moisture loss can be expected between different types of nests, due to differences in temperature.

Temperature of hatching eggs between oviposition and storage is reported to influence hatching results. A "physiological zero", defined as the minimum temperature for embryo development, of 25-27°C has been reported by Funk and Biellier (1944) and Lundy (1969). Proudfoot and Hulan (1983) suggested that chicken eggs should not be stored above 20-21°C to maintain dormancy of the embryo. However, short-term heating of the eggs before storage has been reported to be beneficial for hatching results. Becker and Bearnse (1958) and Kosin (1956) showed an improvement in hatchability when eggs were heated for 1 to 5 h at 37°C following oviposition. Lancaster and Jones (1986) reported a decrease in hatchability when pre-warming was done for more than 5 h. Middelkoop (1972), however, reported no negative effects on hatchability when eggs were stored at 41°C for 10.5 h directly following oviposition. Coleman and Siegel (1966)

reported data suggesting that beneficial effects of pre-storage heating can be expected when blastoderms are in an early embryonic stage at oviposition.

Moisture loss before pipping is reported to influence hatchability (Tullet, 1981; Meir et al., 1984). Christensen and McCorkle (1982) and Meir and Ar (1987) reported that regulating incubator humidity to obtain a moisture loss between setting and pipping of 12% of the initial egg weight improved hatchability. In these experiments moisture loss before and during storage was not incorporated in the total moisture loss. If moisture loss in one of these periods is high, the effect on total moisture loss can become relatively important.

The objectives of this study were (1) to determine the influence of the type of nest on the temperature pattern and on the moisture loss of eggs and (2) to compare the results of the measurements with theoretical calculations.

## MATERIALS AND METHODS

### *Theoretical calculations*

Halfcooling time, defined as the time required to reduce the temperature difference between object and cooling medium to halve of the initial difference, is often used to quantify a cooling process. The halfcooling time of a sphere can be calculated when the radius, the convective heat transfer and the thermal conductivity is known. Calculation of the surface temperature of a cooling egg, imbedded in litter as well as surrounded by air is done with analytical equations for a sphere, described by Luikov (1968). The relation between surface temperature and internal temperature of eggs is described by Meijerhof and van Beek (1993). For eggs surrounded by air the heat transfer coefficient, which depends largely on the air velocity, is calculated with a Nusselt-Reynoldts-Prandtl relation given by Bird et al. (1960). The approach for calculations for eggs in litter holds for infinitive heat transfer between litter and surroundings, so is only valid for relatively thick layers of litter around the cooling egg. The calculations are based on an equal initial temperature of litter and egg. However, litter temperature changes fast to air temperature because of the low heat capacity of the litter.

The effective heat transfer coefficient in a layer of nesting material can be calculated with equation 1 when the thermal conductivity of the material is known.

$$\alpha = \frac{\lambda}{x} \quad (1)$$

Moisture loss is related to the driving force for moisture loss and the porosity of the shell (Fick's first law of diffusion; Ar et al., 1974) and can be calculated with the differential equation 2, based on the surface of the egg:

$$\frac{\partial m}{\partial t} = -K_a \cdot A \cdot (P_{object} - P_{ambient}) \quad (2)$$

or with equation 3, based on the mass of the egg:

$$\frac{\partial m}{\partial t} = -K_m \cdot m \cdot (P_{object} - P_{ambient}) \quad (3)$$

which is more practical and commonly used (Meijerhof and van Beek, 1993). With these methods moisture loss of uncovered eggs can be calculated. The water vapour pressure deficit can be calculated from the temperature of the egg and the water vapour pressure of the surrounding air.

It can be assumed that in a litter nest a boundary layer of stagnant air is formed around the egg. The relative humidity in this layer will be increased because of the constant release of moisture from the egg shell. To be able to calculate moisture loss of eggs that are partly imbedded in litter, it is necessary to introduce a correction for the protection against moisture loss by the litter. This correction factor B is defined as:

$$B = \frac{\text{moisture loss protected egg}}{\text{moisture loss unprotected egg}} \quad (4)$$

#### *Experiment 1*

This experiment was conducted in a climatic chamber with a constant temperature of 22°C and relative humidity of 55%. The average air velocity in the compartment was about 0.2 m/s.

Individual laying nests of roll-away type and litter type were placed in the chamber. Each type of nest had a specific type of bedding material or nest bottom. The materials used were:

- wood shavings
- chopped straw
- oat hulls
- artificial grass (Astroturf, type HPNP5, AstroTurf Industries, Monsanto Europe N.V., Brussel, Belgium)
- jute egg belt

The bedding material was used in a layer of approximately 10 cm thickness. Eggs (weight range 57-65 g, no cracks) were placed halfway in the layer of litter (defined as: in the litter) or gently on the surface of the litter (defined as: on the litter). The eggs that were placed on the artificial grass were placed in the middle of the astroturf. On the egg belt, eggs were placed in the middle or against a vertical side of the nest. Eggs were warmed in a stove to a temperature of approximately 41°C. After warming, eggs were weighed and placed in the nests.

Surface temperature was measured by sticking a calibrated flat K-thermocouple on the surface of the egg at the longitudinal side. The thermocouple was placed at the top side of the egg and therefore not imbedded in the litter or in contact with astroturf or egg belt. The thermocouple wire was attached to the surface of the eggs over half of the radius to minimize deviation by conduction of heat through the wires. The contact potential of the thermocouple was measured with an electronic compensation circuit, the "wheatstone bridge" (Cameron 1986), to be able to measure without electric current. The deviation of the temperature measured by this method is less than 0.1°C (ASHRAE, 1972). The thermocouples were connected with a printer and temperature was recorded constantly. When the cooling experiment was completed, eggs were weighed again. For every type of nest 2 eggs with a known transpiration coefficient were used to determine cooling rate and moisture loss. At least 3 halfcooling times were established for one cooling experiment.

The measured moisture loss of the eggs in the experimental situations is calculated towards a moisture loss after four half cooling times and after a fixed period of 360 minutes. In these calculations all eggs were standardised towards an egg weight of 65 g and a specific moisture loss of  $0.2 \cdot 10^{-10}$  kg/(kg.Pa.s) to be able to compare the results obtained in the experimental situations. Calculations were done by the methods described by Meijerhof and van Beek (1993). To calculate

the average water vapour pressure of the air during the experiment the weight loss of five eggs with a known transpiration coefficient was measured as a function of time.

To determine the actual (momentane) halfcooling time in this experiment, surface temperature was measured as a function of time and halfcooling time was calculated with equation 5.

$$t_{0.5} = \ln 2 \cdot \frac{(T_{\text{ambient}} - T_{\text{object}})}{(\partial T / \partial t)} \quad (5)$$

To calculate the protection factor B, eggs with a known transpiration coefficient and a temperature of 22°C were placed in the nests for several hours and weight loss was determined. Two measurements were done for every experimental situation.

### *Experiment 2*

Temperature development of eggs in litter nests was measured in a flock of Ross broiler breeders. 100 females and 12 males were housed in a floor pen of 4.35 x 4.70 m with a room temperature of approximately 12°C. Artificial lighting was used from 05.00 am until 21.00 pm. 24 individual litter nests were available. The bottom of the nests was covered with a layer of approximately 5 cm of wood shavings. During the experiment, birds were 48 weeks of age with a rate of lay of approximately 60%. At 05.00 am, all eggs were collected from the nests. From this moment, every first egg produced in an individual nest was collected immediately, a temperature detector was attached on the surface of the egg and the egg was directly replaced in the original nest. The eggs were left in the nests until 13.30 h, at what time temperature recording was stopped.

Temperature development of the eggs was recorded with a MRL 244 recorder, using 12 calibrated YSI model 427 temperature detectors (Esterline Angus, Indianapolis, USA). Temperature of the individual detectors was recorded every minute. A temperature detector was attached on longitudinal side of the egg with a small piece of tape, using thermal paste to ensure good heat conductivity. At least 2 cm of wire was led over the surface of the egg to prevent heat loss through the wire. Together with the temperature detector, an egg with a known transpiration coefficient was placed in the nest, and weight loss during the temperature recording period was determined.

## RESULTS

*Experiment 1*

In table 1 the temperature development and moisture loss of the eggs in the experimental situations is given. The moisture loss is presented as percentage of the initial egg weight and as absolute moisture loss.

Table 1: Halfcooling time ( $t_{0.5}$ ) in minutes (surface temperature), moisture loss (dm) after four half-cooling times and after 360 minutes and degree of protection (B) against moisture loss at constant temperature of eggs placed in different types of laying nests. Moisture loss standardised to an egg weight of 65 g, specific moisture loss of  $0.2 \cdot 10^{-10}$  kg/(kg.Pa.s), initial temperature of 40°C and air of 22°C and 55% R.H..

type of nest	$t_{0.5}$ (min)	m after $4 \cdot t_{0.5}$		m after 360 min		B
		g	% of egg weight	g	% of egg weight	
roll away nest with						
artificial grass	26.6	0.019	0.030	0.044	0.068	1.00
jute egg belt	25.0	0.019	0.029	0.043	0.066	1.01
jute egg belt, egg						
against side of nest	26.3	0.019	0.029	0.043	0.066	1.03
litter nest, egg						
in wood shavings	64.4	0.045	0.070	0.055	0.085	1.08
on wood shavings	33.7	0.024	0.037	0.044	0.067	1.08
in short straw	66.7	0.047	0.073	0.056	0.086	1.08
on short straw	32.9	0.023	0.035	0.043	0.066	1.09
in oat husks	57.5	0.042	0.064	0.054	0.083	1.06
on oat husks	30.3	0.022	0.033	0.043	0.067	1.05

The halfcooling time of the surface of an egg placed in a roll-away nest is about 26 minutes, with little difference between astroturf and jute egg belt as bottom. When the egg is placed gently on the litter, the half cooling time is about 32 minutes. When the egg is imbedded halfway in the litter, the half cooling time increases to 63 minutes.

The validity of these results can be estimated by calculation of the theoretical halfcooling time. The theoretical halfcooling time at the surface of a sphere (radius 2.5 cm, thermal properties of an

egg) surrounded by air with a velocity of 0.2 m/s (heat transfer coefficient  $11 \text{ W/m}^2\cdot\text{K}$ ) is 30 minutes (Meijerhof and van Beek, 1993). The theoretical halfcooling time of a sphere half enhanced in litter can be calculated with the mean heat transfer coefficient of air and litter. The thermal conductivity of the litter can be calculated from the chemical composition and the structure of the material (McAdams, 1954) or empirically determined and is about  $0.07 \text{ W/m}\cdot\text{K}$  for wood shavings, resulting in a heat transfer coefficient of  $0.7 \text{ W/(m}^2\cdot\text{K)}$  (thickness of layer of litter 10 cm) and a mean heat transfer coefficient for a sphere half enhanced in litter of  $(11 + 0.7)/2 = 5.9 \text{ W/(m}^2\cdot\text{K)}$ . This results in a halfcooling time of 54 min for the surface temperature, which is in accordance with the measurements.

During the cooling process the temperature at the germ position is not equal to the surface temperature. Calculations (Meijerhof and van Beek, 1993) showed that theoretically the first half cooling time of a sphere (radius 2.5 cm, thermal properties of an egg) is 18 minutes at the surface and 24 minutes at the germ position. The second half cooling time is 24 and 26 minutes, respectively.

Table 1 shows that during the cooling process eggs imbedded in litter loose about twice as much moisture during the cooling process than eggs placed on the litter or on the bottom of roll-away nests. This difference is mainly caused by the difference in cooling rate, which is expressed as difference in half cooling time. Moisture loss is high at the beginning of the cooling process, due to a high water vapour pressure deficit, as explained by Meijerhof and van Beek (1993).

Part of the difference in moisture loss between the types of nest can be explained by the protection of the nests against moisture loss. The table shows that eggs produced in roll-away nests are less protected against moisture loss than eggs produced in litter nests. This means that eggs produced in roll-away nests loose more moisture than eggs in litter nests, when the cooling process is completed. The reduction of moisture loss of eggs in nests is less than 10% compared with uncovered eggs when temperature is constant. Eggs in litter have lost approximately 25% more moisture than eggs in roll-away nests or eggs on the litter at 360 minutes after the start of the cooling process.

### Experiment 2

In figure 1 the average ( $n=12$ ) egg surface temperature recorded in the litter nests is presented. The figure shows that the mean temperature of the eggs in the nests varied between 30°C and 35°C during the first 6 hours of the light period. This is due to warming of the eggs by the birds visiting the nests. The variation in egg surface temperature indicates that nests were not constantly visited by the birds. The average egg temperature decreased with time. This is probably caused by the decreasing number of nest visits. During the last part of the recording period the average egg surface temperature remained between 25°C and 30°C.

The weight loss of the eggs with a known transpiration coefficient indicated that during the experimental period an average water vapour pressure deficit of 1910 Pa occurred. Calculations as presented for experiment 1 show that eggs produced in litter nests visited by birds (average nest temperature of 30°C, average R.H. in the nest of 55%, water vapour pressure deficit 1910 Pa, egg weight 65 g, specific moisture loss of eggs  $0.2 \cdot 10^{-10}$  kg/(kg.Pa.s)) an average moisture loss of 0.008 g or 0.012% per hour can be expected. The protection of the nests against moisture loss is not included in these calculations, because it is imbedded in the estimation of the water vapour pressure deficit. Calculation of moisture loss of eggs produced in roll-away nests under comparable conditions (20°C, 55% R.H., egg weight 65g, specific moisture loss  $0.2 \cdot 10^{-10}$  kg/(kg.Pa.s)) indicate a moisture loss of 0.005 g or 0.008% per hour once the cooling process is completed.

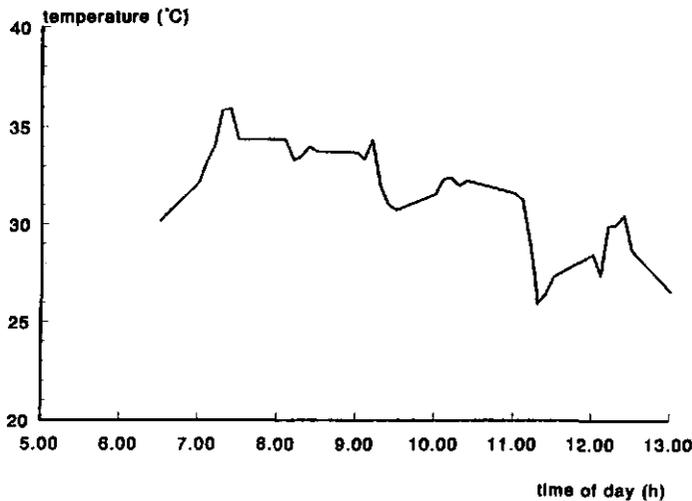


Figure 1: Average surface temperature of eggs ( $n=12$ ) produced in litter nests with birds having access to the nests

## DISCUSSION

The results of the study presented indicate that eggs produced in litter nests experience a high environmental temperature between oviposition and collection. This is partly due to the differences in cooling rate, which is twice as long for eggs imbedded in litter nests as for eggs in roll-away nests. The main reason for the high temperature, however, is the warming of the eggs by the birds in the nest. Even during low environmental temperatures, as shown in experiment 2, the average surface temperature of eggs in litter nests remains above the reported temperature at which chicken embryos remain in dormancy. This is confirmed by the experiments of Fasenko et al. (1991, 1992a), who reported that embryos of eggs that remained in litter nests for 6 to 7 hours were significantly more developed than embryos in eggs that remained in the nests for 0 to 1.5 hour.

Eggs produced in roll-away nests experience a cooling rate that is approximately twice as high as eggs imbedded in litter nests and cannot be warmed by the birds once they are produced. This means that variability of temperature development and therefore embryonic development in eggs produced early in the morning or just before collection in roll-away nests will be less than in eggs produced in the same period in litter nests.

North (1984) stated that eggs should be collected at least four times daily for optimum embryo viability and therefore hatchability. Kirk et al. (1980), however, reported a slightly reduced hatchability when eggs were collected hourly compared with collection five hours after lay and setting within five days. They concluded that this was probably caused by differences in cooling rate. This suggests that the temperature development of the egg in the nest and therefore the embryonic stage influences hatchability. Fasenko et al. (1992a), however, reported no differences in hatchability and viability of eggs left for 6 to 7 hours in litter nests under high environmental temperatures compared with collection within 1.5 hours after oviposition, although embryo's were significantly more developed when the eggs were left in the nest.

The embryonic stage at oviposition is related with several aspects as age of the birds (Mather and Laughlin, 1979; Fasenko et al., 1992b), hatching record of the individual hens (Hays and Nicolaidis, 1934), genotype (Coleman et al., 1964; Kosin and Arora, 1966) and rank of the egg in the sequence (Bernier et al., 1951; Fasenko et al., 1992b). Experimental results suggest that effects of pre-storage environmental temperatures on hatchability may interact with egg and embryo

characteristics.

The results of the study presented indicate that surface temperature development of eggs produced in litter nests without birds having access to the nests and in roll away nests can be calculated rather accurately with mathematical equations as described by Meijerhof and van Beek (1993). When birds have access to litter nests, calculation of the temperature development is difficult and less accurate because of the variability in number and duration of nest visits and therefore warming of the eggs.

Moisture loss between oviposition and collection is higher for eggs in litter nests than in roll-away nests. When the eggs are not warmed by the birds, the total moisture loss per half-cooling time is about twice as high for eggs produced in litter nests than in roll-away nests, due to a slow cooling process resulting in a high water vapour pressure deficit for a longer period of time. When the cooling process is completed, eggs in litter nests experience a slightly lower moisture loss, because of protection of the nesting material. As a result the total moisture loss during the first five hours after oviposition is approximately 25% higher for eggs produced in litter nests than in roll-away nests, when the nests are not visited by the birds. Visiting of the nests by the birds results in a high egg temperature and therefore an increased average water vapour pressure deficit and moisture loss. In this situation the total moisture loss is about 50% higher for eggs produced in litter nests than in roll-away nests. In roll-away nests some air movement over the eggs may occur. This does not influence the moisture loss, as is demonstrated by Kaltofen (1969) and Spotila et al. (1981).

During incubation weight loss of the egg occurs, which can be completely explained by the loss of water (Ar and Rahn, 1980). Christensen and McCorkle (1982a), Meir et al. (1984) and Hulet et al. (1987) reported an optimum water loss between setting and external pipping of about 12% for eggs from chicken, duck and turkey. Christensen and McCorkle (1982b) and Meir and Ar (1987) demonstrated that hatchability can be significantly reduced when water loss is above or below the prescribed amount.

A high level of R.H. during storage increases hatchability when compared with a low level (Cooney, 1943; Funk and Forward, 1951 and Proudfoot, 1976), probably due to a limited water loss. Mayes and Takeballi (1984) concluded that attempts should be made to prevent any weight loss during storage because it increases the total moisture loss between laying and pipping and,

therefore, affects hatchability. Meijerhof (1992) suggested that compensation during the incubation process for the water lost during storage might be beneficial for hatching results.

The results of the experiments presented indicate that eggs produced in litter nests lose more moisture than eggs produced in roll-away nests. However, the total moisture loss during the nesting period is relatively small when compared with the total moisture loss during the incubation period. In this experiment moisture loss of eggs produced in litter nests which were visited by the birds and collected eight hours after oviposition was approximately 0.1% of the egg weight or 0.8% of the suggested optimum total moisture loss during incubation. It is unlikely that hatching results will be influenced by the differences in moisture loss that occur in different types of laying nests. It seems also unlikely that correction during incubation for the amount of moisture lost in the nesting period will be beneficial for hatching results.

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Symbol	Unit	Property
$\alpha$	W/(m <sup>2</sup> .K)	heat transfer coefficient
$\lambda$	W/(m.K)	thermal conductivity
x	m	thickness of the layer
k <sub>a</sub>	kg/(m <sup>2</sup> .Pa.s)	transpiration coefficient
k <sub>m</sub>	kg/(kg.Pa.s)	transpiration coefficient
A	m <sup>2</sup>	surface
m	kg	mass
p	Pa	water vapour pressure
T	°C	temperature
t	s	time

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## CHAPTER 4

# TEMPERATURE DEVELOPMENT AND MOISTURE LOSS OF HATCHING EGGS DURING STORAGE

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## ABSTRACT

An experiment was conducted to investigate the influence of storage method and storage treatment on temperature development and moisture loss of hatching eggs. Eggs were stored on cardboard trays, plastic trays or incubator trays and placed at two levels of air velocity or under plastic coverages at high air velocity. Eggs were warmed from 15°C to 25°C or cooled from 25°C to 15°C. Results were compared with calculations based on analytical equations. It was demonstrated that warming takes more time than cooling when air velocity is low. At high air velocity or when coverages are used, no clear distinction between time needed for cooling and time needed for warming could be made. Eggs placed in side position in the containers experience an increased cooling and warming rate when compared with eggs placed in centre positions. Eggs placed on cardboard trays and stored under low air velocity conditions experience a slow temperature development when compared with eggs placed on plastic trays or incubator trays or with eggs stored under high air velocity conditions. Moisture loss of eggs during the first 48 h of temperature development was slightly decreased when coverages were used. For all storage methods, the observed moisture loss was lower than expected based on temperature development of the eggs and climatic conditions during storage. Analytical calculations based on thermal conductivity and convective heat transfer could not be made accurate enough to estimate the temperature development for every spot in the container, probably due to the presence of natural convection in the container.

## INTRODUCTION

Storage time, storage temperature and moisture loss during storage are reported to influence hatchability of hatching eggs (for review, see Meijerhof, 1992). In general, hatchability decreases when storage periods are extended beyond 7 days, especially with increased storage temperature. According to Proudfoot and Hulan (1984), storage temperature should not exceed 20-21°C to maintain dormancy of the embryo. Kaltofen and El Jack (1972) and Kirk et al. (1980) reported a negative correlation between storage time and optimum storage temperature.

During storage, a constant moisture loss of the eggs occurs. Becker et al. (1968) reported that eggs stored in the temperature range 11.7°C-13.9°C and at relative humidity of 85% lost on average 0.04g per 24 h of storage. The level of moisture loss is directly related with conductance of the

eggshell, temperature and relative humidity. Proudfoot (1976) reported that reduction of moisture loss by increasing relative humidity during storage had beneficial effects on hatchability. Mayes and Takeballi (1984) concluded that moisture loss during storage should be prevented for optimum hatching results.

Temperature development and moisture loss of eggs during storage is influenced by storage method, storage duration and climatic conditions during storage. Commercially hatching eggs are collected at least once a day and normally stored for a period less than a week. Storage is done in containers, with eggs placed on cardboard trays, plastic trays or incubator trays. Occasionally eggs are stored in cardboard boxes. After storage, eggs are transported to the hatchery and set immediately or after a short storage period. During transportation, plastic coverages are sometimes used to protect eggs against environmental changes.

The purpose of this study was to determine the temperature development and moisture loss of eggs in containers with and without coverage and to compare the results with theoretical calculations.

## MATERIAL AND METHODS

### *Theoretical calculation*

For theoretical calculation of the cooling and heating process three units can be distinguished: the complete container, a single drawer and a stack of six trays. The analytical method to calculate temperature development has been explained by Meijerhof and van Beek (1993), for calculation of temperature development in spherical bodies. To calculate the rate of cooling or heating, the following equations (Luikov, 1964) can be used for rectangled bodies:

$$\frac{T - T_{\text{ambient}}}{T_{\text{initial}} - T_{\text{ambient}}} = \frac{PO}{2} \left( 1 - \frac{x^2}{X^2} + \frac{2}{Bi} \right) + \sum_{n=1}^{\infty} \left( 1 - \frac{PO}{\mu_n^2} \right) \cdot A_n \cdot \exp(-\mu_n^2 FO)$$

(1)

where:

$$A_n = \frac{2 \sin(\mu_n)}{\mu_n + \sin(\mu_n) \cdot \cos(\mu_n)}$$

(2)

The dimensionless numbers used in these equations are:

$$Bi = \text{Biot} = \alpha \cdot X / \lambda \quad (3)$$

$$Fo = \text{Fourier} = (\lambda / (p \cdot c)) \cdot (t / X^2) \quad (4)$$

$$Po = \text{Pomeratsev} = \frac{q \cdot X^2}{\lambda \cdot (T_{\text{initial}} - T_{\text{ambient}})} \quad (5)$$

### Experiment

An experiment was conducted in a climatic chamber at air temperature of 15°C or 25°C and relative humidity of 70%. Every temperature setting was used for 48 h, after which the temperature was changed to the opposite set point. After changing of the set point, room temperature reached set point level during warming experiments within 2 h. During cooling experiments, set point levels were reached in 5-10 h. Half times of eggs during cooling and heating were calculated with equation 6, to be able to correct for the time lap mentioned. With equation 6, half times of objects in changing air temperature can be calculated only if the change in air temperature is rapid compared to the change of the object temperature.

$$t_{0.5} = \ln 2 \cdot \frac{(T_{\text{ambient}} - T_{\text{object}})}{(\partial T / \partial t)} \quad (6)$$

Two hatching egg containers and an incubator trolley were used for the experiment. The containers were divided in 5 drawers containing 2 x 3 stacks of 6 trays each, in total 5400 eggs per container. The incubator trolley (Pas Reform, Zeddum, Holland) contained 2 stacks of 15 incubator trays, with 10 x 15 eggs on each tray. In the trolley, the stacks were placed with the short side of the trays against each other. Eggs with an average egg weight of 62 g were used and placed on disposable cardboard trays, plastic trays (Econoom, Putten, Holland) or on incubator trays (Pas Reform, Zeddum, Holland). During the experiment, three different storage methods were used, e.g. two air velocities and the use of coverage. The average air velocity caused by the ventilation system

was approximately 0.06 m/s in a mainly horizontal direction (air velocity: low). To increase the air velocity, one circulating fan was used, positioned to provide a horizontal air stream in the chamber with an average velocity of 0.7 m/s (air velocity: high). The experiments were done with uncovered containers and with containers covered with black polyethene foil coverage. Coverages were open at the bottom side. Air velocity was set at either high or low level during cooling and warming experiments without coverage. During the cooling and warming experiments with coverages, air velocity was set at high level.

In every container, eight calibrated thermocouples were connected to eggs in different positions. In the incubator trolley, seven thermocouples were used. The thermocouples were attached to the longitudinal side of the eggs, with at least 3 cm of wire attached to the surface of the eggs to prevent heat loss through the wires. The thermocouples were connected with a data-taker and temperature of all thermocouples was recorded every 15 minutes. In the containers, three eggs with thermocouples were placed in the upper drawer and five in the middle drawer. The position of these eggs is shown in figure 1 (upper drawer) and figure 2 (middle drawer). The position of the eggs in the incubator lorry is shown in figure 3. At the opposite position of every thermocouple connected

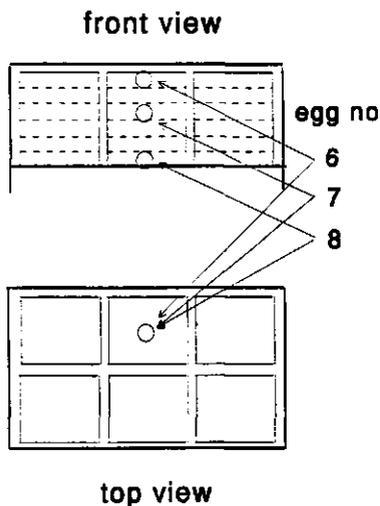


Figure 1: Position of the thermocouple connected eggs placed on the upper drawer of the hatching egg container.

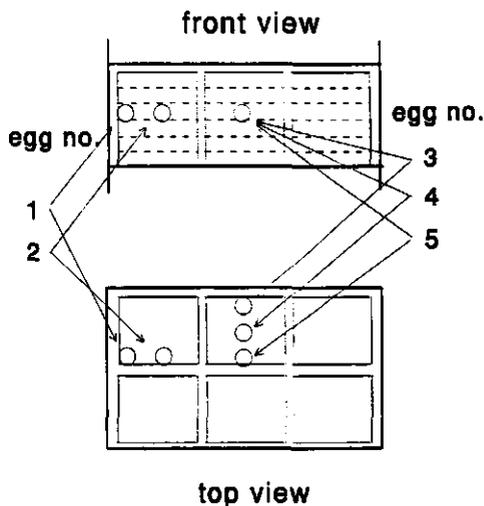


Figure 2: Position of the thermocouple connected eggs placed on the middle drawer of the hatching egg container.

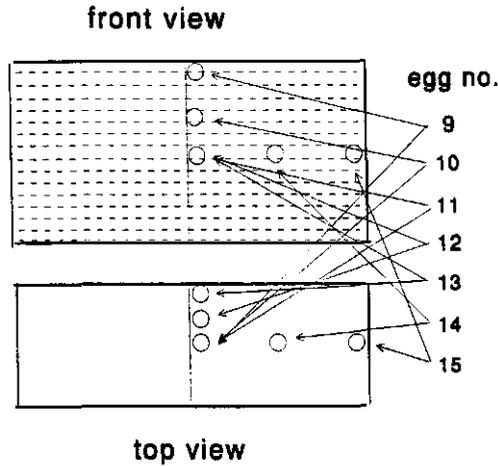


Figure 3: Position of the thermocouple connected eggs on the incubator loric.

egg, an egg with a known specific moisture loss was placed to determine moisture loss. The specific moisture loss of the eggs was determined in 48 h prior to the experiment, by leaving them uncovered under controlled temperature and relative humidity conditions and determining their weight loss in this period. Three extra eggs with a known specific moisture loss were left uncovered in the climatic room to determine the average water vapour pressure and therefore relative humidity (temperature constantly recorded).

During the experiment, all eggs with known specific moisture loss were weighed to the nearest 0.001 g at 0, 24 and 48 h after the start of every cooling or heating period. Moisture loss of each recorded egg was corrected for its specific moisture loss and standardised to a specific moisture loss of  $0.2^{-10}$  kg/(kg.Pa.s). The moisture loss of the eggs is given as the average moisture loss during the first 48 h of the cooling or heating period in percentage of the initial egg weight. Based on the temperature and relative humidity of the environment and the temperature development of the eggs a water vapour pressure deficit can be calculated for each period and each egg. An estimation of the moisture loss of the eggs is calculated from the water vapour pressure deficit and the specific moisture loss of the individual eggs. In this calculation no influence of storage method or tray type on moisture loss other than by temperature is included. To be able to estimate the direct influence

of storage method and tray type on moisture loss the ratio (degree of protection) between estimated moisture loss and recorded moisture loss is calculated.

Temperature and moisture loss results of eggs are combined to calculate the average results for eggs in side position and in centre position in the containers. For the eggs on cardboard trays and plastic trays, egg no. 1, 3 and 6 are combined to an average result for the side position, and egg no. 2, 4, 5, 7 and 8 are combined to an average result for the centre position. For the eggs on incubator trays, egg no. 9, 13 and 15 are combined to an average result for the side position, and egg no. 10, 11, 12 and 14 are combined to an average result for the centre position.

## RESULTS

During the experiments, the average relative humidity in the experimental room was 67%, varying between an average relative humidity during 24 h of 65% and 71%.

In Figure 4 the recorded half time for the individual eggs stored on pulp trays during cooling are presented. This figure shows that a distinction can be made between eggs in side position (eggs no. 1, 3 and 6) and eggs in centre position (eggs 2, 4, 5, 7 and 8), especially for storage treatments with low air velocity and with coverages. For the high air velocity storage treatment, differences

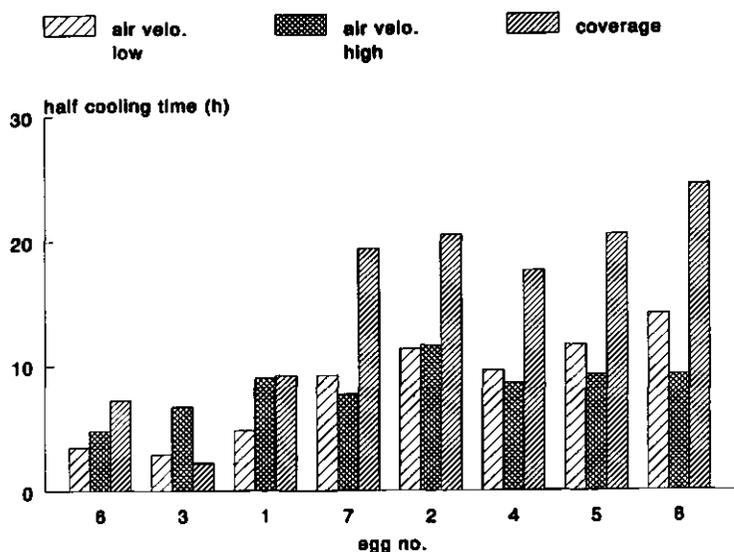


Figure 4: Half cooling time of eggs stored on cardboard trays

between eggs in centreposition are small and not consistent.

In Table 1 the observed cooling and warming characteristics, expressed as half times, are presented. The cooling rate of the eggs on incubator trays with low air velocity could not be calculated accurately because differences in temperature pattern between the eggs and the environmental temperature were too small to apply equation 6. Although no accurate calculation could be done, the temperature recorded showed that half cooling time was shorter for eggs placed on incubator trays than for eggs placed on the other tray types. With low air velocity and no use of coverage, warming time was longer than cooling time. With high air velocity and with the use of coverages, differences in cooling rates and warming rates were small and not consistent. Half times were highest for eggs stored on cardboard trays and smallest for eggs stored on incubator trays, regardless of storage conditions. Half times of eggs stored on plastic trays were intermediate. For all storage treatments and tray types, cooling and warming of eggs in centre positions took more time than cooling and warming of eggs in side positions. Differences between side position and centre position were higher for storage with low air velocity than for storage with high air velocity or with the use of coverages. For all storage treatments, differences between eggs in centre position and in side position were the highest for eggs stored on cardboard trays and lowest for eggs stored on incubator trays. Results of eggs stored on plastic trays were intermediate.

Table 1: Halftimes during cooling and heating of eggs stored on three types of tray under different conditions

		air velocity low (0.06 m/s)		air velocity high (0.7 m/s)		air velocity high (0.7 m/s) + coverage	
		warming	cooling	warming	cooling	warming	cooling
cardboard tray	centre	27.5	11.0	9.0	9.5	19.5	20.5
	side	5.0	3.5	7.0	7.0	10.0	6.0
plastic tray	centre	13.5	7.5	6.5	7.5	13.0	16.5
	side	5.5	5.0	5.0	6.5	10.0	6.5
incubator tray	centre	7.0	--	2.5	4.5	9.5	9.5
	side	3.0	--	1.5	4.0	6.0	4.5

In Table 2 the calculated temperature development of eggs expressed as half time is given, based on equations 1 to 5 and calculated for different levels of heat transfer coefficients and thermal conductivity. In the calculations a heat production of  $-13 \text{ W/m}^3$  is introduced for moisture loss of the eggs, based on an average moisture loss of  $3.5 \text{ mg/egg/h}$ . This table shows that the calculated half warming time is longer than the calculated half cooling time. When the heat transfer coefficient increases, the calculated half times decreases. Increase of the thermal conductivity resulted in an increase in calculated half times. The sensitivity of the theoretical temperature development for heat transfer coefficient is rather small compared to the influence of the position of the eggs in the container and the thermal conductivity.

In Table 3 the observed moisture loss during the first 48 h of the cooling and warming process is given as percentage moisture loss of initial egg mass, corrected for differences in conductance between the individual eggs. This table shows that, on average, eggs placed at side position experienced a higher moisture loss during warming than during cooling, regardless of storage method or storage conditions. Differences for eggs placed at centre position were smaller and not

Table 2: Calculated halftimes of eggs in centre position and side position in a hatching egg container during cooling and heating of eggs with four levels of convective heat transfer coefficient and three levels of thermal conductivity

		convective heat coefficient							
		10 $\text{W}/(\text{m}^2.\text{K})$		20 $\text{W}/(\text{m}^2.\text{K})$		50 $\text{W}/(\text{m}^2.\text{K})$		100 $\text{W}/(\text{m}^2.\text{K})$	
		warming	cooling	warming	cooling	warming	cooling	warming	cooling
0.2 $\text{W}/(\text{m.K})$	centre	27	16	24	15	23	14	22	14
	side	3	2	2	2.0	1	1	1	1
0.3 $\text{W}/(\text{m.K})$	centre	14	10	12	9	10	8	10	8
	side	3	2	1	1	1	<1	<1	<1
0.4 $\text{W}/(\text{m.K})$	centre	10	8	8	7	7	6	6	6
	side	3	2	1	1	<1	<1	<1	<1

Calculations based on equation 1 to 5, heat production caused by moisture loss  $-13 \text{ W/m}^3$  (moisture loss  $3.5 \text{ mg/egg/h}$ )

Table 3: Weight loss loss of eggs (% of initial egg weight) during the first 48 h of cooling (25°C-15°C) or heating (15°C-25°C) of eggs stored on three types of tray under different conditions

		air velocity low (0.06 m/s)		air velocity high (0.7 m/s)		air velocity high (0.7 m/s) + coverage	
		warming	cooling	warming	cooling	warming	cooling
cardboard tray	centre	0.15	0.36	0.37	0.41	0.12	0.25
	side	0.31	0.34	0.46	0.37	0.25	0.22
plastic tray	centre	0.32	0.35	0.41	0.42	0.13	0.40
	side	0.48	0.34	0.49	0.37	0.24	0.33
incubator tray	centre	0.50	0.35	0.54	0.36	0.21	0.37
	side	0.54	0.35	0.56	0.35	0.33	0.33

Weight loss corrected to an equal specific moisture loss for all eggs of  $0.2 \cdot 10^{-10}$  kg/(kg.Pa.s)

consistent. Especially during warming, eggs placed at side position experienced a higher moisture loss than eggs placed at centre position. Eggs placed on incubator trays experienced a higher amount of moisture loss, especially during warming, than eggs placed on plastic or cardboard trays. Differences in moisture loss between storage treatments with high and low air velocity were relatively small and not consistent. The use of coverages resulted in a decrease in moisture loss when compared with storage at low and high air velocity without coverage.

Table 4 shows that moisture loss of the eggs during cooling and warming was smaller than can be expected based on temperature development of the eggs and the climatic conditions outside the containers. In general, the degree of protection was higher during cooling than during warming. When coverages were used, for all tray types the average degree of protection was higher than without coverages, regardless of level of air velocity.

Table 4: Degree of protection against moisture loss (ratio estimated moisture loss based on temperature development / recorded moisture loss) during the first 48 h of cooling (25°C-15°C) or heating (15°C-25°C) of eggs stored on three types of tray under different conditions

		air velocity low (0.06 m/s)		air velocity high (0.7 m/s)		air velocity high (0.7 m/s) + coverage	
		warming	cooling	warming	cooling	warming	cooling
cardboard tray	centre	1.24	1.62	1.24	1.45	2.68	2.69
	side	1.18	2.04	1.18	1.40	1.62	2.45
plastic tray	centre	1.24	2.19	1.24	1.46	1.26	2.08
	side	1.27	1.66	1.27	1.54	1.90	2.38
incubator tray	centre	1.20	1.35	1.18	1.38	1.72	2.06
	side	1.18	1.39	1.20	1.35	1.69	2.03

## DISCUSSION

### *Temperature*

Meijerhof and van Beek (1993) demonstrated that theoretically the half-cooling time of an egg is approximately 30 minutes when air velocity is low. The results presented in this experiment indicates that the rate of temperature changes for eggs stored in containers is much slower than these theoretical calculations for individual eggs suggest. This is in agreement with the results of Fasenko et al. (1991), who reported that hatching eggs that were stored in stacks of cardboard trays before cooling contained more developed embryos than eggs left unpacked. Czarick and Savage (1993) reported that eggs placed in centre positions in boxes or stacks experience very long halftimes during cooling. They demonstrated that the actual temperature development of the eggs was dependent on the place of the eggs in the stack and the method of storage.

The observed halfcooling time was approximately 60% of the halfwarming time when the air velocity was low. This difference can be partly explained by the negative heat production caused by moisture loss of the eggs during cooling, resulting in an increased cooling rate. As explained by Meijerhof and van Beek (1993), negative heat production as a result of moisture loss is higher during cooling than during warming. However, the moisture loss as recorded during the cooling

process is too small to solely explain the observed differences in half time, according to the results of the theoretical calculations presented in table 2. An increase in heat transfer coefficient as a result of condensation of moisture on the cold surfaces of eggs and trays during the warming process is more likely to be the cause of the observed difference. The exact distinction between the effect of the negative heat production caused by moisture loss and the effect of condensation on heat transfer coefficient on the observed temperature development can not be done accurately by the methods described.

It can be suggested that air movement inside a container will be lowest when eggs are stored on cardboard trays with low outside air velocity, especially when coverages are used. This is in agreement with the results obtained in the experiments and calculations. However, when the air velocity is increased or when the storage method allows more air movement within the container, the actual temperature development observed in the experiments is much faster than expected based on the calculations. At the level of air movement used in the experiments, the convective heat transfer coefficient will not exceed a value of  $50 \text{ W}/(\text{m}^2 \cdot \text{K})$ , which is too low to explain the observed half times. The results of measurements and theoretical calculations indicate that the presented equations, based on thermal conductivity and conductive heat transfer, are not accurate enough to estimate the temperature development in these situations. This is probably caused by internal air movement in the container between eggs and trays. That means that heat transport from the centre of the container to the outside layer is not only influenced by thermal conductivity but also by natural convection. Similar effects are observed during storage of horticulture products in containers (Beukema, 1980). This is in agreement with the differences in temperature development observed between egg positions. As a result of an increased air movement within the container, an increased heat flow to the outside layer of the container will occur, resulting in an increased half time of the eggs in side position compared to theoretical calculations. This means that the Biot number, being the ratio between convective heat transport and heat conductivity, appears to be estimated too low. This is confirmed by the results of the analytical equations presented. These results indicate that theoretically the influence of the thermal conductivity on temperature development in containers is more important than the influence of the convective heat transport. In practical situations the thermal conductivity will be below the maximum used in the calculations. However, when natural convection occurs, the apparent conductivity will be increased.

The presented equations are based on thermal conductivity through the container and convective heat transport from container to environment. To be able to calculate the temperature development of eggs in containers accurately, convective heat transport through the container must be incorporated in these equations. The rate and direction of this heat transport is highly dependent on the conditions in the container. Analytical solutions to calculate natural convection under conditions similar to storage conditions and to calculate temperature development in situations where high levels natural convection occurs are not to the authors knowledge. The results presented indicate that with the analytical methods presented no accurate estimation of the temperature development for every spot in hatching egg containers can be made. This means that the effect of egg storage methods on temperature development can not be estimated by analytical calculations but must be evaluated by measurements.

#### *Moisture loss*

Moisture loss of eggs is dependent on climatic conditions and the resistance of the egg shell against moisture loss (Simkiss, 1980) In hatching egg containers, these physical conditions will be determined by the temperature development of the egg, the conditions of the surrounding air and the conditions of the air between the eggs. Meijerhof and van Beek (1993) demonstrated that theoretically moisture loss is higher during cooling than during warming when half times are equal. The results presented show an opposite tendency. For every storage method and storage condition, moisture loss was higher during cooling than during warming. This can be partly explained by the differences in half time. Storage of eggs under low air velocity conditions resulted in a longer half time for warming than for cooling. Eggs will lose more moisture at high temperature conditions than at low temperature conditions when relative humidity is equal in both situations, as a result of differences in water vapour pressure deficit (Meijerhof and van Beek, 1993). This means that eggs that experience an increased half time are expected to lose more moisture during cooling and less moisture during warming when compared with eggs that experience a reduced half time. The results obtained in this experiment indicate that during warming of the eggs, the expected tendency can be observed for the eggs placed in centre position of the containers, especially for the storage treatment with low air velocity. The expected pattern is not observed during cooling of eggs or for eggs placed in side position during cooling and warming. For the eggs in side position, this can be

caused by the fact that half times are not long enough to provide any detectable difference.

The presented experiment indicates that the moisture loss of the eggs can not be fully explained by the temperature of the egg and the conditions of the surrounding air. The storage method itself contributes to the conditions influencing moisture loss, expressed in the degree of protection. This degree of protection is likely to be caused by the limitations that the storage method provides for transport of the moisture through the container. Also the buffer capacity of the trays and the container may influence the moisture loss. As a result, the relative humidity in the boundary layer around the egg will be higher than the relative humidity in the storage room, resulting in a reduced moisture loss.

Condensation on eggs during warming experiments is not likely to be of influence on the observed moisture loss and degree of protection, because the total moisture loss is calculated during the first 48 h. After this period, temperature of all eggs was above dewpoint.

The results obtained indicate that during cooling and warming the moisture loss of eggs stored in hatching egg containers will be lower than can be expected based on temperature development of the eggs, temperature in the storage room and relative humidity. From our results it can be suggested that at storage conditions where egg temperature is constant, a certain degree of protection will occur. This means that in practical situations the moisture loss of eggs during storage will be lower than expected based on climatic conditions.

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Symbol	Unit	Property
t	s	time
T	°C	temperature
x	m	distance to centre
X	m	smallest distance from centre to surface
c	J/(kg.K)	specific heat
q	W/m <sup>3</sup>	heat production
α	W/(m <sup>2</sup> .K)	convective heat transfer coefficient
λ	W/(m.K)	thermal conductivity
p	kg/m <sup>3</sup>	density
n		roots of transcendental equation

## CHAPTER 5

### INFLUENCE OF PRE-INCUBATION TREATMENT ON HATCHING RESULTS OF BROILER BREEDER EGGS PRODUCED AT 37 AND 59 WEEKS OF AGE

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**ABSTRACT**

1. The influence of temperature in the nest box, temperature during storage, storage time and pre-setting temperature on hatchability of broiler breeder eggs produced by birds of 37 and 59 weeks of age was examined.
2. All treatments that can be characterized as being less optimal for embryo survival than the control treatment affected hatch of fertile eggs more in the case of eggs produced by older birds.
3. A higher temperature in the nest box, longer storage periods, higher storage temperature, especially at longer storage periods, and higher presetting temperature significantly reduced hatch of fertile eggs from the older birds.
4. For the younger birds a significant reduction of hatchability was found only for the longest storage period.

**INTRODUCTION**

After oviposition, the embryonic development is highly influenced by environmental temperature (Kaplan et al., 1978). Broiler breeders are normally housed on litter floors with laying nest boxes available. The nest boxes can be of a litter type or of a roll-away type. Fasenko et al. (1991, 1992) reported an increased embryonic development when broiler breeder eggs were left in litter nest boxes for 6 to 7 h before collection when compared with eggs collected within 1.5 h after oviposition. This indicates that the average egg temperature in nest boxes is above the "physiological zero" for embryo development of 25-27°C (Funk and Biellier, 1944; Lundy, 1969). This is confirmed by the experiments of Meijerhof and van Beek (unpublished data), who found that average egg temperature in nest boxes exceeded 30°C during the period that the boxes were frequently visited by the birds.

The influence of nesttype and temperature in the box on hatchability is not clear. Although North (1984) stated that eggs should be collected at least four times a day to provide optimum viability of the embryo, Fasenko et al. (1992) were not able to demonstrate a significant relationship between embryo development in the nest box and hatchability.

In the modern poultry industry it is usual not to extend the pre-setting storage period beyond 7 days and many operations aim to set eggs within 4 days of production. It is reported that the optimal storage temperature is between 10°C and 17°C (Mayes and Takeballi, 1984). Proudfoot (1976) reported that the optimum temperature for storage periods extending beyond 1 week is 11

to 12°C. Kaltofen and El-Jack (1972) and Kirk et al. (1980) reported that optimum storage temperature is higher for shorter storage periods than for longer periods.

Pre-warming of hatching eggs prior to setting is often assumed to be beneficial when storage time is extended (Mayes and Takeballi, 1984). Proudfoot (1966) reported a reduced decline in hatchability when eggs were warmed at 23°C for 18 hours prior to setting after storage for more than 2 weeks.

It is well known that production characteristics of a flock changes with increasing bird age. Production level decreases with increasing bird age as well as the percentage of fertile eggs produced if no artificial insemination is used. Hatch of fertile eggs also decreases with increasing bird age (Tomhave, 1956), although the main reason for this decrease is not clear. The objectives of this study were (1) to determine the effect of differences in nest box temperature, storage time, storage temperature and pre-setting temperature, respectively, on hatchability of eggs produced by broiler breeders of two different ages and (2) to determine interactions between treatments.

#### MATERIAL AND METHODS

A flock of approximately 3000 hens of a commercial broiler breeder strain (Ross x Ross) was used for production of the eggs. At placement of the birds (20 weeks of age) a male:female ratio of 1:10 was used. Birds were housed in a light controlled (16 h light, 8 h dark) darkhouse, containing 16 separate floor pens. Community roll-away nests (Jansen, Barneveld, Holland) with artificial grass (Astroturf, Monsanto, Brussels, Belgium) bottom were available. The experiment was done at 37 weeks of age and repeated when birds were 59 weeks of age. The feeding program was in accordance with the management guide of the breeding company and feed level was adjusted for each pen separately based on production rate. Average production rate of the flock was 75% at 37 weeks and 52% at 59 weeks of age. Level of production, fertility and hatchability was similar for each pen.

#### *Egg collection and simulated nest box temperature*

At both ages, eggs were collected during four days, at day 1, 5, 8 and 11 of the experiment. On each day, 1080 eggs were collected. Visually dirty, misshapen or cracked eggs were excluded from the experiment. Egg collection started 1 h after lights on and continued until a total of 1080 eggs was collected, approximately 8 h and 11 h after start of collection at 37 weeks and 59 weeks of age,

respectively. Every egg was removed from the nest box as soon as possible by frequent (at least every ten minutes) inspection of the nest box and collection of the eggs. Within 10 minutes of collection of the first egg of a group, eggs were placed on plastic trays (30 eggs per tray, Econoom, Putten, Holland) in one of three temperature controlled compartments. Each tray was considered an experimental unit and during the complete experiment data were collected at the level of trays. Stacks were limited to 3 trays each, to enable air circulation around the eggs. Temperature in the compartments was set at 10°C, 20°C and 30°C to simulate temperature conditions in roll away nests under low environmental temperature, average temperature conditions in roll away nests and temperature conditions in a litter nest, respectively. Temperature recordings showed that air temperature fluctuated less than 1°C in the compartments during the experiment. 12 h after start of collection, all eggs were transported to the storage rooms, situated near the poultry house. Transportation was done in a closed vehicle, to prevent uncontrolled temperature shocks.

#### *Egg storage*

Immediately after transportation, the eggs were placed in a disinfection chamber and fumigated for 20 minutes by heating 7 g of paraformaldehyde per m<sup>3</sup> of chamber. After fumigation, eggs were placed in three storage rooms with temperature setting of 10°C, 15°C and 20°C, respectively. Eggs were placed on tables and stacks were limited to 3 trays, to provide air circulation around the eggs. Relative humidity was set at 60%, 71% and 79%, respectively, to maintain a constant water vapour pressure deficit of 490 Pa for all treatments.

Temperature during storage varied less than 0.5°C, except storage temperature of the lowest temperature group when birds were 59 weeks of age. For this treatment, storage temperature differed from 11.0°C to 12.5°C because of high environmental temperatures.

#### *Pre-setting treatment*

Approximately 16 hours before setting, on day 11 of the experiment, all eggs were transported to the hatchery and placed on incubator trollies. Half of the eggs were placed at a temperature of 20°C, the other eggs were placed at a temperature of 27°C. On day 12 all eggs were placed in an incubator (Econoom, Putten, Holland) and fumigated for 20 minutes with formaldehyde gas after which the incubation process was started.

### Incubation

All eggs were incubated at 37.5°C with relative humidity of 55%. At day 7 of incubation (day 19 of the experiment), all eggs were candled and eggs detected as non-fertile or containing dead embryos were opened and macroscopically examined to determine true fertility and mortality. At day 18 of the incubation process (day 30 of the experiment) eggs were candled to determine mortality rate. Eggs containing dead embryos were removed and remaining eggs were transferred to the hatcher. On day 22 of the incubation process (day 34 of the experiment) the numbers of live chicks, dead in shell and culls were determined.

### Statistical analysis

Differences in treatment means were determined by subjecting data to analysis of variance in a split-plot arrangement. Treatment factors were bird age, nest temperature, storage time, storage temperature and pre-setting temperature. At both ages, nest temperature treatment, storage time treatment, storage temperature treatment and pre-setting temperature treatment were randomized over trays of eggs (sub plots). Least significant differences ( $p \leq 0.05$ ) between treatment means have been calculated. The split-plot model used was:

$$Y_{ijklm} = \mu + A_i + N_j + S_k + T_l + P_m$$

+ two and three factor interactions (ANSTP)

$$+ e_{ijklm}$$

where:

$\mu$	overall mean
$A_i$	age
$N_j$	simulated nest temperature
$S_k$	storage time
$T_l$	storage temperature
$P_m$	pre-setting temperature
$e_{ijklm}$	error term (sub plots)

Because of the experimental design, no statistical analysis on the main effect of bird age can be done. A choice in presentation of the results was made based on the presence or absence of

meaningful differences.

## RESULTS

In Table 1 the main effects of the treatments are presented. A decrease in fertility was observed with increasing age of the flock. Fertile eggs originating from the older birds had a higher rate of mortality during the whole incubation process, resulting in a lower number of chicks hatched from fertile eggs. Because of the experimental design, the observed differences between bird ages can not be validated by means of statistical analyses. Fertility was not significantly influenced by the other treatments.

The effect of simulated nest temperature on hatching results was small. However, a significant increase in mortality in the period from 0-7 days could be detected for the 30°C treatment when compared with the 10°C treatment. Table 2 shows that a significant interaction of age and nest temperature on hatching results was present. For the eggs produced at 37 weeks of age, no

Table 1: Influence of bird age, simulated nest temperature, storage temperature, storage time and pre-setting temperature on fertility, mortality (percentage of fertile eggs) and chicks hatched (percentage of fertile eggs)

		% fertility	% mortality			% chickens
			0-7 days	8-18 days	19-22 days	
bird age (weeks) <sup>1</sup>	37	98.1	3.4	0.6	3.2	92.1
	59	94.9	5.1	2.1	4.3	87.0
"nest" temp (°C)	30	96.8 <sup>a</sup>	4.9 <sup>a</sup>	1.5 <sup>a</sup>	3.6 <sup>a</sup>	88.9 <sup>a</sup>
	20	96.6 <sup>a</sup>	4.1 <sup>ab</sup>	1.1 <sup>a</sup>	3.7 <sup>a</sup>	90.4 <sup>a</sup>
	10	96.1 <sup>a</sup>	3.7 <sup>b</sup>	1.5 <sup>a</sup>	4.0 <sup>a</sup>	89.4 <sup>a</sup>
	30	96.2 <sup>a</sup>	4.9 <sup>a</sup>	1.4 <sup>a</sup>	4.3 <sup>a</sup>	88.1 <sup>a</sup>
storage temp (°C)	15	96.6 <sup>a</sup>	4.2 <sup>ab</sup>	1.3 <sup>a</sup>	4.1 <sup>a</sup>	89.4 <sup>a</sup>
	10	96.7 <sup>a</sup>	3.6 <sup>b</sup>	1.4 <sup>a</sup>	2.9 <sup>b</sup>	91.2 <sup>b</sup>
	12	96.2 <sup>a</sup>	6.7 <sup>a</sup>	1.9 <sup>a</sup>	5.5 <sup>a</sup>	84.6 <sup>a</sup>
storage time (d)	9	97.2 <sup>a</sup>	3.4 <sup>b</sup>	1.4 <sup>ab</sup>	3.5 <sup>b</sup>	90.2 <sup>b</sup>
	6	96.3 <sup>a</sup>	3.4 <sup>b</sup>	1.2 <sup>ab</sup>	3.2 <sup>b</sup>	91.3 <sup>bc</sup>
	2	96.3 <sup>a</sup>	3.4 <sup>b</sup>	0.9 <sup>b</sup>	2.8 <sup>b</sup>	92.2 <sup>c</sup>
	27	96.8 <sup>a</sup>	4.6 <sup>a</sup>	1.7 <sup>a</sup>	4.1 <sup>a</sup>	88.3 <sup>a</sup>

Means within columns and treatments with no common superscripts are significantly different ( $P < 0.05$ ).

<sup>1</sup> not statistically analysed

significant difference in hatchability of fertile eggs could be found. At 59 weeks of age the percentage of chickens hatched from fertile eggs was significantly reduced with 2.4% for the 30°C group when compared with the 20°C. This decrease was a result of a small, non-significant increase of mortality both from 0-7 days and from 8-18 days of incubation. The 10°C treatment did not differ significantly from both other treatments.

Table 2: Mortality and chicks hatched (percentage of fertile eggs) at two ages and three simulated nest temperature treatments.

age (weeks)	"nest" temp (°C)	% mortality			% Chickens
		0-7 days	8-18 days	19-22 days	
37	30	4.0 <sup>a</sup>	0.6 <sup>a</sup>	2.9 <sup>a</sup>	91.6 <sup>a</sup>
	20	3.1 <sup>a</sup>	0.6 <sup>a</sup>	3.1 <sup>a</sup>	92.3 <sup>a</sup>
	10	3.0 <sup>a</sup>	0.6 <sup>a</sup>	3.5 <sup>a</sup>	92.3 <sup>a</sup>
59	30	5.8 <sup>a</sup>	2.4 <sup>a</sup>	4.4 <sup>a</sup>	86.1 <sup>a</sup>
	20	5.0 <sup>a</sup>	1.6 <sup>a</sup>	4.3 <sup>a</sup>	88.5 <sup>a</sup>

Means within columns and bird age treatment with no common superscripts are significantly different ( $P < .05$ ).

Table 1 shows that hatching results decreased with increasing storage time, resulting in a significant reduction of percentage of chickens hatched from fertile eggs after 8 and 12 days of storage when compared with 2 and 5 days. In Table 3 the interaction between age and storage time is given. At 37 weeks of age, a significant reduction in percentage of chickens hatched was observed after 12 days of storage compared with 2 to 9 days of storage. The decline between 2 and 12 days of storage was 5.1%. At 59 weeks of age a significant decline was observed after 8 days of storage. The decline between 2 and 12 days of storage at this age was 10.1%. At both ages the decline was mainly due to an increased mortality between 0-7 days, although mortality in the other periods tended to be increased as well.

Table 1 shows that storage at 10°C resulted in a significant increase of hatchability of fertile eggs when compared with storage at 15°C and 20°C. This was mainly due to a decreased mortality in the first 7 days of the incubation process and after transferring the eggs to the hatcher. In Table 4 the interaction between age, storage time and storage temperature is given. At both ages, storage

Table 3: Mortality and chicks hatched (percentage of fertile eggs) at two ages and four storage time treatments

age (weeks)	storage time (d)	% mortality			% Chickens
		0-7 days	8-18 days	19-22 days	
37	12	4.9 <sup>a</sup>	1.1 <sup>a</sup>	4.3 <sup>a</sup>	88.8 <sup>a</sup>
	9	2.6 <sup>b</sup>	0.5 <sup>a</sup>	2.8 <sup>ab</sup>	93.0 <sup>b</sup>
	6	2.9 <sup>b</sup>	0.4 <sup>a</sup>	3.6 <sup>a</sup>	92.7 <sup>b</sup>
	2	3.0 <sup>b</sup>	0.4 <sup>a</sup>	2.0 <sup>b</sup>	93.9 <sup>b</sup>
59	12	8.5 <sup>a</sup>	2.8 <sup>a</sup>	6.8 <sup>a</sup>	80.3 <sup>a</sup>
	9	4.3 <sup>b</sup>	2.4 <sup>ab</sup>	4.2 <sup>b</sup>	87.4 <sup>b</sup>
	6	3.9 <sup>b</sup>	2.1 <sup>ab</sup>	2.8 <sup>b</sup>	90.0 <sup>bc</sup>
	2	3.7 <sup>b</sup>	1.4 <sup>b</sup>	3.5 <sup>b</sup>	90.4 <sup>c</sup>

Means within columns and bird age treatment with no common superscripts are significantly different ( $P < 0.05$ ).

temperature had no significant influence on hatchability for storage periods up to 9 days. For birds at 37 weeks of age, hatchability was increased when eggs were stored for 12 days at 15°C or 10°C when compared with storage at 20°C. For birds at 59 weeks of age, hatchability was increased when storage for 12 days was done at 10°C as compared to the 15°C and 20°C treatment. The differences in hatchability after 12 days of storage between low and high storage temperature were higher for eggs originating from older birds than for eggs originating from younger birds. Table 1 shows that warming the eggs to 27°C for 16 h prior to setting resulted in a reduction of the

Table 4: Interaction between age, storage time and storage temperature on percentage hatch from fertile eggs

age (weeks)	storage time (°C)	storage time (days)			
		12	9	6	2
37	20	85.3 <sup>a</sup>	94.2 <sup>b</sup>	90.5 <sup>b</sup>	93.5 <sup>b</sup>
	15	90.8 <sup>b</sup>	92.3 <sup>b</sup>	93.7 <sup>b</sup>	95.2 <sup>b</sup>
	10	90.5 <sup>b</sup>	92.4 <sup>b</sup>	93.8 <sup>b</sup>	93.1 <sup>b</sup>
59	20	77.0 <sup>a</sup>	86.8 <sup>b</sup>	89.2 <sup>bc</sup>	88.3 <sup>bc</sup>
	15	78.0 <sup>a</sup>	86.5 <sup>b</sup>	88.8 <sup>bc</sup>	90.0 <sup>bc</sup>
	10	85.9 <sup>b</sup>	89.0 <sup>bc</sup>	92.0 <sup>c</sup>	92.8 <sup>c</sup>

Means within columns and bird age treatment with no common superscripts are significantly different ( $P < 0.05$ ).

Table 5: Interaction between age and pre-setting temperature on mortality and chickens hatched (percentage of fertile eggs)

age (weeks)	pre-setting temp (°C)	% mortality			% Chickens
		0-7 days	8-18 days	19-22 days	
37	27	3.9 <sup>a</sup>	0.6 <sup>a</sup>	3.3 <sup>a</sup>	91.6 <sup>a</sup>
	20	2.8 <sup>a</sup>	0.6 <sup>a</sup>	3.1 <sup>a</sup>	92.6 <sup>a</sup>
59	27	4.8 <sup>a</sup>	2.8 <sup>a</sup>	4.9 <sup>a</sup>	85.1 <sup>a</sup>
	20	3.8 <sup>a</sup>	1.4 <sup>b</sup>	3.8 <sup>a</sup>	89.0 <sup>b</sup>

Means within columns and bird age treatment with no common superscripts are significantly different ( $P < 0.05$ ).

percentage chickens hatched from fertile eggs. In Table 5 the interaction of pre-setting temperature of the eggs and age of the birds is given. This table shows that a significant detrimental effect was observed in the eggs originating from the older birds. For the younger birds no significant influence was observed.

## DISCUSSION

The results presented indicate that fertile eggs from broiler breeders of 59 weeks of age had a reduced hatching potential when compared with fertile eggs of the birds at 37 weeks of age. This is in agreement with the results of Tomhave (1956) who reported a decreasing hatch of fertile eggs with increasing flock age.

Our results showed no significant relation between hatching results and simulated nest temperature when the birds were 37 weeks of age. This is in agreement with the results of Fasenko et al. (1992), who reported no differences in hatchability of eggs left in litter nests for 6 to 7 hours when compared with eggs collected within 1.5 hours after oviposition. Similarly, Middelkoop (1972) did not find significant differences in hatchability when eggs were kept at 40°C for several hours after oviposition. At a bird age of 59 weeks, a significant reduction in hatchability was observed for the 30°C nest temperature treatment when compared with the 20°C treatment. This suggests that eggs produced by older birds are more sensitive to high temperatures in the laying nest. It also indicates that an increased temperature of the eggs during the nesting period should be considered a negative factor for maintaining hatchability. This is in agreement with North (1984),

who stated that eggs should be collected at least four times a day to prevent embryo development after oviposition and to maintain viability of the embryo.

Hatchability of fertile eggs decreased with increasing storage time. On average the decline started in the period between 6 and 9 days of storage. This is in agreement with earlier work, as reviewed by Meijerhof (1992), although several workers (Bohren et al., 1961; Byng and Nash, 1962) reported a decline in hatching results even before 7 days. Mayes and Takeballi (1984) stated that reduction of hatchability after 7 days of storage could be as much as 1% per day. In our experiment the average reduction of hatchability of fertile eggs from 6 to 12 days was 1.1%, which is well in agreement with the literature. The detrimental effect of the extended storage periods on hatching results were more pronounced for eggs produced by birds at 59 weeks of age than for birds at 37 weeks of age. This is in agreement with the work of Kirk et al. (1980). The results show that the hatchability declined more and that the decline started earlier for the eggs from the older birds when compared with those from the younger birds. In the period from 6 to 12 days of storage, hatch of fertile eggs decreased with 0.7% and 1.6% per day for the eggs originating from the younger and the older birds, respectively.

It is often reported (e.g. Meijerhof, 1992) that temperature during storage should be decreased with increasing storage time. Our results indicate that this treatment was more beneficial for the eggs produced by the older birds than for the eggs of the younger birds. At both ages significant differences could only be observed after 12 days of storage but the reduction in hatchability was higher for the eggs of the older birds. Contrary to the results of Kaltofen and El Jack (1972) and Kirk et al. (1980) an increased storage temperature for a short storage period did not result in an increase in hatchability. On the contrary, storage at 10°C for short storage periods tended to be beneficial for hatching results of the older birds, although the differences were not significant.

Hatchability of eggs from younger birds was not significantly influenced by pre-warming at 27°C for 16 h when compared with prewarming at 20°C for the same period. Hatchability of eggs from older birds was significantly reduced by pre-warming at 27°C. No significant interaction with storage time or storage temperature was observed. This is not in agreement with results presented in the literature. Mayes and Takeballi (1984) concluded that pre-warming improves viability and hatchability when low storage temperatures were used. No beneficial effects were reported when eggs were stored for a short period at 15-16°C. The differences between our results and the literature cited can perhaps be partly explained by the temperature used. In our experiment,

pre-setting temperature was set at 27°C and compared with leaving the eggs at 20°C, while in experiments of Proudfoot (1966) pre-setting for 18 h at 23°C was compared with setting eggs directly after storage at 11-12°C. Under these conditions pre-warming of eggs before setting can prevent condensation of moisture on the eggs.

The observed interactions between bird age and nest temperature, storage time, storage temperature and pre-setting temperature might explain some of the reported differences in the literature. Research on factors influencing hatchability is often done with hatching eggs originating from birds in or shortly after peak production, to provide maximum egg production and fertility. The results of our experiment indicate that eggs from these birds are less sensitive for non-optimal treatments and therefore will show less differences in hatchability when compared with eggs originating from older birds.

There are several mechanisms that can be involved in the reported relations between pre-incubation treatment and bird age. Fasenko et al. (1992) reported that the first egg in a sequence has a reduced fertility and hatchability. Because sequence length decreases with flock age, this might provide an explanation for the reported decrease in hatchability. The same authors discussed some of the factors possibly involved in the relation of bird age and hatchability. Reduced fertility and hatchability with increasing bird age might be related to a decline in the ability of the hens to retain spermatozoa in the sperm host glands, a lower quality follicle or preovulatory aging of the follicle.

It can be suggested that the decrease in hatchability of fertile eggs from older birds is related with the increased sensitivity of those eggs for non-optimal pre-incubation treatments.

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## CHAPTER 6

### MOISTURE LOSS OF HATCHING EGGS DURING STORAGE AND INCUBATION, THEIR INTERACTION AND INFLUENCE ON HATCHING RESULTS

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## ABSTRACT

1. The moisture loss of hatching eggs during storage and incubation, the interaction between them and the effects on hatching results were investigated.
2. Eggs produced by birds of 33 and 55 weeks of age were stored for 7 days at a high and low level of humidity, resulting in a moisture loss of 0.45% or 1.09%, respectively. During incubation, relative humidity levels were set to obtain moisture loss at standard levels or below standard levels during three different time periods.
3. Eggs with a high moisture loss during storage showed less moisture loss during incubation, when compared to eggs with a low moisture loss during storage. These effects were more pronounced for eggs produced by the older flock.
4. A significant influence of the storage treatment on hatching results was not observed.

## INTRODUCTION

The amount of weight lost during incubation influences hatchability (Lundy, 1969) and chick size (Tullett and Burton, 1982; Burton and Tullett, 1985). This weight loss is completely due to the evaporation of water from the egg, because the respiratory gas exchange of the embryo involves no change in mass (Drent, 1975). During the first half of the incubation process, the amount of moisture loss is constant. In the second half, the moisture loss is slightly increased as a result of the increased egg temperature (Ar, 1991; Meijerhof and van Beek, 1993). Meir et al. (1984) reported that hatchability of turkey eggs was optimal, when 12% of the initial egg mass was lost as moisture during the incubation process. Hulet et al. (1987) showed that hatchability of turkey eggs can be improved when the relative humidity during incubation is set to obtain 12% moisture loss instead of incubating at a fixed relative humidity level. Data presented by Tullett (1990) suggest that the negative influence of non-optimal moisture loss on hatchability of turkey eggs is more pronounced for moisture losses below than above the reported optimum.

Moisture loss during storage is normally not included when studying the influence of moisture loss on hatchability. It is often reported that relative humidity during storage should be maintained at a high level to achieve maximum hatching results (for a review, see Meijerhof, 1992). Mayes and Takeballi (1984) concluded that the moisture loss during storage adds up to the total moisture loss during incubation. They suggested that therefore attempts should be made to prevent moisture loss during storage. No other mechanisms regarding the influence of moisture loss during storage on

hatchability have been reported. Meijerhof (1992) suggested that correction during the incubation process for moisture loss during storage might limit the possible negative influences of low relative humidity during storage on hatching results.

The objective of the study reported here was to investigate (1) the moisture loss of hatching eggs during storage and incubation, (2) the interactions of moisture loss during storage and incubation and (3) the influence on hatching results.

## MATERIAL AND METHODS

### *Egg production and storage*

A total of 26,400 broiler type hatching eggs (Ross x Ross) was used for the experiment. Half of the eggs were produced by a flock at 33 weeks of age, the other eggs were produced by a flock at 55 weeks of age. Both flocks were fed a commercial broiler breeder diet and housed in a litter type housing, with roll-away laying nests. The eggs were produced in two days preceding the experiment, collected on pulp trays and stored at 18°C and 70-80% relative humidity. On the third day after start of collection, eggs were transported to the research station. There the eggs were placed on incubator trays, weighed and stacked in incubator lorry's, in order to provide adequate air circulation around the eggs. The eggs were then stored for 7 days in two climate controlled rooms. In both rooms, temperature was maintained at 16°C. In one room relative humidity was set at 85%. In the other room, relative humidity was set at 45%. After storage, trays were weighed, transported to the hatchery and fumigated for 20 minutes by heating 7 g of paraformaldehyde per m<sup>3</sup> before the incubation process started.

Moisture loss during storage was calculated as percentage weight loss from egg weight at the start.

### *Incubation*

Eggs were placed in four incubators (model 8400, Petersime, Belgium) and incubated at 37.5°C. The first 17 days of the incubation process was divided in three periods (day 1-7, 7-12 and 12-17). During these periods, relative humidity in the incubator was set at either the Control level (55% R.H.) or at the High level (70%), resulting in four different relative humidity treatments, one for each incubator (table 1). The weight loss during the first 7 days of incubation showed, that the difference between high relative humidity treatment and low relative humidity treatment was too

Table 1: levels of relative humidity set during incubation

treatment	days of incubation				
	1-7	7-12	12-17	17-20	21
*CCC	55 %	55 %	55 %	55 %	70 %
*HCC	70 %	55 %	55 %	55 %	70 %
*CHC	55 %	65 %	55 %	55 %	70 %
*CCH	55 %	55 %	65 %	55 %	70 %

\*CCC = R.H. set at level: Control - Control - Control

\*HCC = R.H. set at level: High - Control - Control

\*CHC = R.H. set at level: Control - High - Control

\*CCH = R.H. set at level: Control - Control - High

large to obtain the desired differences in weight loss of the hatching eggs. Therefore the level of the high relative humidity treatment was decreased with 5% to 65% from day 7 of incubation on.

Eggs were weighed prior to incubation and at 7, 13 and 17 days of incubation. At 17 days, all eggs were candled and divided into unfertile eggs, dead before 7 days of incubation, dead between 7 and 17 days of incubation and eggs containing a living embryo. Eggs classified as containing a living embryo were transported to the hatcher and fumigated with formaldehyde. In the hatcher, relative humidity was set at 55% until 20 days. During pipping of the chicks, relative humidity was set at 70%. At 21 days the hatch was pulled and the number of live chickens, dead in shell embryos and culled chickens was determined.

Moisture loss during incubation was calculated as the percentage of weight loss compared to the egg weight at day 0 of incubation.

### *Statistical analysis*

Moisture loss and hatchability were statistically analysed by means of analysis of variance in a completely randomised design within incubation treatment (split plot model). As a result of the experimental design incubation, humidity treatment is confounded with incubator and therefore a correct statistical analysis of the results of incubation treatment is not possible. The model used for analysing the egg weight loss was:

$$Y_{ijk} = \mu + I_i + e_i + A_j + S_k + IAS_{ijk} + e_{ijk}$$

where:

$\mu$	overall mean
$I_i$	incubation treatment
$e_i$	error term main plot
$A_j$	age
$S_k$	storage treatment
$IAS_{ijk}$	2-way and 3-way interaction
$e_{ijk}$	error term

Due to the low hatching results of the eggs from the flock at 33 weeks of age, these results were excluded from the analysis of the hatching results. The model used for analysing hatching results and moisture loss of the eggs produced by the flock at 55 weeks of age was:

$$Y_{ij} = \mu + I_i + e_i + S_j + SI_{ij} + e_{ij}$$

where:

$\mu$	overall mean
$I_i$	incubation treatment
$e_i$	error term
$S_j$	storage treatment
$e_{ij}$	error term

## RESULTS

The average egg weight and standard deviation before storage was 58.5 g 4.5 g for the eggs of the young birds and 64.9 g 5.1 g for the eggs of the older birds. In table 2 the mean observed moisture losses of the eggs during storage, during incubation (day 1 to 17) and the total moisture loss during storage and incubation until 17 days are presented. To obtain the moisture loss at 21 days of incubation, 2.1% and 2.3% moisture loss must be added to the data at 17 days for the eggs of the young birds and older birds, respectively. These percentages are based on the conductance of the shell, the moisture loss between day 13 and day 17 and the conditions in the incubator and hatcher. During storage, no significant differences in average moisture loss could be observed

Table 2: Moisture loss (percentage of the initial egg mass before storage) during storage, day 1-7, day 7-13, day 13-17 and day 1-17 and total moisture loss during storage and incubation (day 1 to 17)

age (weeks)	storage humidity (R.H.)	incubation treatment	% moisture loss of initial egg mass during:					
			storage	day 1-7	day 7-13	day 13-17	day 1-17	total
33			0.75 <sup>a</sup>	3.41 <sup>a</sup>	2.98 <sup>a</sup>	2.10 <sup>a</sup>	8.52 <sup>a</sup>	9.27 <sup>a</sup>
55			0.78 <sup>a</sup>	3.56 <sup>b</sup>	3.14 <sup>b</sup>	2.21 <sup>b</sup>	8.92 <sup>b</sup>	9.70 <sup>b</sup>
	high		0.45 <sup>a</sup>	3.57 <sup>a</sup>	3.12 <sup>a</sup>	2.19 <sup>a</sup>	8.88 <sup>a</sup>	9.33 <sup>a</sup>
	low		1.09 <sup>b</sup>	3.41 <sup>b</sup>	3.00 <sup>b</sup>	2.12 <sup>b</sup>	8.55 <sup>b</sup>	9.64 <sup>b</sup>
		*CCC	0.75	3.80	3.33	2.24	9.37	10.13
		*HCC	0.77	2.67	3.09	2.10	7.87	8.64
		*CHC	0.78	3.83	2.74	2.33	8.89	9.68
		*CCH	0.76	3.66	3.08	1.95	8.73	9.49

Means within columns and treatments with different superscripts are significantly different ( $p < 0.05$ ).  
(differences between incubation treatments are not statistically analysed)

\*CCC = R.H. set at level: Control - Control - Control

\*HCC = R.H. set at level: High - Control - Control

\*CHC = R.H. set at level: Control - High - Control

\*CCH = R.H. set at level: Control - Control - High

between eggs of flocks of different ages. The moisture loss during incubation is higher for the eggs originating from older birds than for the eggs produced by the younger birds.

Eggs stored at low relative humidity lost 0.64% (p) more moisture during the storage period than eggs stored at high relative humidity. Differences in relative humidity during storage resulted in a significant difference in moisture loss during incubation. Eggs stored at low relative humidity and thus had a high moisture loss during storage, experienced a 0.33% (p) lower moisture loss during incubation. In total, eggs stored at low relative humidity lost 0.31% more moisture during storage and incubation (day 1 to 17) than the eggs stored at high relative humidity. During incubation, an increased relative humidity during one of the experimental periods, resulted in a significant reduction of moisture loss during that period. Treatments with an equal relative humidity setting for the same period, sometimes showed a significant difference in moisture loss for that period, probably due to differences between incubators.

In table 3, moisture loss for each treatment is given during storage and during incubation. This table shows, that within a storage treatment the moisture loss differed not significantly between ages. For both ages, moisture loss of eggs during incubation was influenced by storage treatment. The differences in moisture losses during incubation was higher for eggs produced by the flock at 55 weeks of age than for eggs produced by the birds at 33 weeks of age. Moisture loss during incubation was significantly affected by the storage treatment for the eggs produced by the older birds. For the younger birds, differences are significant only for the HCC incubation treatment. Within the control incubation treatment (CCC) differences in moisture losses during incubation between storage treatments were 0.25% for the eggs of the flock at 33 weeks of age and 0.51% for eggs of the flock at 55 weeks of age.

Table 4 shows the moisture loss during storage and incubation (day 1 to 17) and the hatching results of the eggs of the older birds. Due to the influence of storage humidity on moisture losses during incubation, differences in cumulative moisture losses between storage treatments were smaller than the differences in moisture losses during storage. No significant differences in hatching results within incubation treatments were observed between the storage treatments.

Table 3: Moisture loss (percentage of the initial egg mass before storage) during storage and incubation (day 1 to 17)

age (weeks)	storage humidity (R.H.)	% moisture loss during storage				% moisture loss during incubation				
		incubation Treatment	*CCC	*HCC	*CHC	*CCH	*CCC	*HCC	*CHC	*CCH
33	high		0.43 <sup>a</sup>	0.42 <sup>a</sup>	0.49 <sup>a</sup>	0.42 <sup>a</sup>	9.25 <sup>a</sup>	7.85 <sup>a</sup>	8.74 <sup>ab</sup>	8.64 <sup>a</sup>
	low		1.02 <sup>b</sup>	1.10 <sup>b</sup>	1.08 <sup>b</sup>	1.08 <sup>b</sup>	9.00 <sup>a</sup>	7.57 <sup>a</sup>	8.62 <sup>a</sup>	8.46 <sup>a</sup>
		difference	0.59	0.68	0.59	0.66	0.25	0.28	0.12	0.18
55	high		0.45 <sup>a</sup>	0.46 <sup>a</sup>	0.46 <sup>a</sup>	0.44 <sup>a</sup>	9.88 <sup>b</sup>	8.31 <sup>b</sup>	9.23 <sup>b</sup>	9.16 <sup>b</sup>
	low		1.10 <sup>b</sup>	1.10 <sup>b</sup>	1.11 <sup>b</sup>	1.10 <sup>b</sup>	9.37 <sup>c</sup>	7.73 <sup>a</sup>	9.00 <sup>ab</sup>	8.67 <sup>a</sup>
		difference	0.65	0.64	0.65	0.66	0.51	0.58	0.23	0.49

Means within columns without common superscripts are significantly different ( $P < 0.05$ )

\*CCC = R.H. set at level: Control - Control - Control

\*HCC = R.H. set at level: High - Control - Control

\*CHC = R.H. set at level: Control - High - Control

\*CCH = R.H. set at level: Control - Control - High

Table 4: % moisture loss during incubation, % moisture loss during incubation and storage, % fertility, mortality from 1-7 days, 7-17 days and 17-21 days as % of fertile eggs and % of chickens hatched from fertile eggs produced by birds at 55 weeks of age

incubation treatment	storage humidity (R.H.)	% moisture loss during storage and incubation	% fertility	percentage of fertile eggs			
				mortality day 1-7	mortality day 7-17	mortality day 17-22	chicks
*CCC	high	10.34 <sup>a</sup>	80.6 <sup>a</sup>	4.8 <sup>a</sup>	0.1 <sup>a</sup>	17.5 <sup>a</sup>	77.7 <sup>a</sup>
	low	10.47 <sup>a</sup>	79.1 <sup>a</sup>	6.1 <sup>a</sup>	0.2 <sup>a</sup>	19.6 <sup>a</sup>	74.1 <sup>a</sup>
*HCC	high	8.76 <sup>a</sup>	81.9 <sup>a</sup>	5.8 <sup>a</sup>	0.1 <sup>a</sup>	16.5 <sup>a</sup>	77.7 <sup>a</sup>
	low	8.83 <sup>a</sup>	81.0 <sup>a</sup>	4.9 <sup>a</sup>	0.2 <sup>a</sup>	15.8 <sup>a</sup>	79.1 <sup>a</sup>
*CHC	high	9.68 <sup>a</sup>	81.8 <sup>a</sup>	6.4 <sup>a</sup>	0.2 <sup>a</sup>	16.8 <sup>a</sup>	76.5 <sup>a</sup>
	low	10.10 <sup>b</sup>	80.7 <sup>a</sup>	7.2 <sup>a</sup>	0.2 <sup>a</sup>	15.7 <sup>a</sup>	77.0 <sup>a</sup>
*CCH	high	9.62 <sup>a</sup>	81.3 <sup>a</sup>	7.0 <sup>a</sup>	0.2 <sup>a</sup>	17.5 <sup>a</sup>	75.3 <sup>a</sup>
	low	9.78 <sup>a</sup>	81.6 <sup>a</sup>	8.5 <sup>a</sup>	0.1 <sup>a</sup>	17.8 <sup>a</sup>	73.6 <sup>a</sup>
avg.	high	9.60 <sup>a</sup>	81.4 <sup>a</sup>	6.0 <sup>a</sup>	0.2 <sup>a</sup>	17.1 <sup>a</sup>	76.8 <sup>a</sup>
	low	9.79 <sup>b</sup>	80.6 <sup>a</sup>	6.7 <sup>a</sup>	0.2 <sup>a</sup>	17.2 <sup>a</sup>	75.9 <sup>a</sup>

Means within columns and incubation treatments without common superscripts are significantly different ( $p < 0.05$ )

\*CCC = R.H. set at level: Control - Control - Control

\*HCC = R.H. set at level: High - Control - Control

\*CHC = R.H. set at level: Control - High - Control

\*CCH = R.H. set at level: Control - Control - High

## DISCUSSION

Ar (1991) stated that water movements between egg and environment is essentially a physical process. Moisture loss of eggs through evaporation, both during storage and incubation, is related to egg shell conductance, egg and ambient temperature and relative humidity and can be calculated by Fick's law (Simkiss, 1980). The observed differences in moisture losses during storage in this experiment can completely be explained by the differences in relative humidity during storage. The total amount of moisture lost during storage is small compared to the moisture losses during incubation, even when storage is extended. For longer storage periods, a lower storage temperature is advised (see for review Meijerhof, 1992). This results in a reduction of water vapour pressure deficit and thus in a reduction of moisture loss (Meijerhof and van Beek, 1993).

Our results show, that the amount of moisture lost by the hatching eggs during storage

influences the moisture loss during incubation. Due to this compensation-like mechanism, the total moisture loss during storage and incubation differs less than the differences in moisture loss during storage. This correction-like mechanism is at least present from the beginning of the incubation process until 17 days of incubation. Weight loss was not measured in the final part of the incubation process, but there are no indications for the absence of the reported mechanism in this period. If the mechanism does also occur in the final parts of the incubation process, differences between storage treatments in total moisture loss during storage and incubation will be smaller than those in this experiment.

The eggs produced by the older birds experience during incubation a higher compensation for high moisture loss during storage than the eggs produced by the younger birds. Also, the amount of compensation was not equal in each incubation treatment. Especially eggs with a low moisture loss in the period from day 7 to day 13 of incubation, had a smaller difference in total moisture loss during incubation between storage treatments, regardless of bird age.

The mechanism responsible for this effect remains to be clarified. If moisture loss during incubation is a strictly physical process, the explanation must lie in changes in the egg shell or shell membranes, resulting in a difference in conductance, or changes in the egg albumen, resulting in a difference in water vapour pressure deficit. If changes in within-egg water movements are responsible for the effect, then moisture loss from eggs is not strictly a physical process, but physiological processes are also involved. The moisture loss during storage and incubation indicates, that differences in conductance between the age groups is limited. The observed differences in compensation effects for moisture loss during storage between age groups are therefore probably not solely due to differences in conductance. Also the difference in compensation effects between incubation treatments can not be explained by differences in conductance. Seymour and Piper (1988) reported that during early incubation albumen increases in osmolarity. When a similar effect occurs during storage, this might give an explanation for the compensating influence reported. However, the reported changes in osmolarity are rather small and do not provide an explanation for differences between age groups and incubation treatments as found in this experiment. No experiments reporting similar results or indications for physiological processes were found in the literature. The results obtained, indicate that the involvement of physiological processes in the moisture loss of eggs needs to be considered further.

The average hatchability from fertile eggs of 76% obtained in our experiment was well below

the practical standards of about 90%. This was mainly due to a high mortality occurring after transportation to the hatcher. The reason for this high mortality is not clear, although a restricted ventilation rate during hatching could be causal. It is assumed, that the conclusions drawn from the results of the experiment are not altered by the low hatching results.

A high relative humidity and, therefore, a low moisture loss during storage is reported to improve hatching results when compared to low relative humidity (for review, see Meijerhof, 1992). Mayes and Takeballi (1984) stated that moisture loss during storage should be limited in order to provide an optimum amount of moisture loss at hatch and, thereby, improve hatching results. In our experiment, the total moisture loss during incubation was limited. Based on the moisture loss from 13 to 17 days of incubation, an average total moisture loss during incubation of the eggs of the older birds in the CCC incubation treatment of 11.9% was calculated. The average moisture loss of the other incubation treatments was lower. Data presented by Tullett (1992) suggest, that moisture loss below the reported optimum of 12% decreased hatchability. Although moisture loss during incubation in our experiment was limited, especially for incubation treatments with a period of reduced moisture loss, no significant differences in hatching results were observed between storage treatments (*p*-value percentage chickens from fertil eggs between storage treatments: 0.48). This is not in agreement with results of Funk and Forward (1951), Kosin and Konishi (1973) and Proudfoot (1976), who reported a decreased hatchability when eggs were stored under low relative humidity conditions. In their experiments, eggs were stored for longer periods, what might be of influence on the mentioned differences in hatching results. In the present experiment, differences in total moisture loss are smaller than differences in storage moisture loss, what leads to the suggestion that the influence of moisture loss during storage on hatching results is, under practical conditions, limited. This is in agreement with the results of Kaufman (1939) who concluded that dehydration is not the main cause for the increase of embryo mortality after prolonged storage.

Snyder and Birchard (1982) concluded, that desiccation in the first part of incubation is more harmful than desiccation in the later part of incubation, when both are corrected to a similar moisture loss during total incubation. Although no statistical analysis can be presented, the results of our experiment do not confirm this conclusion. Correction of desiccation during a certain part of the incubation process by an increased moisture loss during storage, did not significantly affect hatchability for any of the incubation treatments.

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## GENERAL DISCUSSION

The pre-incubation treatment of hatching eggs influences mortality of embryos between oviposition and hatching. To maximize hatchability of fertile eggs, pre-incubation conditions must be adjusted towards the demands of the embryo. The aim of this study was (1) to formulate analytical equations to describe the temperature development and moisture loss of hatching eggs in the pre-incubation period, (2) to determine the actual conditions in this period that occur in field situations and (3) to determine the influence of these conditions on hatchability.

The results of the experiments reported in this thesis indicate that temperature before and during storage and storage time have a major impact on hatching results, depending on bird age. Interaction between storage temperature below the physiological zero and storage length indicates that presence or absence of detectable embryonic development during storage is not the only factor influencing hatchability. The reported positive effect of low storage temperature during long storage periods on hatchability can be explained by assuming that these conditions decrease the rate of decline of the quality of the embryo or egg content.

To explain the positive effect of high storage temperature during short storage periods on hatchability as reported in the literature, the assumption must be made that the embryo or the egg content has to reach a certain stage of development during storage to obtain maximum hatchability results.

A possible explanation for the reported effects can be changes in the quality of the albumen, expressed by pH of the albumen.

### *Albumen pH*

During storage of the egg, albumen pH increases from approximately 7.6 to a level of approximately 9.5, associated with a reduction in albumen height. The increase in albumen pH is due to reduction of the bicarbonate buffering system as a result of carbondioxide loss. The rate of increase and the resulting pH level at the start of incubation is a function of storage time, storage temperature, transport of carbondioxide through the albumen, conductance of the shell and membranes for carbondioxide and carbondioxide concentration outside the egg. The pH of the yolk remains at approximately 6.5. As a result, difference between albumen pH and yolk pH can be as high as 3 pH units at the start of the incubation process. This means that at the start of incubation the blastoderm, being one layer of cells thick and separated from the albumen only by the vitelline membrane, experiences a very alkaline environment on the dorsal side of the epiblast, while its

basal surface experiences a slightly acid environment. A gradient of this level over tissues is rather unique in adult animals. Only highly specialised cells as found in the stomach experience gradients of this magnitude.

One of the functions of the high pH level of the albumen can be related with protection against microbial contamination. The highly alkaline level of the albumen will form a line of defense against micro-organisms before the development of any immune and phagocytic system of the embryo (Board and Fuller, 1974).

The magnitude of the pH gradient over the blastoderm influences the physiological status and transportation of inorganic ions and the electrical potentials that they generate. It has been reported that a certain gradient is important for early development of the embryo. One of the functions of this gradient seems to be connected with the organisation of the cells to form the dorsal-ventral polarity of the epiblast and therefore with the organisation of the early development, especially of the primitive streak (for review, see Stern, 1991). Gillespie and MacHanwell (1987) reported that intra-embryonic pH within the early chick embryo between the 8- and 20-somite stages is approximately 8.3. The same authors reported that at this stage of development a gradient of approximately 0.5 pH units is present in the embryo between the head and the tail region.

From the results presented in the literature it can be assumed that a specific pH gradient over the blastoderm will be optimal for early embryo development and therefore hatchability. This gradient will probably be reached at a pH level of the albumen of approximately 8.2, according to the results of Sauveur et al. (1967). If this hypothesis is correct, storage conditions can be evaluated by their influence on pH level of the albumen at the moment of setting. In chapter 1 the mechanisms influencing the pH level of the albumen during storage are discussed. The results of the experiments presented in this thesis show that hatchability decreases with increasing storage length, especially at low storage temperatures. The results obtained in the experiments can be partly explained by differences in pH of the albumen.

It is reported (see chapter 1) that albumen pH reaches a certain plateau after a period of storage, with the level of the plateau depending on storage temperature. During storage at low temperature, the final level of albumen pH remains lower than during storage at high temperature. This means that at low storage temperature the obtained albumen pH is closer to the optimum than at high storage temperature, what can explain the reported hatchability results. Another explanation can be that the decrease in viability of the embryo or the decrease in suitability of the egg content for

embryo development as a result of aging will be retarded when eggs are stored at low temperature, because of a decrease in the rate of biochemical processes. The decrease in hatching results as a result of storage beyond seven days can not be explained by the increase of albumen pH, because after seven days of storage no changes in albumen pH occur. It is more likely that the reported decrease in hatchability after prolonged storage periods is due to retardation of the viability of the embryo or decrease in the egg characteristics for embryo development, as mentioned earlier. Pre-setting treatments that will result in lowering the albumen pH before setting after prolonged storage might be beneficial for hatching results, according to the literature (chapter 1). In the experiments reported in this thesis (chapter 5), a decrease in hatchability was found when eggs were warmed for 16 h at 27°C before setting. No interaction with storage time was observed. The temperature treatment used will result in some metabolic activity and carbon dioxide production, what will result in a reduced albumen pH. According to the hypothesis mentioned, this is expected to result in an increased hatchability after prolonged storage, in accordance with the results mentioned in the literature. This is not confirmed by our results.

The albumen height, expressed as Haugh units, of day old eggs decreases with increasing age of the flock (Williams, 1992). This is in agreement with Sauter et al. (1954), who reported that during storage the increase of albumen pH and decrease of albumen height was more rapid in eggs of older birds than in eggs of younger birds. Sauter et al. (1954) reported that the final level of albumen pH after storage was higher in the eggs of older birds than in the eggs of younger birds. This observation explains the differences in hatchability after storage between the age groups, as observed in our experiments. An increased development of albumen pH means that the pH level will be above the optimum sooner than when the development of the albumen pH is more slowly. This means that eggs produced by older birds will experience a decrease in hatchability after a shorter storage period than eggs from younger birds. It also means that storage for longer period at low temperature will be more beneficial for eggs from older birds than for eggs from younger birds, because the limitation of the increase in albumen pH is more important for hatchability for eggs of older birds. This is in agreement with the results reported in this thesis.

The reason for the differences between age groups in albumen pH and albumen quality after storage is not clear. The increasing egg weight is not likely to be the cause, because Haugh units are corrected for egg weight. The correlation between conductance and age indicates that the release of carbon dioxide from the egg content will be more rapidly for eggs from older birds. However, it

can be questioned if differences between age groups in conductance, corrected for egg mass, will be high enough to explain the differences observed. Also the difference in final pH level indicates that the albumen pH is not influenced by conductance only.

After storage periods of 0-1 days, the pH level will be below the value of 8.2, according to Sharp and Powell (1931). If a pH level of 8.2 must be reached before setting, this means that setting of eggs within 2 days after production will result in a reduced hatchability. This is confirmed by field observations (Brake, 1993; personal communication). Theoretically, storage of eggs for short periods at higher temperatures will result in an increased pH level at setting and therefore hatchability, which is in agreement with the literature (see chapter 1). However, this is not confirmed by the results of the experiments presented. A possible explanation can be that the storage period of 2 days was just enough to obtain an adequate pH level, even at low storage temperatures. This is in agreement with the results of Goodrum et al. (1989), who reported that pH value of albumen of table eggs was 8.4 after 1 day of storage at 4°C. Storage for 1 day at 24°C resulted in pH value of the albumen of 8.5.

In general, the influences of the pre-incubation treatments on hatching results were not limited to the start of the incubation process. Treatments resulting in a reduced hatchability increased mortality throughout the incubation process. This indicates that before incubation vital changes can occur within the egg or embryo. The nature of these changes are not known. One explanation can be that the environment for development or the embryo itself is negatively influenced by the storage conditions, resulting in non-optimal growing conditions that persist during the incubation process. Another explanation can be that the initiation the development of the embryo is retarded by the storage treatments. This means that the initiation of the development of the embryo is not complete and fully successful, which will influence the viability of the embryo during the entire incubation period. This also can explain the reported effects of storage conditions of hatching eggs on chick quality after hatching and during the growing period.

If pH value of the albumen at setting is an important factor for hatchability, it can be assumed that also the ratio thick albumen/thin albumen, albumen height or Haugh unit score can be used as an indicator to predict hatchability and susceptibility of eggs for storage length and storage conditions.

*Temperature in the nest*

Because rate of development and plateau level of albumen pH are influenced by temperature conditions, an effect of temperature during the nesting period on albumen pH can be questioned. If this effect is present, it might contribute to an explanation for the reported effects of nest temperature on hatchability. No experimental data on this subject are to the authors knowledge, although Skala and Swanson (1962) stated that albumen quality in freshly laid eggs is not associated with pH value of the albumen.

Several authors reported a relation between embryonic development at oviposition and hatching results. In general, it is reported that an advanced development before storage is beneficial for hatching results, especially when increased storage periods are applied. If this is correct, temperature treatments simulating the conditions in litter nests and therefore resulting in an advanced embryonic stage before storage are expected to be beneficial for eggs to sustain storage conditions. This is not confirmed by the results of the experiments reported in this thesis, because no interactions between nest temperature treatment and storage conditions have been observed. On the contrary, temperature treatments resulting in an advanced embryonic stage resulted in a slightly reduced hatchability, indicating a decreased viability of the embryo.

A possible explanation for the differences between the literature cited and the results presented in this thesis might be the embryonic development at oviposition. The results presented in the literature are often obtained from experiments with Leghorn type birds. If the modern breeder type birds produce eggs with more advanced embryos at oviposition, the effects of pre-incubation treatments resulting in an advanced embryonic stage during storage will be limited. No data on this subject are to the author's knowledge. When a specific embryonic stage is optimum for embryo development during storage, temperature conditions as reported in litter nest might cause an embryonic development beyond that specific embryonic stage, resulting in a reduced hatchability. However, if there is a specific optimum embryonic stage for storage of hatching eggs in the range that is reached in the experiments reported in this thesis, some interaction between nest temperature treatment and storage conditions or storage length could be expected. This is not confirmed by the results reported in this thesis.

Decreased hatching results after high temperature treatment during the nesting period can also be explained by a negative influence of high temperature in the laying nest on quality of the egg content and therefore the growing conditions for the embryo. This might explain the absence of

interaction with storage conditions or storage time. It might also explain the reported relation between bird age and nest temperature treatment, because it is reported that albumen quality of freshly laid eggs decreases with increasing bird age (Hill and Hall, 1980). This hypothesis does not explain the differences between the literature cited and the results presented in this thesis.

Because time lap between oviposition and collection is not equal for all eggs produced, variation in embryo development will occur when the eggs are collected from litter nests. Eggs produced in roll-away nest are not protected against heat loss or warmed by the birds and will therefore directly start to cool to the environmental temperature. Calculations as described in chapter 2 indicate that under average climatic conditions in the Netherlands, these eggs will reach a temperature below the physiological zero within 30 minutes, depending on the actual climatic conditions in the nest. It therefore can be expected that eggs produced in roll-away nests have a more similar rate of embryo development when collected than eggs produced in litter nests. As a result of the expected difference in variation of embryonic development between nest types, it can be expected that variation in stage of embryonic development within a batch of eggs during incubation is higher for eggs produced in litter nests than in roll-away nests. Theoretically this will result in an increased variation in hatching time for the eggs produced in litter nests. No experimental data on this subject are to the author's knowledge.

#### *Temperature during storage*

The temperature development of eggs during storage depends on the method of storage, storage conditions, initial egg temperature and place of the egg in the container. Approximately 30% of eggs placed in a container are located in the outside layer of the container. The remaining 70% of the eggs are located in centre position. It is demonstrated (chapter 4) that eggs stored on cardboard trays centre position in egg containers experience a longer half time during both cooling and warming than eggs stored at the outside layer of the container. As a result, the temperature of the eggs collected from litter nests and stored directly on cardboard trays in the centre of the container will be above the physiological zero for a longer period than the temperature of the same eggs stored on plastic trays or incubator trays or at the outside layer of the container. The results described in chapter 3 show that after oviposition temperatures above the physiological zero have a slightly detrimental effect on hatchability. This indicates that storage of hatching eggs from litter nests before cooling on cardboard trays in containers must be considered as having a negative

influence on hatchability, because similar conditions as observed in litter nests will occur. Detrimental effects under these conditions will be increased when coverages are used and decreased when air movement in the storage room is increased. Detrimental effects in identical situations will be limited or non-existing when plastic trays or incubator trays are used, depending on storage conditions.

Warming of eggs stored in containers takes longer than cooling of eggs. Differences in half warming time between storage methods and storage conditions are in the same range as differences in half cooling time. Because hatching eggs are commercially stored at temperatures below the physiological zero, this phenomenon will not influence the stage of embryonic development during storage. However, as a result of the slow adaptation to environmental temperatures, eggs collected from roll-away nests under low environmental temperature conditions and stored on cardboard trays will experience a slow warming rate. This results in an increased risk of condensation on the eggs while unpacking them at the hatchery, which will have a negative influence on hatching egg quality because of increased risk of micro-biological contamination and mould growth.

#### *Moisture loss during storage and incubation*

It has been reported that, for maximal hatchability, moisture loss until pipping must be approximately 12%-14% of the initial egg mass (Meir et al., 1984; Hulet et al., 1987). It is not clear whether this percentage must be realised during incubation, or that moisture loss before incubation must be added to the total moisture loss. The results reported in this thesis indicate that under normal conditions the moisture loss before incubation will be relatively small compared to the total moisture loss. No influence of moisture loss before incubation on hatchability was observed. To be able to evaluate the significance of moisture loss before incubation on hatchability, the function of moisture loss for embryo development must be evaluated.

The initial water fraction of the egg is equal to that of the fresh hatchling. Due to metabolic activity, approximately 15% of the initial egg mass must be lost until pipping to obtain this level. According to Decuyper et al. (1979), energy production through metabolic activity is approximately 10 times as high on day 19 of incubation when compared with energy production on day 10 of incubation. This means that, in the first part of the incubation process, metabolic rate and, therefore, metabolic water production is low. When moisture loss functions as a method to dispose of metabolic water, it can be suggested that a constant level of water fraction in the egg

will be beneficial for the development of the embryo. This means that, due to the increase in metabolic activity in the last part of the incubation process, a proportional amount of moisture loss would be beneficial for hatchability. It is normally advised to set the relative humidity during incubation at a constant level to reach a constant level of moisture loss. As a result of the metabolic activity, an increase in egg temperature and, therefore, a slight increase in water vapour pressure deficit and moisture loss in the second half of the incubation process occur. This increase is by far not enough to match the increase in metabolic activity. No evidence for beneficial effects of increasing the rate of moisture loss at the end of the incubation process on hatchability is to the authors knowledge. When the primary function of moisture loss is to create a constant water fraction during the incubation process, moisture loss during storage can have a negative influence on hatching results, because metabolic activity and therefore metabolic water production does not occur during storage. However, because correction for the moisture loss according to the metabolic water production has not been reported to be beneficial for embryo development, it is likely that especially the total amount of moisture loss at the end of the incubation process is important for embryo development. This would mean that moisture loss during storage must be added to the total moisture loss.

Severe desiccation during the early stages of incubation is reported to be more harmful for embryo development than desiccation in the later part of the incubation process (Snyder and Birchard, 1982). Because albumen increases in osmolarity with evaporation, it is suggested that the very young embryo is more susceptible for osmotic changes in its surrounding than mature embryos (Ar, 1991a). Another explanation can be that the relative high moisture loss compared to the metabolic water production during early incubation, as explained earlier, provides a relative imbalance in water content during early incubation. Desiccation will add to this imbalance and, therefore, might have a negative influence on embryo development. In the last part of incubation, metabolic water production is high compared to the moisture loss and hence desiccation will not add to the imbalance. When desiccation during early embryonic development is harmful, moisture loss during storage must be avoided. However, it can be questioned if under normal storage conditions moisture loss before incubation will be high enough to create an environment during incubation that will influence the osmotic balance of the embryo.

For the expansion of the respiratory airsacs and the initiation of lung respiration, an air cell must be formed by pipping time. To create an adequate air cell volume, a minimum of 10% moisture

loss must occur at the moment of pipping (Ar, 1991b). When the primary function of moisture loss is to create an adequate air cell at the time of pipping, moisture loss during storage must be added to the total moisture loss.

In the experiments presented in this thesis, a high amount of moisture loss during storage was followed by a reduced amount of moisture loss during the incubation period. The nature of this correction-like mechanism is not known. However, it seems that the incubated egg or the developing embryo can respond in some way to an advanced moisture loss during storage. This suggests that, although moisture loss is a very important factor during the complete incubation process, under commercial conditions a slightly increased moisture loss during storage can be accepted.

#### *Effect of bird age*

The results presented in this thesis indicate that eggs produced by older birds are more susceptible for non-optimal pre-incubation conditions than eggs produced by birds during the peak period of lay. It was mentioned before that a possible explanation can be found in the decrease of the albumen quality and the increase of pH of the albumen.

Another explanation might be the decrease of the quality of the embryo. Mather and Laughlin (1979) reported a slightly higher number of embryo abnormalities in eggs of very young and very old birds than in eggs of birds of 31-49 weeks of age. Fasenko et al. (1992) hypothesized that the increasing number of first-of-sequence eggs, defined as the first egg after a nonlaying day, with increasing flock age might be related with decreasing hatching results. However, these authors were not able to demonstrate this effect with broiler breeder eggs. Petersen (1984) reported that extreme large eggs had a low hatchability what could be an explanation for the observed differences because egg size increases with bird age. Landauer (1967), however, stated that decreased hatchability due to egg weight was more related with a high variation in egg weight, resulting in sub-optimal incubation conditions than with the increased average egg weight itself.

#### RECOMMENDATIONS FOR FUTURE RESEARCH

The temperature development of eggs in containers cannot be calculated accurately with the methods presented in this thesis. To be able to evaluate new methods of egg storage regarding to their influence on temperature development, more knowledge about heat transport by combined

free and forced convection must be egged on.

It is shown in this thesis that the influence of pre-incubation treatments on hatchability is related with the age of the breeder flock. In this thesis, the effect of the treatments was studied, not the mechanisms involved. To be able to understand the mechanisms involved in the retardation of the environment for the embryo or the embryo itself, research on the physiological differences between eggs produced by birds at different ages is necessary. More research into the function of the pH gradient in the egg for the embryo and its relation with the quality of the medium for embryo development is necessary for a better understanding of the mechanisms involved.

Moisture loss during storage influences the moisture loss during incubation. The mechanisms involved in this compensation like effect are not understood. It is not clear whether the effect is caused by the developing embryo or by changes in the albumen or shell membranes. To be able to evaluate the basic function of the moisture loss during incubation and to quantify the significance of the moisture loss during storage for the incubation process, research involving this mechanism has to be carried out.

#### PRACTICAL IMPLICATIONS OF RESULTS

The results presented indicate that roll-away nests must be preferred over litter nests to obtain maximum hatching results. Storage on incubator trays or plastic trays is advisable for optimum temperature development during storage, especially when litter nests are used. The frequency of transportation of the eggs to the hatchery must be adjusted to the age of the breeder flock. Eggs produced by older flocks must be gathered twice a week, to prevent long storage times. It can be questioned whether transportation of eggs produced by younger flocks to the hatchery twice a week is advisable. When the presented hypothesis about the decline of the albumen quality is correct, setting within two days after production is not advisable. Less frequent transportation to the hatchery will result in a smaller part of the eggs set within two days after production. Also for hygienic standards and prevention of uncontrolled temperature shocks, less frequent transportation to the hatchery is advisable.

In general, storage temperatures can be lowered when outside temperature conditions are low. This will result in a reduction of energy costs because of reduction of heating capacity and reduction of added moisture.

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## SUMMARY

In the Netherlands, approximately 800 million hatching eggs per year are produced on highly specialized broiler breeder farms. On these farms, the eggs are produced and stored for several days. Normally once or twice a week the eggs are collected from the farms and transported to the hatchery, where they are set and incubated.

Between production of the eggs and setting, a certain amount of mortality occurs in the eggs. As a result of this mortality and because some embryos are not able to complete the pipping process in time, about 10% of the fertile eggs do not hatch. A certain percentage of these eggs are not hatching because the conditions in the period before incubation are not set at a level that is optimal for embryo survival. To minimize the number of non-hatching fertile eggs, the conditions during the pre-incubation period must be set at a level that meets the demands of the embryo. In commercial situations, the management decisions concerning the egg handling that have to be taken in the pre-incubation period are mainly influencing the effects of time, temperature and relative humidity.

The aim of the studies presented in this thesis was (1) to formulate analytical equations to calculate the temperature development and moisture loss of hatching eggs, (2) to determine the climatic conditions in the pre-incubation period that can occur in field situations and (3) to determine the influence of these conditions on hatchability.

In chapter 1 a review of the literature concerning the treatment of eggs in the pre-incubation period is presented. From this review, it can be concluded that the majority of the research on this subject is done in the period before 1970. The availability of experimental results on this subject with eggs produced by the modern type broiler breeder is limited. In chapter 1, some hypotheses are formulated to explain the influence of storage time and storage temperature on hatchability.

With the analytical equations formulated in chapter 2, the effect of climatic conditions on the temperature development and moisture loss of eggs can be calculated. Therefore, climatic conditions that occur in field situations can be evaluated on their influence on the eggs. One of the conclusions from these calculations is that air velocity will have a major influence on internal egg temperature in the second half of the incubation process.

In chapter 3 and 4, the results of the calculations based on the equations are compared with measurements. The results show that temperature development and moisture loss of eggs can be predicted rather accurately when eggs are placed in litter nests and roll away nests. When birds have access to litter nests, eggs are warmed by the birds. Under these conditions, accurate calculation of

temperature and moisture loss is not possible. During storage, no accurate calculation of temperature and moisture loss could be made, because of natural convection in hatching egg containers and protection of the storage system against moisture loss.

In chapter 3, the temperature and moisture loss of eggs during their period in the nest boxes is examined. The results indicate that eggs produced in litter nests are warmed by the birds to a temperature above the minimum level for embryo development. Eggs produced in roll-away nests are not warmed by the birds and their cooling process starts immediately after production, with the cooling rate and final temperature level depending on the climatic conditions in the house. Moisture loss of eggs in produced in litter nests is higher than in roll-away nests, but relatively low compared to the total moisture loss during incubation.

During storage, temperature and moisture loss characteristics are dependent on the position of the eggs in the containers, the method of storage and the storage conditions (chapter 4). Eggs placed in centre position of containers experience a slow warming and cooling process when compared with eggs in side positions. When eggs are stored on incubator trays, cooling and warming of eggs is more rapidly than when eggs are stored on cardboard trays. The rate of cooling and warming of eggs stored on plastic trays is intermediate. When air velocity is increased, the cooling and warming rate of eggs increases. The use of coverages over containers decreases the rate of temperature change.

Moisture loss of eggs in containers can not be calculated directly from temperature and relative humidity of the air surrounding the container. The containers provide a protection against moisture loss, probably due to the presence of a boundary layer around the eggs, which forces the eggs to loose less water than is expected from the climatic conditions.

The influence of temperature during the pre-incubation period on hatchability is examined in chapter 5. The results indicate that the influence of storage time and pre-incubation temperature on hatchability is related with age of the flock. Pre-incubation treatments that can be classified as having a negative influence on hatchability were more detrimental for eggs of older birds than for eggs of younger birds.

The results presented in chapter 5 indicate that hatchability of eggs that has experienced a high temperature during the nesting period is lower than of eggs that has experienced a low temperature in this period. This indicates that eggs produced in litter nests will have a lower hatchability than eggs produced in roll away nests, due to differences in egg temperature. From the results presented

in this chapter it can be concluded that eggs of younger birds can be stored for longer periods than eggs of older birds. For both age groups, the combination of longer storage periods and low storage temperature is beneficial for hatchability compared to longer storage periods and high storage temperature. According to the results presented in this chapter, low storage temperatures can also be used when eggs are stored for relatively short periods.

In chapter 7, some possible explanations for the reported relations between storage time, storage temperature and bird age are discussed. It is suggested that the development of albumen pH determines the suitability of eggs for storage and that storage conditions can be evaluated on their influence on the albumen pH development.

In chapter 6, the influence of moisture loss during storage on moisture loss during incubation and hatchability is reported. The results presented in this chapter indicate that moisture loss during storage influences moisture loss during incubation. As a result, relative humidity during storage has a smaller effect on total amount of moisture lost between oviposition and hatching than would be expected from the amount of moisture lost during storage. Moisture loss during storage had no significant effect on hatchability.

## SAMENVATTING

In Nederland worden per jaar ongeveer 800 miljoen broedeieren van slachtrassen geproduceerd op gespecialiseerde vermeerderingsbedrijven. Op deze bedrijven worden de eieren na productie gedurende enige dagen bewaard. Veelal worden de eieren één à twee keer per week opgehaald en getransporteerd naar de broederij, waarna het broedproces wordt gestart.

Tussen productie van de eieren en het inleggen in de broederij treedt een zekere hoeveelheid sterfte van embryo's in de eieren op. Hierdoor en omdat een aantal embryo's niet in staat is het uitkomstproces tijdig af te ronden, levert gemiddeld ongeveer gemiddeld 10% van de bevruchte eieren geen kuiken op. Een zeker percentage van deze eieren komen niet uit omdat de condities in de periode voor het eigenlijke broedproces niet goed afgestemd zijn op de behoeften van het embryo. Op commerciële bedrijven beperkt de invloed van de behandeling van broedeieren in de periode voor het broeden zich vooral tot de invloed van tijd, temperatuur en relatieve luchtvochtigheid.

Het doel van het gepresenteerde onderzoek is (1) het opstellen van analytische vergelijkingen waarmee de temperatuur en vochtverlies van eieren berekend kan worden, (2) het vaststellen van de klimaatscondities zoals die in de periode voor het broedproces in praktijksituaties voorkomen en (3) het bepalen van de invloed van deze klimaatscondities op broedresultaten.

In hoofdstuk 1 is een overzicht gegeven van de literatuur die betrekking heeft op de behandeling van broedeieren voor het broedproces. Uit dit overzicht kan geconcludeerd worden dat het merendeel van het betreffende onderzoek uit de periode voor 1970 is. Resultaten met broedeieren geproduceerd door het moderne type moederdier zijn slechts beperkt beschikbaar. In hoofdstuk 1 zijn een aantal hypothesen geformuleerd die de invloed van bewaartijd en bewaar temperatuur op broedresultaten kunnen verklaren.

Met de analytische vergelijkingen zoals die gepresenteerd zijn in hoofdstuk 2 kan de temperatuur en het vochtverlies van eieren worden berekend. Hiermee kunnen klimaatscondities die optreden in praktijksituaties worden beoordeeld op hun invloed op de eieren. Een van de conclusies uit deze berekeningen is dat gedurende de tweede helft van het broedproces luchtsnelheid een relatief grote invloed zal hebben op de temperatuur in het ei.

In hoofdstuk 3 en 4 zijn de resultaten van berekeningen, gebaseerd op de vergelijkingen in hoofdstuk 2, vergeleken met meetresultaten. Uit de resultaten blijkt dat de temperatuur en het vochtverlies van eieren in strooiselnesten en wegrolnesten met de gebruikte methode goed kunnen worden berekend. Als de dieren toegang tot de nesten hebben, worden de eieren in strooiselnesten

voortdurend door de dieren opgewarmd. Onder deze omstandigheden kan de berekening van temperatuur en vochtverlies niet nauwkeurig worden uitgevoerd. Gedurende bewaring van de eieren is nauwkeurige berekening van temperatuur en vochtverlies ook niet mogelijk gebleken, omdat in broedecontainers natuurlijke stroming van warmte optreedt en de bewaarmethode de eieren enigszins tegen vochtverlies beschermt.

In hoofdstuk 3 is het temperatuurverloop en vochtverlies van eieren gedurende hun periode in het legnest onderzocht. Uit de gegevens blijkt dat eieren die geproduceerd worden in strooiselnesten, door de dieren worden opgewarmd tot een temperatuur die boven het minimum niveau voor embryo ontwikkeling ligt. Eieren in wegrolnesten worden niet opgewarmd door de dieren en de afkoeling van deze eieren begint direct na het leggen. De snelheid van afkoelen en de uiteindelijke temperatuur van deze eieren is afhankelijk van de condities in het hok. Het vochtverlies van eieren die geproduceerd zijn in strooiselnesten is hoger dan het vochtverlies van eieren in wegrolnesten, maar is laag in relatie tot het totale vochtverlies tot het moment van uitkomen.

Gedurende de bewaring is het vochtverlies en de temperatuur van de eieren afhankelijk van de positie van de eieren in de containers, van de wijze van bewaren en van de condities in de bewaar ruimte (hoofdstuk 4). Eieren die in het centrum van eicontainers zijn geplaatst hebben een langzaam opwarm- en afkoelproces in vergelijking met eieren die aan de buitenzijde zijn geplaatst. Temperatuursveranderingen van eieren die bewaard worden op voorbroedladen verlopen sneller dan van eieren die bewaard worden op pulp trays. De snelheid van temperatuursveranderingen van eieren bewaard op plastic trays ligt tussen deze twee in. Bij een hoge luchtsnelheid verlopen temperatuursveranderingen sneller, bij het gebruik van hoezen langzamer.

Het vochtverlies van eieren in broedecontainers kan niet worden berekend uitgaande de temperatuur en het relatieve luchtvochtigheid van de omringende lucht. De container veroorzaakt een bescherming tegen vochtverlies, waarschijnlijk als gevolg van een constante luchtlaag om de eieren, welke een lager vochtverlies tot gevolg heeft dan verwacht zou worden op basis van de klimaatscondities.

In hoofdstuk 5 is de invloed van temperatuur tijdens de periode voor het eigenlijke broedproces op de broedresultaten nader onderzocht. Uit de resultaten blijkt dat de invloed van bewaartijd en bewaar temperatuur van de eieren op broedresultaten mede afhankelijk is van de leeftijd van de ouderdieren. Behandelingen die een negatieve invloed hebben op broedresultaten beïnvloedden de

eieren van oudere moederdieren sterker dan de eieren van jonge moederdieren.

De resultaten van het onderzoek gepresenteerd in hoofdstuk 5 geven aan dat de broedresultaten negatief worden beïnvloed als de eieren in het nest een hoge temperatuur meemaken. Dit geeft aan dat eieren die geproduceerd worden in strooiselnesten een slechter broedresultaat zullen geven dan eieren die geproduceerd worden in wegrolnesten, als gevolg van verschillen in temperatuur.

Uit de resultaten blijkt tevens dat eieren van jonge moederdieren langer kunnen worden bewaard dan eieren van oudere dieren. Voor beide leeftijdsgroepen geldt, dat een combinatie van lange bewaartijd en lage bewaartemperatuur beter is voor het broedresultaat dan een lange bewaartijd en hoge bewaartemperatuur. De resultaten geven tevens aan dat lage bewaartemperaturen ook gebruikt kunnen worden wanneer eieren voor korte perioden worden bewaard.

In het laatste hoofdstuk worden een aantal mogelijke verklaringen voor de gevonden relaties tussen bewaartijd, bewaartemperatuur en leeftijd van de ouderdieren gegeven. Geconcludeerd wordt dat de ontwikkeling van de pH van het eiwit wellicht een indicatie kan geven voor de bewaarbaarheid van de eieren en dat bewaarcondities beoordeeld kunnen worden op hun invloed op de pH ontwikkeling van het eiwit van de eieren.

In hoofdstuk 6 wordt de invloed van het vochtverlies tijdens de bewaring op het vochtverlies tijdens het broeden en op de broedresultaten onderzocht. Uit de resultaten blijkt dat het vochtverlies tijdens het bewaren van invloed is op het vochtverlies tijdens het broedproces. Het gevolg van deze beïnvloeding is dat de invloed van relatieve luchtvochtigheid tijdens de bewaring op het totaal vochtverlies tussen het moment van leggen en uitkomst van het kuiken kleiner is dan verwacht kan worden op basis van het vochtverlies tijdens het bewaren. Het vochtverlies tijdens de bewaring had geen significante invloed op de broedresultaten.

Ron Meijerhof is op 28 februari 1960 geboren in Zaandam. Na het doorlopen van de lagere school in Westzaan behaalde hij in 1976 het diploma van de Lagere Agrarische school in Purmerend, in 1979 het diploma van de Middelbare Agrarische school in Alkmaar en in 1983 het diploma van de Hogere Agrarische school in Leeuwarden. Na zijn militaire diensttijd en een functie als leraar akker- en weidebouw aan de Lagere Land- en Tuinbouw school in Nijmegen volgde op 1 februari 1986 een tijdelijke aanstelling als technisch medewerker bij de toenmalige Stichting Pluimveeteeltproefbedrijven, gevestigd op "Het Spelderholt" te Beekbergen. Per 1 april 1987 werd hij aangesteld als assistent-coördinator bij dezelfde stichting. Sinds 1 januari 1990 is hij in vaste dienst bij het ministerie van Landbouw, Natuurbeheer en Visserij, aangesteld als wetenschappelijk onderzoeker vermeerdering, werkzaam voor het Praktijkonderzoek Pluimveehouderij op eerder genoemd instituut. In het kader van de werkzaamheden als wetenschappelijk onderzoeker is in het voorjaar van 1990 een promotie-onderzoek gestart.