



REPUBLIC OF TURKEY
SELÇUK UNIVERSITY
GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES



**THE EFFECTS OF DIFFERENT TEMPERATURE
APPLICATIONS DURING LONG STORED EGGS OF OLD
LAYER HENS AND BROILER BREEDER EGGS ON THE
HATCHING RESULTS, CHICK QUALITY AND GROWTH
PERFORMANCE**

Abdoulaziz HAMISSOU MAMAN

DOCTORATE (Ph.D.) THESIS

DEPARTMENT OF ANIMAL SCIENCE

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ABSTRACT

Ph.D. THESIS

THE EFFECTS OF DIFFERENT TEMPERATURE APPLICATIONS DURING LONG STORED EGGS OF OLD LAYER HENS AND BROILER BREEDER EGGS ON THE HATCHING RESULTS, CHICK QUALITY AND GROWTH PERFORMANCE

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**THE GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES OF
SELÇUK UNIVERSITY
THE DEGREE OF DOCTOR OF PHILOSOPHY
IN ANIMAL SCIENCE**

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Effects of short periods of incubation during egg storage (SPIDES) on eggshell microbial population, internal egg quality, embryonic mortality, hatchability, and chick quality of eggs from Babcock white (Layer) and Broiler parent stock flocks were examined in these studies. Three independent trials were carried out to determine the effects of SPIDES on eggshell microbial population, internal egg quality characteristics, hatching results, chick quality, and growth performances from 59-(trial 1) and 72-(trial 2) weeks Babcock white and 40 weeks (trial 3) Broiler age parents' stocks breeders. In the first and third trials, it was determined the effects of SPIDES on hatching results, eggshell microbial population, chick quality, and growth performances while in the second trial; it was determined the effects of SPIDES on internal egg quality characteristics, hatching results, chick quality, and growth performances. The eggs were stored for 21 d in trials 2 and 3, while in trial 1 for 28 d. The eggs were divided into 5 experimental groups as follow: control incubation "(CO) treatment": the eggs were stored 3 d and then incubated; non-SPIDES control eggs "(NS)" indicating the eggs were stored 28 d (trial 1) or 21 d (trial 2 and 3), without any heat treatment; "S1"= eggs were treated three times at 36°C until EST reached 32-34°C, delivered at 6, 12 and 18 d over of 28 d (trial 1) or 21 d (trial 2 and 3) of storage; "S2" = eggs were treated three times at 36°C until EST reached 28-30°C, delivered at 6, 12 and 18 d over of 28 d (trial 1) or 21 d (trial 2 and 3) of storage; "S3" = eggs were treated twice at 36°C until EST reached 32-34°C, delivered at 8 and 17 d over of 28 d (trial 1) or 21 d (trial 2 and 3) of

storage. The SPIDES treatments had a positive reducing effect on the eggshell microbial population by lowering total mesophilic aerobic bacteria (TMAB), total coliform bacteria (TC), and total yeast and mold (YMR) compared to the NS group ($P \leq 0.05$). The results of this study have highlighted that pre-incubation heat treatment did not have any negative effect on internal egg characteristics ($P > 0.05$). The results of all experiments showed that the hatchability of fertile eggs (HOF) was increased in S1 by 8.43% (trial 1), 7.69% (trial 2) and 3.15% (trial 3), and in S3-treated eggs by 14.44% (trial 1), 13.38% (trial 2) and 10.76% (trial 3) compared to the NS eggs ($P < 0.05$) because of low total embryonic mortalities ($P \leq 0.05$). The positive effects of SPIDES on HOF were more evident in S3 treated eggs than those from S1. No difference was found between S2 and NS groups for HOF ($P > 0.05$). All SPIDES treatments have improved the chick quality in terms of both chick length and Tona score quality ($P < 0.05$). However, chick weight, yolk-free body weight, and organs weight were not significantly affected by SPIDES treatments ($P > 0.05$) at hatch. The SPIDES treatment did not have any effect on the performances of layer chicks (Trial 1 and 2). However, it had an effect on broiler chicks (trial 3) and had improved 2 weeks' chick performances by increasing body weight and relative growth ($P < 0.05$) compared to the NS treatment group. According to the results, it can be concluded that applying two times heat treatment during storage at 36°C until EST reached 32-34°C, delivered at 8 and 17 d over of 21 d or 28 d of storage (S3) can be an effective method for improving HOF and chick quality by minimizing the detrimental effects of long term stored of hatching eggs.

Keywords: Storage period, Short incubation during storage, hatching results, Broiler, Layer, chick quality, Eggshell microbial population, internal egg quality

ÖZET

DOKTORA TEZİ

UZUN SÜRELİ DEPOLANAN YAŞLI DAMIZLIK YUMURTA TAVUĞU VE ETLİK PİLİÇ YUMURTALARINDA FARKLI SICAKLIK UYGULAMALARININ KULUÇKA SONUÇLARI CİVCİV KALİTESİ VE BÜYÜME PERFORMANSINA ETKİLERİ

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Çalışmada uzun süreli yumurta depolamada kısa süreli ısıtmanın (SPIDES) Babcock White ve Broiler ana damızlık sürülerinden elde edilen yumurtaların yumurta iç kalitesi, kuluçka sonuçları, civciv kalitesi ve civciv performansı üzerine etkileri incelenmiştir. Bu amaçla SPIDES'in yumurta kabuğu mikrobiyal popülasyonu, yumurta iç kalite özellikleri, kuluçka sonuçları, civciv kalitesi ve büyüme performansları üzerindeki etkilerini belirlemek amacıyla 59-(deneme 1) ve 72-(deneme 2) haftalık yaştaki Babcock White ve 40 haftalık yaştaki (deneme 3) etlik piliç ebeveynleri olmak üzere üç bağımsız deneme yapılmıştır. Birinci ve üçüncü denemelerde, SPIDES'in kuluçka sonuçları, yumurta kabuğu mikrobiyal popülasyonu, civciv kalitesi ve büyüme performanslarına etkisi belirlenirken üçüncü denemede SPIDES'in yumurta iç kalite özellikleri, kuluçka sonuçları, civciv kalitesi ve büyüme performansları üzerine etkileri belirlenmiştir. Yumurtalar, ikinci ve üçüncü denemelerde 21 gün depolanırken birinci denemede ise 28 gün depolanmıştır. Denemelerde yumurtalar 5 gruba rastgele ayrılmıştır. Deneme grupları; CO: Yumurtalar 3 gün süre ile depolanıp doğrudan kuluçka makinesine yüklenmiştir; NS: 21 ya da 28 gün depolanıp, depolama sırasında ısıtma uygulanmayan kontrol grubu; S1: depolama sırasında 6, 12, 18 günlerinde kabuk sıcaklığı 32-34°C'ye ulaşana kadar 36°C'de ısıtma uygulandı; S2: depolama sırasında 6, 12, 18 günlerinde kabuk sıcaklık 28-30°C ulaşana kadar 36°C'de ısıtma uygulandı; S3 depolama sırasında 8, 17 günlerinde kabuk sıcaklık 32-34°C'a ulaşana kadar 36°C'de ısıtma uygulandı. SPIDES uygulanan grupları, NS grubuna ($P \leq 0.05$) kıyasla mezofilik aerobik bakterileri (TMAB), toplam koliform bakterileri (TC) ve toplam maya ve küfü (YMR) düşürerek yumurta kabuğu mikrobiyal popülasyonu üzerinde olumlu bir etkiye sahip olduğu tespit edilmiştir. SPIDES'in yumurta iç kalite özellikleri üzerinde herhangi bir etkisi bulunmamıştır ($P > 0.05$). Deneme sonuçlarına göre, çıkış gücü (HOF)

bakımından S1 uygulanan yumurtalarında % 8.43 (deneme 1), %7.69 (deneme 2) ve %3.15 (deneme 3) ve S3 ile muamele edilmiş yumurtalarda %14.44 (deneme 1), %13.38 (deneme 2) ve %10.76 (deneme 3) oranlarında arttığını göstermiş olup ve her iki muamele grubunda (S1 ve S3) düşük toplam embriyonik ölüm oranlarına gerçekleşmiştir ($P \leq 0.05$). NS grubunda daha düşük çıkış gücün hesaplanmıştır ($P < 0.05$). S3 ile muamele edilen yumurtalar S1'den daha yüksek çıkış gücüne sahiptir. Ayrıca, S2 ve NS grupları arasında fark bulunmazken ve en düşük çıkış gücü bu iki gruptan elde edilmiştir ($P > 0.05$). Tüm SPIDES uygulamaları civciv kalitesini civciv uzunluğu ve Tona, skor kalitesi açısından bir iyileştirmeye neden olduğu tespit edilmiştir ($P < 0.05$). SPIDES uygulamalarının civciv ağırlığı, sarısız civciv ağırlığı ve organ ağırlıkları üzerine bir etkisi ($P > 0.05$) tespit edilememiştir. SPIDES uygulamasının yumurtacı civcivlerin performansları üzerinde herhangi bir etkisi olmamıştır (Deneme 1 ve 2). Ancak, etlik civcivler (deneme 3) üzerinde NS muamele grubuna kıyasla vücut ağırlığını ve büyüme oranı artırarak ($P < 0.05$) 2 haftalık civciv performanslarını iyileştirdiği tespit edilmiştir. Elde edilen sonuçlara göre, 36°C'de kabuk sıcaklığı 32-34°C 'ye ulaşana kadar 21 günlük depolama sırasında, depolamanın 8. ve 17. günlerinde iki kez kısa süreli ısıtma uygulanmasının (S3), kuluçkalık yumurtaların uzun süreli depolanmasının olumsuz etkilerini en aza indirerek HOF ve civciv kalitesini iyileştirmek için etkili bir yöntem olabileceği ortaya çıkmıştır.

Anahtar Kelimeler: Depolama süresi, depolama sırasında kısa inkübasyon, kuluçka sonuçları, broiler, yumurtacı, civciv kalitesi, yumurta kabuğu mikrobiyal popülasyonu, yumurta iç kalite

FOREWORD

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ABBREVIATIONS AND ACRONYMS

PI:	Pre-incubation
SPIDES:	Short Period of Incubation during Egg Storage
RH:	Relative Humidity
CFU:	Colony Forming Unit
ANOVA:	Analysis of Variance
GLM:	General Linear Model
CO ₂ :	Carbon dioxide
HU:	Haugh Unit
h:	hour
d:	day
Wk.:	Week
EST:	Eggshell Temperature
EWL:	Egg Weight Loss
IEW:	Initial Egg Weight
EWLS:	Egg Weight Loss during Storage
EWLI:	Egg Weight Loss during Incubation
TEWL:	Total Egg Weight Loss
TMAB:	Total mesophilic aerobic bacteria
YMR:	Total yeast and mold
SL:	Salmonella spp.
TC:	Total bacteria Count
CF:	Total coliform bacteria
HOF:	Hatchability of Fertile eggs
NS:	Non SPIDES
CW	Chick Weight
ICW:	Initial Chick Weight
CWG:	Chick Weight Gain
RG:	Relative Growth
BPW:	Buffered Peptone Water
PW:	Peptone Water
EM:	Embryonic Mortality
EEM:	Early Embryonic Mortality
MEM:	Middle Embryonic Mortality
LEM:	Late Embryonic Mortality
E6:	day 6 of incubation
E18:	day 18 of incubation
mm:	Millimeter
g:	gram
ml:	milliliter
RYS:	Residual Yolk Sac
HW:	Heart Weight
LW:	Liver Weight
GW:	Gizzard Weight
PrW:	Proventriculus Weight
FPC:	Feed intake Per Chick
FCR:	Feed Conversion Ratio
MJ:	millijoule
cm:	Centimeter

nd:	no detected
L:	light
D:	Dark
CL:	Chick Length
°C:	Degree Celsius
°F:	Degree Fahrenheit
PBS:	Phosphate Buffered Saline
PS	Parent Stock
YFBW	Yolk Free Body Weight



1. INTRODUCTION

After oviposition, hatching eggs are first stored in the breeding farm, then transported to the hatchery and stored again before finally being placed in the incubator. Normally, commercial hatcheries set their eggs after 3-5 days of storage to minimize the negative effects of egg storage on hatchability and chick quality. However, in broiler parent and grandparent hatcheries due to a surplus of hatching eggs or a varying market demands for day-old chicks in the poultry industry and the maximum capacity of hatcheries, the egg storage time varies from a few days to several weeks.

It is well known that an increase in the storage period has some negative effects such as delays hatching (Mather and Laughlin 1976), decreases hatchability of fertile eggs (Becker, 1964; Bohren et al., 1961; Fassenko et al., 2001; Tona et al., 2004), negatively affects the quality of chicks (Reijrink et al., 2009; Tag EL-Din et al., 2017; Tona et al., 2003), increased early embryonic mortality (Funk, 1934; Scott, 1933), reduce hatchability by 0.7% per day (Heier and Jarpe, 2001) and 0.5% per day (Hodgetts, 1981), increased the hatching time (Becker et al., 1968; Bohren et al., 1961), decreased embryo livability (Christensen et al., 2002; Elibol et al., 2002; Van de Ven, 2004) and decreases subsequent growth performance (Becker, 1960; Merritt, 1964; Tona et al., 2003; Tona et al., 2004).

Longer egg storage can also increase albumen pH and Haugh units or lower albumen high than fresh eggs (Benton Jr and Brake, 1996; Lapao et al., 1999; Silversides et al., 2001; Van den Brand et al., 2008), deteriorates albumen-yolk ratio (Decuyper and Bruggeman, 2007; Tona et al., 2004). In addition, long period storage can increase the probability of bacterial contamination as the quality of the cuticle deteriorates (De Reu et al., 2006). Wang and Slavik (1998) reported that for a storage period of 21 days, the rate of bacterial penetration increased.

The role of incubation in bacterial movement on eggshells has been corroborated in subsequent studies (Grizard et al., 2014; Grizard et al., 2015; Potter et al., 2013; Ruiz-de-Castañeda et al., 2011).

Incubation is thought to reduce or limit bacterial growth by altering the microbial populations associated with eggshells (Cook et al., 2005; Cook et al., 2003) by maintaining the dryness of the shell (D'Alba et al., 2010) and controls bacterial affluence (Shawkey et al., 2009).

Incubation decreased the abundance of eggshell microbes (Baggott and Graeme-Cook, 2002) and influenced the composition of the eggshell microbial population (Potter et al., 2013), leading to a less pathogenic microbiota (Brandl et al., 2014; Lee et al., 2014).

Numerous methods have been investigated to minimize the hatchability losses and to reduce the second-grade chick due to long-period storage eggs. For instance, among these methods, we have the short periods of incubation during eggs storage (SPIDES) which has proven successful in terms of improving hatchability and also in reducing embryonic mortality.

Several studies have been carried out with this method in the term to prevent the detrimental effects of long-period storage eggs by authors such as (Dymond et al., 2013; French et al., 2011; Garip and Dere, 2011; Gucbilmez et al., 2013; Meir and Ar, 1998; Nicholson et al., 2013; Özlü et al., 2021a; Özlü et al., 2021b), who carried out with success the SPIDES treatment during several trials. Furthermore, the best results were obtained when applying four (4) preincubations (PI) heat treatments at 5, 10, 15, and 18 of storage than three (3) or five (5) PI heat treatments (Nicholson et al., 2013), El-Menawey (2019) stated that heating eggs two times on days 5 and 10 of storage hatchability were higher than heating the eggs one time on day 5 of storage. Also, Özlü et al. (2021a) reported that turning eggs 4 hours per day and one SPIDES treatment at day 5 of storage during 14 days' storage better improves hatchability in both young and old broiler grandparent flocks. SPIDES heating time and heat repetition have been investigated and the influence of the effect of low (28-30°C) and high (32-34°C) eggshell temperature and eggshell microbial population during SPIDES is largely unknown.

Therefore, this research project was initiated with the following objectives.

1. Investigate the effect of heating period and repetition during long egg storage
2. Investigate the effect of eggshell temperature during short period incubation on hatching results
3. Investigate the effects of pre-incubation heat treatment on eggshell microbial population
4. Investigate the effects of pre-incubation heat treatment on internal egg quality

5. Investigate the effects of pre-incubation heat treatment on hatchability and chick quality
6. Investigate the effects of pre-incubation heat treatment on chick growth performance



2. LITERATURE REVIEW OVERVIEW

The goal of this literature review is to provide background information, summarize previous research, and highlight voids in the topics of the effect of pre-incubation heat treatments on eggshell microbial population, egg storage, internal egg quality, hatching results, chick quality, and performances.

2.1. Length of Storage on Hatchability

Several studies have shown that stored eggs for more than seven days can be detrimental to the embryo livability and hatchability. Nowadays, a lot of attention has been given to examine the detrimental effects of long term storage periods on hatchability and this have been revealed by numerous studies to begin declining even before 7 days of storage. This was confirmed by Waite (1919), Scott (1933) and Funk (1934) who reported that hatchability will begin to decline after 7 days of storage under optimal conditions. Similarly, these findings were confirmed by Funk et al. (1950).

For instance, according to Bohren et al. (1961) and Byng and Nash (1962) the hatchability began to drop 2-3 days after oviposition and continued to drop with length of storage. On the other hand, Becker (1964) reported that excessively prolonged storage prior to incubation causes a decrease in hatchability. Furthermore, Heier and Jarp (2001) also reported that during each day of storage there was a reduction of 0.7% of hatchability.

As a result of Elibol et al. (2002) when eggs were stored for 14 days a decrease in hatching caused by an increase in early and late embryonic mortality compared to those stored for 3 days. Similarly, Yassin et al. (2008) found a reduction of hatchability by 0.2% per storage day when eggs stored for a period of 7 days while, during a storage period of more than 7 days, the hatching was decreased by 0.5% per storage day.

In addition, prolonged storage of fertile eggs has been reported to be the source of tissue death and regressive changes in the blastoderm (Arora and Kosin, 1966; Kosin and Pierre, 1956). Similarly, Mather and Laughlin (1977) have shown that the embryogenesis and embryonic life of chicks deteriorate with egg storage.

2.2. Temperature and Relative Humidity during Storage

For a long storage period of hatching eggs, it is essential to control certain factors such as temperature and humidity because the correct application of these two factors prevent the loss of the weight of the egg which when this loss is high, can lead to the early death of the embryo.

Optimal egg storage time and environmental conditions are suitable for embryonic diapause and maximize hatchery capacity. In general, as the duration of egg storage increases, the temperature in the storage room should be decreased and the relative humidity increased to avoid dehydration (Christensen et al., 2002; Elibol et al., 2002).

Landauer (1967) reported that the ambient temperature of hatching eggs should be below physiological zero to maintain embryo dormancy during storage. This is found by Edwards (1902) to be between 20 and 21°C. However, for Funk and Biellier (1944) this is approximately 27°C, and for Lundy (1969) at 28 to 29°C. Funk et al. (1950) reported that storage eggs at a temperature above 21.1°C had detrimental effects on hatchability. Numerous researchers reported that storage temperature affects hatchability of fertile eggs (McDonald, 1960; Olsen and Haynes, 1948).

Funk and Forward (1960) came out that the ideal temperature during short period eggs storage of 7 days should be between 16-17°C. However, the optimum storage temperature for long eggs storage period for more than 1 week should be between 10-15°C. This has been shown by many researchers like Kaltoven and El Jack (1972) and Kirk et al. (1980) who reported that the best hatching results are related to short storage period and high optimum storage temperature.

Ruiz and Lunam (2002) reported that both hatchability and chick hatch weight during long-term storage eggs (9 to 11 d) at 16.5°C are reduced compared to the eggs stored at 10°C. In other hand, El Jack and Kaltoven (1969) and Kirk et al. (1980) reported that during a short storage period of up to 3 days at a storage temperature above 18°C increased hatchability whereas, Meijerhof et al. (1994) reported that no improvement in hatching from fertile eggs when the eggs were stored for 2 days at 15-20°C

Mayes and Takeballi (1984) reported that the storage temperature ought to be maintained between 18 and 30°C, whereas when eggs are stored beyond 7 days, this temperature ought to be 10 to 12°C. Reis et al. (1997) also found no improvement

during storage periods of 1 or 2 days after increasing the storage temperature from 16 to 21°C.

Mather and Laughlin (1976) and Tona et al. (2003) stated that stored eggs more than 7 days at 10-20°C and 50-80% relative humidity delayed hatching and also decreased the hatching rate (Becker, 1964; Fasenko et al., 2001; Merritt, 1964). While, Fasenko et al. (1992) stated that storage chicken eggs at 14°C for 1 to 21 days did not have any effect on the development of the embryo.

Meijerhof (1992) reported that the storage temperature for eggs stored for 4 to 7 days should be between 16-17°C while storage for more than 7 days should be reduced to 10-12°C. Therefore, the storage temperature of eggs stored for 3 to 4 days should be between 18 and 20°C and about 80% high humidity (Meijerhof, 1995).

In other hand, Brake (1999) recommended that eggs from young flocks (25 and 35 weeks) stored up to 4 days should be stored at 50 to 60% humidity. However, the storage temperature for a storage duration of 2 to 5 days' eggs from broiler breeders flock aged between 36 and 50 weeks should be stored at 18°C and 75% relative humidity and eggs from broiler breeders aged between 51 and 65 weeks for storage duration more than 4 days, the storage temperature ought to be decreased and the relative humidity should be increased.

Reijrink et al. (2008) reported that reduce embryonic development eggs should be stored at temperatures of 10°C whereas, Arora and Kosin (1966) stated that for less advanced changes in blastoderm structure the eggs should be stored at 13°C.

Storage at high relative humidity is not recommended because condensation on the eggs might occur, thus stimulating bacteria and mold to grow (Sharp and Stewart, 1936).

Cooney (1943) reported that during storage of eggs at high relative humidity of 90% hatchability was better than at 60-80% relative humidity. However, Funk and Forward (1951) concluded that high relative humidity at 80 to 90% during 11 to 14 days of holding period improved hatchability.

Özlü et al. (2018a) determine the effects of temperature fluctuation during 7 d of storage on the stage of blastoderm development, embryonic mortality, and hatchability of broiler hatching eggs. They found that fluctuated temperature decreased early embryonic mortality in the eggs from the young flock but increased early embryonic mortality in the eggs from the old flock, which decreased the hatchability of fertile eggs in the old flock.

In general, suggested temperatures during a storage period of fewer than 4 days, 4 to 7 and more than 7 days are 20-25°C, 16-17°C, and 10-12°C, respectively. It can be concluded that during storage of eggs for less than 3 days the storage temperature should be 18 to 30°C. For storage periods, up to 7 days, the storage temperature should be between 16-17°C. Finally, for long storage periods of more than 7 days, lower temperatures of 10 to 12°C are recommended.

2.3. Effect of Storage Temperature and Humidity on Bacterial Population

The storage time and conditions can have an influence by the eggshell microbial population. Numerous research has shown that there is a lag of 10-20 days after bacterial penetration of the shell and before the recovery of an appreciable number of viable organisms or the development of macroscopic changes in the contents of the eggs stored at the temperature range from 15 to 30°C (Bigland and Papas, 1953; Fromm and Monroe, 1960; Garibaldi and Bayne, 1960; Gillespie et al., 1950; Stokes et al., 1956).

McNally (1954) found a significant correlation during storage between bacterial penetration of the shell and the temperature and humidity. Furthermore, Graves and MacLaury (1962) showed that apart from temperature and humidity, vapor pressure also had a significant correlation with bacterial penetration.

Salmonella on eggshells can die off during storage at a very low relative humidity (Simmons et al., 1970) or at low temperatures (Baker, 1990; Rizk et al., 1966).

Messens et al. (2006) carried out research to determine the survival and penetration of *Salmonella enterica* serovar Enteritidis (SE) inoculated on the eggshell during storage for 20 days at a temperature of 15 to 25°C and 45 to 75% relative humidity (RH). They examined that increasing in storage time; decreased the percentage of shells contaminated and was more pronounced at 75% RH. They concluded that survival was inversely proportional to storage temperature and relative humidity.

Kim et al. (1989) and Humphrey (1990) reported that when eggs are stored at a temperature below 10°C, *Salmonella enterica* serovar Enteritidis (SE) does not grow in the egg or egg yolk. Okamura et al. (2007) concluded that storing eggs at temperatures between 10°C and 20°C can control the development of *Salmonella enterica* serovar Enteritidis (SE) over a period of 6 weeks while storing eggs at 30°C increased the number of contaminated eggs after 3 weeks.

Rizk et al. (1966) demonstrated that the number of *Salmonella* that enters an egg will increase dramatically with increasing storage temperature and that this increase is favored by the conditions during egg incubation. In other hand, Braun et al. (1999) showed that salmonella penetration was improved at higher temperatures, although eggshell survival was lower.

Survival of *Salmonella enterica* on eggshells was previously shown to be rare because the pathogen died rapidly during storage (Messens et al., 2005). Lublin and Sela (2008) reported that after storing eggs for 2 weeks at 25°C, *Salmonella Enteritidis* was detected on the shell of one egg of all 3 eggs and this number was 3.6×10^3 CFU / egg. However, no other *Salmonella* cells were detected on the eggshells during a control period of 4 to 8 weeks. No cases of *Salmonella* were detected from the eggshell at 6°C or in the control group at any time.

2.4. The Effect of Incubation on Eggshell Microbial Population

It is evidently clear that long periods of storage can increase the probability of bacterial contamination as the quality of the cuticle deterioration (De Reu et al., 2006).

The early researches conducted by some researchers such as Wang et al. (2011) and Cook et al. (2003) suggest that the second role of incubation is good maintenance of microflora on the eggshell which limits potentially pathogenic bacteria.

The role of incubation in bacterial movement on eggshells has been corroborated in subsequent studies (Brandl et al., 2014; Grizard et al., 2014). These studies suggest that high bacterial densities should correlate with poor hatching and will reduce chick quality. In other hand, incubation can also change the composition of the eggshell microbial population by decreasing the abundance of eggshell microbes (Baggott and Graeme-Cook, 2002). Furthermore, it can also increase the antimicrobial activity of albumen enzymes (Fang et al., 2012; Tranter and Board, 1984).

In this perspective, incubation is thought to reduce or limit bacterial growth by altering the microbial populations associated with eggshells (Cook et al., 2005; Cook et al., 2003) by maintaining the dryness of the shell (D'Alba et al., 2010; Ruiz-de-Castañeda et al., 2011) and controls bacterial affluence by inhibiting the growth of both pathogenic and harmless bacilli on pearly-eyed thrasher eggs (Shawkey et al., 2009).

Additionally, incubation influenced the composition of the eggshell microbial population (Potter et al., 2013), leading to a less pathogenic microbiota (Lee et al., 2014).

During storage, the probability of survival of *Salmonella enterica* on eggshells was previously rare because the pathogen died quickly (Messens et al., 2005). As a result of Bollinger et al. (2018), during oviposition, partial incubation can improve egg viability in eastern bluebirds by decreasing eggshell microbial populations.

Nowadays, a lot of attention has been given to investigate the effect of eggshell bacteria on hatchability and reported that bacterial thickness of the eggshell is the principal cause of decreasing in the hatching rate of species avian (Peralta-Sánchez et al., 2018; Tomás et al., 2018).

Additionally, some authors have suggested that microbes could penetrate the eggshell into the interior of the egg and negatively affect the viability of the embryo (Bruce and Drysdale, 1994; Fonseca et al., 2011; Hansen et al., 2015; Pinowski et al., 1994).

It is evidently clear that incubation of eggs reduces both the number and diversity of bacteria and seems to be especially efficient against enteric Gram-negative bacteria, which are most often isolated from the interior of infected eggs (Houston et al., 1997).

As a result of Wang and Slavik (1998) reported that for a storage period of 21 days, the rate of bacterial penetration increased. On the other hand, Gentry and Quarles (1972) found that incubation at 38°C with 80% relative humidity showed a higher decrease in the number of shell bacteria after 1 to 4 days and was close to 10 percent of the original counts after 7 days.

2.5. Water Loss during Eggs Storage and during Incubation

During storage, water loss from eggs occurs through evaporation under the influence of relative humidity (R.H.), temperature, and shell porosity. The factors that determine the evaporation process are related to the environment and the characteristics of the eggs (Garip and Dere, 2011; Hristakieva et al., 2011).

As a result of Phoudfoot (1967) reported that during storage, the loss of egg weight by evaporation is influenced by relative humidity. In this perspective, some

authors reported that during storage, the water loss from eggs increased with the duration of storage (Fasenko et al., 2001; Goliomytis et al., 2015; Reijrink et al., 2009).

It is evidently clear that embryonic mortality during storage is closely related to egg weight loss and increases in the duration of storage (Nahm, 2001; Reijrink et al., 2010b). Reijrink et al. (2008) reported that weight loss during incubation is not affected by storage time. Therefore, the higher increase in total egg weight loss during incubation observed after longer storage time is mainly attributed to higher egg weight loss during longer storage.

During the egg storage period of 4 to 14 days, the water loss increased by 1.04% (Hristakieva et al., 2011). However, Reijrink et al. (2010a) reported that after 4 and 14 days' storage, the water loss from chicken eggs was lower, ranging from 0.30% to 0.83%. While for a storage period of 7 to 14 days this water loss increased by 1.14% (Alsobayel et al., 2013).

As a result of Senbeta (2016), during the eggs storage periods of 5 and 10 days the weight loss was 1.62% and 3.27%, respectively. In addition to Walsh et al., (1995) storing eggs at 23.9°C for a period of 14 days resulted in significant egg weight loss, which impacted embryonic survival as excessive egg weight loss resulted in less water available to be lost during incubation. According to Landauer (1967), during storage a decrease in the hatching quality of the eggs would probably result in the loss of water by evaporation from the egg.

It is evidently clear that that pre-storage incubation and frequent warming increased egg weight loss during storage by 0.12% (Reijrink et al., 2010a). Whereas, except the frequent warming which increased egg weight loss by 0.28% and total egg weight loss by 0.39%, pre-storage incubation did not affect egg weight loss during incubation and total egg weight loss (Reijrink et al., 2010a). Furthermore, Silva et al. (2008a) found that heating eggs for 6 to 12 hours before storage egg weight loss increased as a function of the storage period at any preheating period and stored for four days, weight loss was higher in eggs heated for 12 hours as compared to the non-heated eggs. However, El-Menawey (2019) reported that SPIDES treatments did not affect the relative egg weight loss.

2.6. The Effect of Storage on Internal Egg Quality

During the period of laying or storage, the characteristics of the egg albumen probably play the most important role in the changes and viability of embryos (Brake et al., 1997; Hurnik et al., 1978; Lapao et al., 1999). In this context, numerous authors have examined the effects of egg storage and the age of flock on the characteristics of egg albumen (Benton Jr and Brake, 1996; Reis et al., 1997; Silversides et al., 2001; Suarez et al., 1997).

It is evidently clear that the negative effects of a long storage period on hatching eggs result from the degradation of the internal quality of the eggs and was most pronounced in eggs from old breeding flocks (Kirk et al., 1980; Meijerhof et al., 1994).

The pH of albumen at egg-laying is approximately that of blood (7.6), which increases from 9.0 to 9.5 with long-term storage (Selleck and Stern, 1991). Furthermore, Lapao et al. (1999) reported that with the time of storage and the age of the breeder, the pH of the albumen increases while the height of the albumen decreases and the elasticity of the yolk sac membrane decreases with storage time (Jones and Musgrove, 2005).

It is evidently clear that the albumen pH at oviposition is about 7.6, which is slightly more basic than the uterine fluid (Arad et al., 1989). In just a few days of storage, the viscosity of the albumen drops considerably (Hurnik et al., 1978).

Burley (1989) reported that with the loss of CO₂ from the egg the pH of the albumen increases. In other hand Goodrum et al. (1989) reported that albumen pH increased rapidly when eggs were stored at elevated temperatures. However, Reijrink et al. (2010a) found that pre-storage incubation did not affect albumen height or albumen pH while frequent warming during storage resulted in a higher albumen height and a lower albumen pH at the end of storage. Furthermore, storage temperature influences the loss of carbon dioxide, and therefore, results in an increase in the albumen pH (Meijerhof, 1992).

It is obvious that for embryo growth, the optimal pH of albumen has been estimated to be between 8.2 and 8.8 (Sauveur et al., 1967; Walsh, 1993). Storage of eggs prior to incubation results in morphological changes in the blastoderm and malformations in the embryo (Arora and Kosin, 1966). Additionally, Brake et al. (1993) found the degradation of the albumen leads the blastoderm to move immediately next to the eggshell during storage.

Cotterill et al. (1959) reported that fresh egg albumen is the weakest buffered between pH 7.0 and 9.0 and strongest between pH 6 and 10. Furthermore, Benton Jr and Brake (1996) reported that the low pH of fresh eggs can have negative effects on the survival and hatching of embryos.

As a result of El-Menawey (2019), at ten (10) and fifteen (15) days of storage, the non-SPIDES group had the highest yolk index as compared to SPIDES groups. However, on the tenth day, SPIDES groups had lower albumen height than that of the non-SPIDES group. On the fifteenth day, the fresh eggs group had significantly higher albumen height than the heat-treated groups and in the SPIDES1 group; albumen height was higher than in the SPIDES2 group. Tona et al. (2004) found that storage duration decreases the depressed egg albumen Haugh units (HU) with 1.75 U/d.

2.7. Flock Age and Albumen Quality during Storage

Albumen quality decreases with the age of the hen (Burley, 1989). The viscosity of albumen is influenced by storage (Walsh, 1993) and the age of the flock (Romanoff and Romanoff, 1949). As a result of Lapao et al. (1999), the increasing in albumen pH during egg storage was higher in old than in young breeders. However, Nasri et al. (2019) reported that after 8 days of egg storage the albumen pH increased more in younger flocks than in older flocks.

According to Reis et al. (1997), the effect of pre-incubation storage of eggs from young breeders had no effect on viability and hatching rate, the viability was even higher in fresh eggs from older hens. Additionally, during long storage periods, albumen characteristics, albumen/yolk ratio deteriorates (Decuypere and Bruggeman, 2007; Tona et al., 2004).

It is evidently clear that stored eggs have higher albumen pH and Haugh units or lower albumen height than fresh eggs (Silversides et al., 2001; Van den Brand et al., 2008). Extended storage and inadequate storage temperatures reduce the albumen Haugh unit and increase the size of the eggshell air cells, resulting in poor hatchability (Samli et al., 2005). Furthermore, Benton Jr and Brake (1996) and Silversides et al. (2001) found a decrease in the values of HU with prolonging pre-incubation storage.

It has been shown that egg storage has an impact on the yolk index. This index decreases with the length of the storage period because the vitelline membrane weakens,

thus decreasing the height of the yolk and increasing its diameter (Kirunda and McKee, 2000).

2.8. Effect of Breeder Age on Hatchability

It is evidently clear that as the age of breeders and the egg storage duration increase, many changes occur; these changes may have an effect on the hatching rate.

As a result of Lapao et al. (1999) reported that the negative effects of a long storage period of fertile eggs on the viability in the older breeding flock are mainly due to an increased incidence of culling and embryo loss at all stages.

In this perspective, it has been related that hatchability was better in old breeders than in young breeder flocks (Elibol et al., 2002; Meijerhof et al., 1994; Tona et al., 2004). However, Yassin et al. (2008) reported that during extended eggs storage for a period of 8 to 14 d, the hatchability from young breeder's eggs was more affected than that of old breeders.

The poor hatchability of eggs obtained by older breeders is partly due to the increase in total embryonic mortality (Almeida et al., 2006; Tona et al., 2001; Vieira et al., 2005).

According to Tanure et al. (2009) stored eggs obtained from 57-week old breeders for 7 days decreased the hatchability. Similarly, Schmidt et al. (2009) reported that in a 39-week old flock hatchability began to be reduced on day 4 of storage, with losses of 1.38% per day up to 8 days of storage.

Thus, it can be said that hatchability of eggs from older flocks decreases more with increasing storage time (Kirk et al., 1980). Furthermore, it has also been suggested that if eggs have to be stored, eggs from younger breeders be stored rather than those from older breeders (Lapao et al., 1999; Reis et al., 1997; Tona et al., 2004).

2.9. Effect of Storage on Hatching Time

It has been shown by several studies that storage eggs for more than seven days can increase the hatching time and increase the hatch windows for a period of more than 24 hours.

Longer storage periods lead to longer incubation (Christensen et al., 2002). As a result of Arora and Kosin (1966), long-term storage of fertile eggs has been reported to

cause delayed embryonic development and increased incubation time. Additionally, Ruiz and Lunam (2002) and Tona et al. (2003) have also reported that stored eggs for a long period cause a delay in hatching. In other hand, a stored egg for more than seven days was found to decrease embryo livability (Elibol et al., 2002; Van de Ven, 2004).

According to Mather and Laughlin (1976) results, stored eggs for a period of 14 days increased the average total incubation time of 0.96 h per storage day. This can be hypothesis by the fact that stored eggs can slow embryogenesis and slowdown in embryonic development (Mather and Laughlin, 1977).

Fasenko et al., (2002) reported that 14 days stored eggs hatched 11 hours later than eggs stored for 1 day. Furthermore, Bohren et al. (1961) found an increased in hatching time when stored eggs for 12 days. In addition, these findings were approved by Becker et al. (1968) who reported that an increase in each day of storage increases the hatching time by 1 hour.

2.10. Effect of Pre-incubation Storage Heating on Hatchability

In order to improve hatchability and decreased embryonic mortality during long fertile eggs storage, several studies have been shown that heating eggs before or during storage can have an effect on hatching results.

Özlü et al. (2018b) concluded that the better hatchability was observed in eggs given 5.5 h SPIDES treatment compared to 3.5 h treatment at 5 days of 14 days' storage in 27 to 50 weeks' flock ages.

As a result, Reijrink et al. (2009) pre-storage incubation has found to reduce the hatchability of set eggs from 59.3 to 51.5% when storage time was 12 days while in another experiment they found an increase in the hatchability of fertile eggs with pre-incubation storage from 80.6 to 85.9% when storage time was 11 days.

According to Fasenko et al. (2001) storage eggs during 14 days decreased the hatchability of set eggs by 58.4% compared to 4 days' storage eggs (88.2%). However, pre-storage incubation of 6 hours improved the hatchability of set eggs from 70.5% to 79.0% when eggs are stored for 14 days.

Jackson (1912) reported that warmed fertile chicken eggs daily for a period between 30 minutes to 2 hours have improved the hatchability. In this context, Funk (1934) suggested that when the eggs were held for six to eight hours at 101°F the hatchability was improved. Additionally, Becker and Bearse (1958) reported that pre-

warmed storage eggs for a period between 1 to 5 hours at 37.5°C improved the hatchability of fertile eggs. Proudfoot (1966) also reported that pre-warmed eggs storage for more than 14 days improved the hatchability of fertile eggs.

On the other hand, Kosin and Pierre (1956) found that daily heating of fertile eggs during 14 days of storage for a period of 1 hour at 37.6°C improved the hatchability of fertile eggs from 64% to 70% and this can be explained by the hypothesis that an advanced stage of blastoderm development is beneficial in helping the embryo to resist storage. According to Nikolaeva (1958), fertile eggs could be maintained up to 20 days when the eggs were heated for a period of 2-4 hours per day at 37.5°C or 39-40°C.

Mayes and Takeballi (1984) concluded that pre-incubation warming is beneficial when stored eggs for a period of 7 days. Similarly, Kan et al. (1962) found that daily heating during eggs storage up to three weeks improved hatchability. However, was detrimental when eggs were stored between three and four weeks.

Additionally, Lancaster and Jones (1986) reported a reduction in hatching when heating eggs for more than 5 hours before storage.

As a result of Dowden (2009) daily warming of fertile eggs from broiler had no beneficial or detrimental effect on the hatchability of fertile eggs which stored for four to six days. However, Kalaba et al. (2017) found that 4 hours pre-heating treatments may increase the viability of stored eggs.

On the other hand, Kollmann Filho et al. (2020) suggest that four to six-hour pre-storage heat treatment at 37.5°C modulates the hatch window; however, did not influence the hatchability. Similarly, Terčič and Pestotnik (2016) who found that heating of hatching eggs for 5 hours at 37.8°C was not an effective way to reduce the loss of hatchability in eggs stored for long periods. However, Proudfoot and Hulan (1982) stated that warming hatching eggs at 38°C for 5 h before incubation can result in substantial gains in hatchability. In this context Silva et al. (2008a) concluded that heating eggs for six-hour before storage improves incubation results as it decreases late embryo mortality.

2.11. Short Periods of Incubation during Eggs Storage

Nowadays, a lot of attention has been given to increase hatchability and chick quality in long-stored broiler eggs. For this purpose, numerous studies were carried out as follow:

Nicholson et al. (2013) carried out four (4) independent types of research about short incubation during storage. In experiment one, the objective was to determine how many heat treatments were needed to give the best hatchability. In this experiment, all eggs were stored for 21 days except for the positive control. The eggs were held in the setter corridor for 4 hours for each heat treatment, with 3, 4, or 5 repetitions as follows: eggs were heated for 4 hours on 8 and 15 days of storage, or heated for 4 hours on days 5, 10, 15 and 18 of storage or 4 hours on days 3, 8, 12, 15 and 19 of storage. They commented that all SPIDES treatments improved hatchability, decreased both early dead embryos and late dead embryos and live pips that the untreated eggs after the 21 days' storage. They concluded that applying four treatments on the 5, 10, 15, and 18th day of storage gave better results than three (3) or five (5) treatments. In another experiment, it is aimed to determine the best combination of treatment duration and treatment frequency. In this experiment, eggs were heated on the 5, 11, and 16th days of storage or on the 4,8,13 and 17th days of storage and on the 3,6,9,12,15, and 18th days of storage during 21 days of eggs storage for either 2, 4, or 6 hours. They found that heating eggs for 2 hours had the best results. However, heating the eggs 6 times on the 3,6,9,12,15, and 18th days of storage for six-hour treatment caused a decrease in hatchability with 1.1% compared to the non-SPIDES group with 37% of hatchability.

Dymond et al. (2013) carried out research about the short periods of incubation during broiler fertile eggs storage. Three independent experiments using eggs obtained from 35, 36, and 33 week-old flocks in experiments 1, 2, and 3 respectively. In this experiment, all eggs were stored for 21 days except for the positive control (eggs were stored for 4 d). Non-SPIDES control eggs were not heated during storage for 21 days, the SPIDES group eggs were heated for 4-hours at 37.5°C on the 4, 8, 14, and 18th days during 21 days of storage in the trial 1 and on the 5, 9, 15, and 19th days during storage in the second and third trials. They found the non-SPIDES group decreased in hatchability by 71% compared to the SPIDES with 84%. The embryonic mortality was higher in the non-SPIDES group with 14% and 14% in early and late embryonic

mortality compared to the SPIDES group with 11% and 5% in early and late embryonic mortality respectively. The hatching weight in the NSP group was similar to the SPIDES group. However, the 7-day chick body weight in the SPIDES group was heavier than the NSP group.

Damaziak et al. (2018) carried out a research to determine the effect of short periods of incubation, egg turning during storage on hatching results, chick quality, and juvenile growth. In their experiment eggs from two broiler breeder flocks with 49- and 52-wk-old in the first flock and 70 and 73 wk-old in the second flock were used. All eggs were stored for 12 days except for the control which was stored for 5 days. The non-SPIDES group eggs were stored for 12 days without heating and turning every 12 h, the SPIDES group eggs were heated for 4-hours at 30°C and 50-55% RH on the 5 and 10th days during 12 days of storage and turning every 12 h. The negative control eggs were not delivered to heating and turning during 12 days of storage. They concluded that SPIDES treatment had no effect on the length of incubation and hatch windows. However, they found that the SPIDES treatment increased hatchability from the set and apparently fertilized eggs and reduced the number of eggs not hatched, and improved the chick quality.

Gucbilmez et al. (2013) carried a research to determine the effects of preincubation heating of broiler hatching eggs during storage on hatchability. In their experiment, eggs were obtained from Ross 344 male × Ross 308 broiler breeders at 28, 38, and 53 weeks (experiment 1), 28 weeks (experiment 2), 29 weeks (experiment 3), and 27 weeks (experiment 4). Eggs were heated for 1- or 2-times during storage at 26°C for 2 h, 37.8°C for 3 h, and 26°C for 2 h. In the first experiment eggs were heated for 1 to 5 times on the 11th day of storage, in the second experiment eggs were heated on the 6th day of storage period, in the third experiment the eggs were heated for 1 to 5 times on the 11-day storage period and in the fourth experiment the eggs were heated for 1 to 5 times on the 11th day storage. In the first experiment they found an increased in hatchability when heating eggs at d 5 of 11-d storage period. In the second experiment, no benefit was found when heating of 28 weeks old flock eggs on the 6th day of storage. In experiment3, hatchability was increased when heating eggs from 29 weeks old flock on day 1 or 5 of 11-days storage period. In the experiment 4, heating eggs obtained from 27 weeks old flock twice during 11th d of storage improved hatchability.

Tag EL-Din et al. (2017) carried out research about the effect of short incubation during storage on hatching results and chick quality. The experiment aimed to determine the role of heating times of 2,5 h and 5 hours during 7, 14,21, and 29 days of storage on hatching results. In each storage period, the eggs were heated for 2, 5 or 5 hours at 99.5°F. They found that heating eggs for 2, 5 hours during SPIDES gave the better hatching results and lowest early embryonic mortality (0-7 day), mid embryonic mortality (8-14 day) and showed highest significant for chick quality when compared with control. They concluded that during 7, 14, 21, and 29 days of storage, heating the eggs for 2.5 hours every five days once, twice or four times could be a method to reduce the detrimental effect of storage on hatchability.

Ebeid et al. (2016) examined the effect of short incubation during storage. Their experiment aimed to determine the effect of pre-storage warming egg weight loss, hatchability, chick quality, and thyroid hormones. In their experiment, eggs were stored at 4 and 10 days and pre-storage heated at 37.5°C for 4, 6 or 8 hours. They found that pre-storage incubation increased egg weight loss during storage, improved hatchability but did not affect the total egg weight loss and the early, intermediate, and late embryonic mortality. They concluded that 6 or 8 hours of pre-storage heating at 37.5°C improved hatchability and chick quality.

Gharib (2013) carried out research to determine the effect of pre-storage warming during long storage periods on hatchability and chick quality. In their experiment, eggs were stored at 4, 7, 10, and 14 days and pre-storage heated at 37.5°C for 6 or 9 hours. They found that pre-storage heating affects the egg weight loss and heating eggs for 9 hours had higher weight loss during storage and incubation and the total weight loss. Pre-storage heating for six hours resulted in higher hatchability by decreasing the early and late embryonic mortality compared to non-heated eggs or heated for nine hours whereas no significant effect was found in the middle embryonic mortality. The pre-storage heating for six hours improved chick quality, increased hatching weight, and chick length. They concluded that pre-storage heating for 6 hours could be used to improved hatchability when stored eggs for seven days or more than seven days.

Seker et al. (2006) also carried out a research about the effect of eggs warming during 21 d storage on hatchability of Japanese quail eggs (*Coturnix coturnix japonica*). In their experiment, five storage warming groups except the control group were used. The treatment groups were as follows: control group; eggs were not heated, in the second group; eggs were heated on day one of storage, eggs were heated on day 1 and 7 of storage for the third group, group four eggs were heated on day 7 of storage, group five eggs were heated on day 7 and 14 of storage, and for the sixth group, eggs were heated on day 14 of storage. The heating procedure occurred by holding the eggs at beginning at 21-23°C for 15 h then heated the eggs at 24-37.5°C for 1.5 hours and at 37.5°C for 3 hours then open the incubator for two hours after that the eggs were returned in the storage room for 15 hours at 21-23°C. The lowest hatchability was found in the control group with 39.9% compared to group 4 and 5 with 56.2% and 57.4%, respectively. The highest early embryonic mortality rate was found in group 1 and the lowest was found in group 5. In the middle embryonic mortality rate, the lowest was found in group 5 while the highest was in group 3. In the late embryonic mortality rate, the lowest was found in group 4 while the highest was in the control group.

Al-Samrai and Al-Dhanki (2017) carried out a research on short periods of incubation during storage and thermal manipulation during incubation on hatching results. In their experiment, eggs were obtained from Ross 308 broiler breeder flock and were stored for 7 and 14 days. Before storage eggs were preheated at 37.5°C and 55-60% RH for four hours. During storage, eggs were heated on day 4 and 7 of storage in the first experiment and on day 4, 7, and 10 in the second experiment. They concluded that SPIDES and thermal manipulation during incubation at 37.5°C to 39.5°C for 3 hours on the 16, 17, and 18 days of incubation improved hatchability of 7 and 14 storage eggs.

Abdel-Halim et al. (2015) carried a research on short periods of incubation during storage to alleviate the negative effects of long storage eggs on hatchability. In their study, eggs were obtained from broiler breeder 29–31-week-old. The eggs were stored for 7, 14, and 21 days and were subdivided into three groups as follows: non-warming group, heating at 37.5°C and 55% RH for 6 hours before storage and 2 hours heating at 37.5°C and 55% RH every three days during the storage period. They found that heating egg continuously for 6 hours before storage increased egg weight loss

percentage during storage and lowers egg weight loss percentage after 18 days of incubation compared to those non-heated. Pre-incubation heating eggs resulted in significantly lower percentages of early and total embryonic mortality when compared to the non-heated eggs. They concluded that pre-incubation heating of Arbor Acres breeder hatching eggs at 37.5°C and 56% RH for 2-hrs every 3 days during storage improved hatchability.

El-Menawey (2019) carried a research on the effect of heating treatment during storage on hatchability and chick quality. In their experiment, eggs were obtained from 49-wk-old Arbor Acres broiler breeders flock and stored for 15 days except for the control group which was stored for 5 days and were subdivided into four groups as follow: control group eggs were stored for 5 days without heating, the second group eggs were stored for 15 days and heated once at 32°C and 55 – 60% for 6 hours during 5th d of storage; the third group eggs were stored for 15 days and heated two times at 32°C and 55 – 60% for 6 hours on day 5 and 10 of storage and the fourth group eggs were stored for 15 days without heating. They found that the hatchability of heating eggs two times on days 5 and 10 of storage were higher than heating the eggs once on day 5 of storage. They also found that heating the eggs during storage at 32°C for 6 hours improved early embryonic mortality, hatchability, and visible fertility. The hatchability of eggs that were two times (5th and 10th of storage) exposed to heat treatment was higher as compared to those exposed one time (at 5th of storage). On the other hand, no differences were observed mid embryonic mortality rate, live pipped and of the different groups.

Özlü et al. (2018b) carried out a research that aimed to determine the effect of SPIDES duration and flock age on embryonic development and hatchability of long-stored grandparent eggs. For this purpose, eggs were obtained from two Ross female line grandparent flocks at prime (37 wk.) and, older (54 wk.) ages and were stored for 14 days at 15°C and 75% RH. During storage, the eggs were heated on day 5 of storage for 3.5 or 5.5 hours above 32°C. They concluded that the better hatchability was observed in eggs given 5.5 h SPIDES treatment compared to 3.5 h treatment on day 5 of 14 days storage in both flock ages.

Özlü et al. (2021a) also carried out new research that aimed to determine the effects of turning and a short period of incubation during long-term egg storage on embryonic development and hatchability of eggs from young and old broiler 6 grandparent flocks. Their study was aimed to investigate the effects of turning and a short period of incubation (SPIDES) during 14 days' storage on the stage of blastoderm development, embryonic mortality, and hatchability of eggs from young (29 wk.) and old (58 wk.) broiler grandparent flocks. The egg storage temperature was 15 °C and rotated 90 ° or 4 times a day during storage. On day 5 of storage, eggs were subjected to a SPIDES treatment. During the SPIDES treatment, the eggshell temperature (EST) was over 32°C for 3.5 h and remained at 35°C for 2.5 h. They found that a better hatchability from 29 weeks' young flock age than 58 weeks old flock age and during storage turning eggs 4 hours/daily increased hatchability when compared to the non-turning eggs due to a reduction of late embryo mortality. SPIDES treatment has decreased the early and late embryonic mortality by increasing the hatchability of fertile eggs and improves the chick quality. They concluded that turning eggs 4 hours per day and one SPIDES treatment on day 5 of storage during a period of 14 days' storage better improves hatchability in both young and old broiler grandparent flocks.

2.12. Effect of Storage on Chick Quality

The quality of day-old chicks is an important criterion for the success of hatcheries and also the profitability of broiler producers. In hatcheries, the primary objective is to maximize the hatching rate with a large number of high-quality, salable chicks that are demanded by broiler producers for their viability and high performance (Decuypere and Bruggeman, 2007).

Some of the quality parameters of the chicks are the day-old chicks' weight, the chicks' length, the length of the legs and toes, the appearance of the chicks, vitality, alertness, the condition of the navel, etc. (Tona et al., 2004; Willemsen et al., 2008). Certain scoring systems have been developed by considering various quantitative and qualitative parameters of researchers, such as the Tona score or the Pasgar score (Boerjan, 2002; Tona et al., 2003).

It is well known that long egg storage adversely affects day-old chick quality (Tona et al., 2003). Reijrink et al. (2010a) found that pre-storage incubation (PSI) did not affect chick quality in terms of chick length or yolk-free body mass. In another

study of Reijrink et al. (2009), PSI also did not affect chick length on the day of the hatch when egg storage was prolonged.

Byng and Nash (1962) reported that a long-term period of egg storage increased the number of second-grade chick's quality. Becker (1960) reported that 8-9 weeks' chicks' body weight was negatively affected by the length of storage.

El-Menawey (2019) reported that heating the eggs during storage at 32°C for 6 hours makes relative yolk sac weight of newly hatched chicks increased significantly with the length of the pre-incubation storage period and chick quality traits (Tona score, chick weight, relative chick weight, and chick length\weight) of the non-SPIDES group had the lowest values as compared to the SPIDES groups. Janikovičová et al. (2019) concluded that pre-incubation increased heart weight.

2.13. Effect of Egg Warming during Storage on Growth Performance

Dowden (2009) concluded that the growth performance of male broilers raised for 13 days was not affected by daily warming during six days of storage. Senbeta (2016) reported that during the eggs storage period of 5 and 10 days no significant difference was found in the hatching weight. The finding that storage had no effect on one-day-old chick weights agrees with the previous reports (Reis et al., 1997; Tona et al., 2004).

Khan et al. (2012) concluded that preheating of hatching eggs before incubation had no effect on day-old chick weight, weight gain in the first and second week of age, feed conversion ratio in the first and second week of age, and early mortality percentages. Kollmann Filho et al. (2020) suggested that four or six-hour pre-storage heat treatment at 37.5°C did not influence the average chick weight.

Tona et al. (2003) reported that eggs stored for 18 days depressed relative growth at the 7 days of performance than those stored for 3 days. Becker (1960) reported that 8-9 weeks' chicks' body weight was negatively affected by the length of storage.

Long storage periods decreased embryonic growth rate, viability, and hatching at all ages of herds and were more pronounced in older herds (Fasenko et al., 2002; Lapao et al., 1999).

Tona et al. (2004) found that weights of 7-d-old chicks, as well as RG, were significantly reduced by the storage time. Tanure et al. (2009) also observed that chicks

from eggs stored for three days resulted in lighter chicks at hatching, compared to those from eggs stored for seven days.

The objective of this thesis was to determine the effects of short periods of incubation during eggs storage (SPIDES) on eggshell microbial population, internal egg quality characteristics, hatching results, and 2 weeks' chick growth performances in layer and Broiler breeder parent stocks flocks' eggs.



3. GENERAL MATERIALS AND METHODS

In the scope of this thesis, it was aimed to determine the effects of short periods of incubation on different periods of eggs storage of Broiler and Layer breeder parent stock (PS) hens on eggshell microbial population, internal egg quality, hatchability, embryonic mortality, chick quality, and chick performance. Accordingly, in layers two trials, and in Broiler one trial were conducted independently:

- a. In layer breeder PS hens the first trial was namely called **"The effects of short periods of incubation during 28 days egg storage on eggshell microbial population, hatchability, chick quality, and two (2) weeks chick performance of eggs from 59 weeks age layer parent stock flock"**, and the second one was entitled **"The effects of short periods of incubation during 21 days egg storage on internal egg quality, hatchability, chick quality, and two (2) weeks chick performance of eggs from 72 weeks layer parent stock flock"**,
- b. In Broiler breeder PS hens, the trial was namely called **"The effects of short periods of incubation during 21 days' egg storage on eggshell microbial population, hatchability, chick quality, and two (2) weeks chick performance of eggs from 40 weeks Broiler breeder's parent stock flock"**.

All these trials have common points and so that they are grouped into the material and method part.

3.1. General Experimental Design

3.1.1. Study site

All the experiments were carried out in the incubation laboratory of Selçuk University, at the Faculty of Agriculture Department of Animal Science (Konya/Turkey).

3.1.2. Egg collection, storage and preincubation warming

The day before starting the experiment in each trial, the eggs laid on the same day were collected at the same time and then transported in a controlled environment

vehicle to the laboratory for incubation. The eggs were randomly divided into 5 treatment groups and were coded: CO indicating that the eggs were stored for 3 d after collection and then incubated to hatch, non-SPIDES control group (NS): eggs were kept in the storage cabinet during the entire storage period without heating them, SPIDES 1 ‘(S1)’ stating that the eggs were three times short period incubated at 6, 12 and 18 d of storage until the eggshell temperature (EST) reached 32-34°C then were directly transferred to the storage machine; SPIDES 2 ‘(S2)’ stating that the eggs were three times short period incubated at 6, 12 and 18 d of storage until the EST reached 28-30°C then were transferred immediately in the storage machine; SPIDES 3 ‘(S3)’, which indicates that the eggs were two times short period incubated at 8 and 17 d of storage until EST reached 32-34°C then were transferred immediately in the storage machine. During the short period of incubation, the EST was determined on 30 eggs (in total 150 eggs) per tray from each treatment group. The EST was measured through the blunt end of the egg with an infrared ThermoScope-HT-F03A (Medicare, Singapore, Malaysia). During the short period of incubation, the temperature in the machine was recorded.

Table 3.1. The experiment heating design

Groups	Storage period (day)	Heating day	Eggshell Temperature during short incubation	Heating repetition during storage
Control (CO)	3	nh		0
non SPIDES (NS)	21 or 28	nh		0
SPIDES1 (S1)	21 or 28	6 th , 12 th , 18 th day of storage	32-34°C	3
SPIDES2 (S2)	21 or 28	6 th , 12 th , 18 th day of storage	28-30°C	3
SPIDES3 (S3)	21 or 28	8 th and 17 th day of storage	32-34°C	2

nh: no heating

3.1.3. Eggshell temperature during SPIDES

The eggshell temperatures (EST) during short incubation in trials I, II, and III are given in Tables 3.2, 3.3, and 3.4 respectively. In layers' breeder PS hens' eggs, the average EST during heat treatment at 36°C were 33.07°C, 28.49°C and 33.06°C in S1, S2, and S3 respectively in trial I (Table 3.2), and were 33.36°C, 29.32°C and 33.39°C in S1, S2 and S3 respectively in trial II (Table 3.3), while in Broiler breeder PS hens eggs the average EST during SPIDES at 36°C were 33.17°C, 28.93°C and 33.20°C in S1, S2 and S3 respectively in trial III (Table 3.4)

Table 3.2. Eggshell temperature during SPIDES (Trial I)

Treatment groups								
Heat repetition	SPIDES 1			SPIDES 2			SPIDES 3	
	EST-6	EST-12	EST-18	EST-6	EST-12	EST-18	EST-8	EST-17
	------(°C)-----							
1	32.6	34.0	33.6	29.2	28.0	28.2	32.3	33.4
2	33.1	33.6	32.2	29.1	28.2	28.5	33.2	34.0
3	33.0	33.9	32.2	29.8	28.5	28.6	32.8	33.6
4	32.7	34.0	32.8	29.1	28.6	28.3	32.9	33.8
5	33.1	32.9	32.5	29.2	28.3	28.1	33.2	33.5
6	33.2	34.0	32.9	29.2	28.1	28.3	32.3	33.1
7	32.5	33.8	33.7	28.8	28.3	28.8	32.6	33.1
8	32.3	32.5	33.2	29.0	28.8	28.6	32.3	33.7
9	32.3	33.8	32.6	29.7	28.6	28.6	32.8	33.4
10	32.5	33.6	32.2	29.0	28.6	28.7	32.2	33.2
11	32.5	33.7	32.7	29.4	28.7	28.0	33.2	33.8
12	32.2	33.0	32.7	29.1	28.8	28.2	33.3	34.0
13	32.2	33.2	32.5	29.3	28.7	28.2	33.0	33.1
14	32.7	33.3	34.0	28.8	28.8	28.0	32.1	34.0
15	33.0	33.1	33.3	28.8	28.2	28.2	33.1	32.9
16	32.6	33.0	32.9	28.6	28.4	28.4	32.5	32.0
17	32.4	33.1	33.5	28.7	28.0	28.1	32.7	33.0
18	32.6	32.4	32.9	29.2	28.9	28.1	32.6	32.0
19	32.3	32.9	33.2	28.6	28.3	28.3	33.5	32.0
20	32.7	32.3	33.0	28.6	28.4	28.3	33.4	33.3
21	32.9	33.0	33.7	28.1	28.5	28.1	33.6	33.0
22	32.8	33.0	33.6	28.0	28.6	28.0	33.5	32.0
23	32.9	32.5	33.5	28.8	28.5	28.0	33.2	32.9
24	32.4	32.6	32.9	28.8	28.1	28.0	32.9	34.0
25	32.4	33.4	33.0	28.1	28.1	28.2	32.1	33.7
26	32.2	33.9	32.8	29.1	28.1	28.4	33.3	34.0
27	33.4	32.7	33.4	28.5	28.0	28.1	32.9	34.0
28	33.1	33.4	33.2	28.3	28.1	28.1	32.6	33.9
29	33.3	33.0	33.7	28.6	28.0	28.3	32.6	32.0
30	33.2	33.6	32.8	29.1	28.1	28.3	32.8	32.2
31	33.4	33.8	34.0	28.0	28.0	28.0	32.7	33.1
32	33.1	34.0	33.3	28.1	29.4	28.0	32.8	33.2
33	32.6	34.0	33.6	28.0	29.1	28.2	32.8	33.6
34	33.1	34.0	33.0	28.2	29.3	28.4	32.8	34.0
35	33.0	33.9	33.6	28.1	28.5	28.1	33.2	34.0
Average±SE	33.07±0.05			28.49±0.05			33.06±0.06	

EST-6: Eggshell Temperature during short incubation at 6th day of storageEST-8: Eggshell Temperature during short incubation at 8th day of storageEST-12: Eggshell Temperature during short incubation at 12th day of storageEST-17: Eggshell Temperature during short incubation at 17th day of storageEST-18: Eggshell Temperature during short incubation at 18th day of storage

Table 3.3. Eggshell temperature during SPIDES (Trial II)

Treatment groups								
Heating Period	SPIDES 1			SPIDES 2			SPIDES 3	
	EST-6	EST-12	EST-18	EST-6	EST-12	EST-18	EST-8	EST-17
	-----(°C)-----							
1	32.9	34.0	34.0	29.3	29.7	30.0	33.0	32.8
2	33.1	34.0	34.0	29.6	29.3	29.5	32.0	33.0
3	32.4	34.0	34.0	29.5	28.4	29.7	33.4	33.0
4	32.7	34.0	34.0	30.0	29.9	29.8	33.0	32.0
5	32.6	33.8	34.0	29.5	29.2	29.3	33.0	34.0
6	32.5	33.8	34.0	29.7	28.5	29.3	33.0	34.0
7	33.0	33.9	34.0	29.8	28.9	30.0	34.0	34.0
8	32.6	34.0	33.4	29.3	29.5	28.5	34.0	34.0
9	32.3	32.5	33.1	29.3	28.2	28.5	34.0	34.0
10	32.3	32.6	33.0	30.0	28.2	28.3	34.0	34.0
11	32.3	33.2	33.2	30.0	28.5	28.4	33.9	34.0
12	33.1	34.0	33.9	29.8	29.5	28.3	33.2	33.2
13	33.2	34.0	34.0	29.7	28.9	28.5	34.0	33.3
14	32.6	33.3	34.0	29.4	28.3	28.7	33.8	33.4
15	32.7	33.2	34.0	30.0	28.5	28.8	33.9	33.1
16	32.4	33.3	34.0	29.3	29.7	28.2	32.9	33.5
17	32.8	33.1	33.9	29.6	29.3	28.3	32.8	34.0
18	33.0	32.4	34.0	29.5	28.3	28.4	33.0	33.2
19	33.7	33.2	33.4	30.0	29.9	29.5	34.0	33.8
20	33.9	33.0	33.2	29.8	29.2	29.1	33.9	33.6
21	32.8	33.6	32.7	29.7	28.3	28.8	34.0	33.7
22	33.8	33.7	32.9	30.0	28.5	28.7	32.2	33.8
23	33.4	33.7	33.1	29.9	29.7	28.8	33.3	33.9
24	33.6	34.0	33.4	29.9	29.3	28.5	32.8	34.0
25	32.9	33.5	34.0	29.7	28.5	28.8	32.8	34.0
26	33.4	34.0	34.0	30.0	29.3	28.6	32.9	33.2
27	33.0	34.0	34.0	29.8	29.6	29.3	32.6	33.3
28	33.2	34.0	34.0	30.0	29.5	29.6	32.6	33.8
29	33.4	34.0	33.0	29.9	30.0	29.5	33.1	33.6
30	33.2	34.0	32.9	29.9	29.5	30.0	33.0	34.0
31	32.5	34.0	33.1	30.0	29.7	29.5	34.0	34.0
32	32.4	34.0	32.4	30.0	29.8	29.7	33.6	33.4
33	32.5	34.0	32.7	29.7	29.3	29.8	33.0	32.6
34	33.2	34.0	32.6	30.0	29.3	29.3	32.9	32.6
35	33.3	34.0	32.5	30.0	30.0	29.3	32.0	33.9
Average±SE	33.36±0.056			29.32±0.056			33.39±0.07	

EST-6: Eggshell Temperature during short incubation at 6th day of storageEST-8: Eggshell Temperature during short incubation at 8th day of storageEST-12: Eggshell Temperature during short incubation at 12th day of storageEST-17: Eggshell Temperature during short incubation at 17th day of storageEST-18: Eggshell Temperature during short incubation at 18th day of storage

Table 3 4. Eggshell temperature during SPIDES (Trial III)

Treatment groups								
	SPIDES 1			SPIDES 2			SPIDES 3	
Heating Period	EST-6	EST-12	EST-18	EST-6	EST-12	EST-18	EST-8	EST-17
	----- (°C) -----							
1	33.6	34.0	33.4	29.9	28.4	28.8	34.0	33.1
2	33.8	33.3	32.8	30.0	28.6	28.1	34.0	33.5
3	32.0	33.4	33.1	29.6	28.5	28.3	33.5	32.4
4	34.0	33.4	32.6	29.9	28.1	28.4	33.1	34.0
5	34.0	34.0	32.5	29.9	28.6	28.0	34.0	33.4
6	34.0	34.0	32.9	30.0	28.5	28.5	33.2	32.6
7	34.0	33.4	32.3	30.0	28.6	28.8	33.2	32.6
8	33.6	33.3	32.8	30.0	28.4	28.1	34.0	32.9
9	34.0	33.4	32.8	30.0	28.5	28.2	32.2	32.9
10	33.1	32.1	32.9	30.0	28.4	28.3	33.3	32.4
11	34.0	33.2	32.4	29.7	28.5	28.5	32.8	32.2
12	33.2	33.5	32.8	29.3	29.5	28.3	32.8	34.0
13	33.4	33.4	32.7	28.1	28.1	28.1	32.9	32.7
14	33.8	32.3	33.4	29.9	28.5	28.0	32.6	32.6
15	33.4	32.1	32.5	29.2	28.9	28.5	34.0	32.4
16	33.8	33.5	32.4	28.5	28.4	28.7	33.1	34.0
17	33.4	33.4	32.3	28.9	28.9	28.8	33.0	34.0
18	33.6	32.4	32.3	29.5	28.4	28.0	34.0	34.0
19	32.8	32.2	32.4	28.2	28.4	28.1	33.6	34.0
20	33.8	32.2	33.9	29.8	28.4	28.0	32.9	34.0
21	33.4	32.3	34.0	29.8	28.6	29.5	32.7	34.0
22	33.6	33.9	34.0	29.5	28.1	29.1	32.6	33.9
23	32.9	33.8	33.6	28.9	29.8	28.8	32.3	33.6
24	33.4	33.1	33.9	28.3	29.5	28.7	32.9	33.7
25	33.0	32.7	34.0	28.5	29.4	28.8	32.8	33.5
26	33.2	32.5	33.9	29.7	30.0	28.5	32.7	33.6
27	33.4	32.6	33.9	29.3	30.0	28.8	32.3	33.5
28	33.2	32.7	33.3	28.1	29.9	28.6	32.5	33.4
29	32.5	33.2	33.8	29.9	29.8	28.6	34.0	33.8
30	32.4	33.9	33.2	29.2	29.6	28.7	32.5	32.6
31	32.5	33.3	33.1	28.5	28.9	28.5	34.0	32.7
32	32.1	33.7	33.8	28.9	29.7	29.1	33.0	32.6
33	32.2	33.5	32.5	29.5	29.9	29.1	34.0	32.4
34	32.3	33.7	32.7	28.9	29.5	28.4	32.5	34.0
35	33.0	33.2	33.2	28.3	29.5	29.0	32.4	33.5
Average±SE	33.17±0.060			28.93±0.060			33.20±0.070	

EST-6: Eggshell Temperature during short incubation at 6th day of storageEST-8: Eggshell Temperature during short incubation at 8th day of storageEST-12: Eggshell Temperature during short incubation at 12th day of storageEST-17: Eggshell Temperature during short incubation at 17th day of storageEST-18: Eggshell Temperature during short incubation at 18th day of storage

3.2. Measurements

3.2.1. Microbiological analysis

The eggshell microbial population at the 18th day of incubation was determined by selecting randomly 6 eggs from each treatment group. The egg samples were transferred to sterile sampling bags (LP Italiana SPA, Milano, Italy) under sterilized conditions and then the contents were subjected to microbial analysis for 30 min, with 50 mL of sterile phosphate buffer saline (PBS) (Merck KGaA, Darmstadt, Germany). Ten-fold serial dilutions of the samples were prepared by using sterile PW (pH 7.0 ± 0.2 , Merck KGaA, Darmstadt, Germany). Total mesophilic aerobic bacteria were enumerated by using Compact Dry TC (Nissui Pharmaceutical Co., Ltd, Tokyo, Japan) and Petri dishes were incubated at 30°C for 24-48 hours. Total coliform bacteria were counted by using Compact Dry CF (Nissui Pharmaceutical Co., Ltd, Tokyo, Japan) and Petri dishes were anaerobically incubated at 30°C for 24-48 hours by means of Anaerocult A (Merck KGaA, Darmstadt, Germany). Total yeast and mold numbers were enumerated by using Compact Dry YMR (Nissui Pharmaceutical Co., Ltd, Tokyo, Japan) and Petri dishes were aerobically incubated at 25°C for 48-72 hours. For *Salmonella* spp. detection, Buffered Peptone Water (BPW) (Merck KGaA, Darmstadt, Germany) was used as pre-enrichment broth and appropriate dilutions from this media were pipetted on Compact Dry SL (Nissui Pharmaceutical Co., Ltd, Tokyo, Japan). Then the Petri dishes were incubated at 37°C for 24-48 h and the means of log colony forming units per g sample (log CFU/g sample) of the eggshells was determined.

3.2.2. Incubation

At the last storage day, for each group of treatment, eggs were removed from the storage machine and disinfected by spraying with 70% ethanol alcohol after 30 minutes of disinfection. The laboratory was ventilated for one (1) hour with fresh air to dry the eggshell and placed it in an incubator (Çimuka Incubator Company Turkey), which were preheated for 6 hours at a temperature of 30°C. A standard single-stage incubation program was used during the setter and the setpoint temperature was 38°C during the first five (5) days of incubation and was reduced gradually to 37.7°C at E6 of incubation

until E18 with a relative humidity of 55%. To apprehend if the air temperature of the machine and the relative humidity were correct, a HOBO MX1101 data loggers (United States) temperature recorder was used to monitor the temperature in the setter and the hatcher has been placed at the top next to the machine temperature sensor. The eggs were turned at an angle of 90° until 18 days of incubation. After the 18th day of incubation, the eggs were transferred to the hatching cabinets where the hatcher's air temperature and relative humidity were kept at 37.2°C and 70% RH, respectively. All the eggs in each treatment were distributed into 35 hatching baskets with ten eggs per replicate according to their group. Each group had a total of 35 replicates with a total of 350 eggs. On the 22nd day of incubation, un-hatched eggs were broken and opened to determine the embryonic mortality stage (EM). The different stages below were used to determine the period at which the embryonic death occurred: Early d 0 to 7 (white membrane over the yolk or blood vessels very visible), intermediated 8-14 (feather tracts saw, upper and lower beak equal in length), and late d 15 to 21 (large embryo with the egg yolk sac outside).

3.2.3. Chick quality

After 512 h of incubation the quality criterion were determined by selecting randomly and weighting 40 chicks from each group. Tona score chick quality was used as chick quality criteria. In the Tona score some quality parameters such as; physical characteristics, movement, and quality of the navel area were determined (Tona et al., 2003); (Table 3.5). After 528 h of incubation the relative organ weights were determined by selecting randomly 20 late hatched chicks from each group and then they were weighed and euthanized by cervical dislocation. The residual yolk sac (RYS), heart weight (HW), liver weight (LW), Gizzard weight (GW), proventriculus weight (PrW), and yolk free bodyweight (YFBW). Chick length was determined by (Hill, 2001; Willemsen et al., 2008) methods that measure the length of the chick from the tip beak to the nail on the middle toe.

Table 3.5. Allocation of scores to different parameter observations

Parameters	Characteristics	Scores
Activity	Good	6
	Weak	0
Downs and appearance	Clean and dry	10
	Wet	8
	Dirty and wet	0
Retracted yolk	Body with normal swallowed yolk	12
	Body with swallowed large yolk and rather hard to touch	0
Eyes	Opened and bright	16
	Opened and not bright	8
	Closed eyes	0
Legs	Normal legs and toes	16
	One infected leg	8
	Two infected legs	0
Navel	Completely closed and clean	12
	Not completely closed and not discolored	6
	Not closed and discolored	0
Remaining membrane	No membrane	12
	Small membrane	8
	Large membrane	4
	Very large membrane	0
Remaining yolk	No yolk	16
	Small yolk	12
	Large yolk	8
	Very large yolk	0

Tona et al. (2003)

3.2.4. Body weight, relative growth, feed consumption, and feed conversion ratio

After 528 hours of incubation, 40 chicks selected from each treatment group were placed in Çımuka grower battery cage. The chicks of each treatment were selected, weighted separately and then distributed among 16 (4 pens × 4 treatments) floor pens (120 cm× 120 cm) and each pen contained 2 nipple-type drinkers. Initial chick weight (ICW), chick weight gain (CWG), and relative growth (RG) were controlled each week for during 14 days of life. During the same period feed consumption and feed conversion ratio (FCR) were determined each week per pen up to the 14th day. The temperature values during rearing in the trials are given in Table 3.6. For all trials, the chicks received 24 h of light (24L: 0D) per day from fluorescent white lamps and the temperature was regulated at 34°C at d (0) then gradually reduces to a room temperature of 27°C at 14 days applied according to the handbook recommendation. The chicks were fed with ad libitum with a commercially produced wheat-maize-soya-based diet. Mortality was collected twice daily and weighed in order to calculate the adjusted FCR. Feed and water were available ad libitum during the entire rearing period, and the diets

were formulated to meet or exceed (Council, 1994) recommendations throughout the grow-out period.

Table 3.6. Temperature values at the floor level of the grower battery cage during the rearing period

Age	Temperature
(day)	(°C)
0	34
1	33
2	32
4	31
7	30
10	29
12	28
14	27

3.3. Calculations

Egg weight loss (EWL) during storage was determined by calculating the difference between the egg weight before storage and the egg weight on the last day of storage. EWL during incubation was determined by calculating the difference between the egg weight on the day of incubation and the egg weight on the 18th day of incubation. The total egg weight loss (TEWL) was determined by adding the EWL during storage with EWL during incubation. Fertility was computed as the percentage of set eggs and hatchability as the percentage of fertile eggs. EM was estimated as the percentage of fertile eggs. Yolk sac weight and chick live weight (CW) values were used to calculate residual yolk sac (RYS) in slaughtered chicks, while yolk sac weight difference from body weight was calculated as yolk free body weight (YFBW). Supply organ weights such as heart, gizzard and liver were calculated as percentages of live weight. In the experiments carried out, broiler performance criteria such as; live chick weight (CW), chick weight gain (CWG), relative growth (RG) feed intake per chick (FPC), and feed conversion ratio (FCR) of the subgroups were calculated. For each chick, 0 day-old weight and 7 and 14 d-old weights were used to calculate the chick relative growth (RG).

$$\text{EWL during Storage (\%)} = \frac{\text{Eggs weight at 0 d} - \text{Eggs weight at 21 or 28 d of storage}}{\text{Eggs weight at 0 d}} \times 100$$

$$\text{EWL during incubation(\%)} = \frac{\text{Eggs weight at set d} - \text{Eggs weight at 18 d of incubation}}{\text{Eggs weight at set d}} \times 100$$

$$\text{TEWL (\%)} = \text{Eggs weight at last d of storage} - \text{Eggs weight at 18 d of incubation}$$

$$\text{Hatchability} = \frac{\text{Number of eggs hatched}}{\text{Number of fertile eggs}} \times 100$$

$$\text{Early Embryonic Mortality} = \frac{0 - 7 \text{ d Embryonic mortality}}{\text{Number of fertile eggs}} \times 100$$

$$\text{Middle Embryonic Mortality} = \frac{8 - 14 \text{ d Embryonic mortality}}{\text{Number of fertile eggs}} \times 100$$

$$\text{late Embryonic Mortality} = \frac{15 - 21 \text{ d Embryonic mortality}}{\text{Number of fertile eggs}} \times 100$$

$$\text{Residual Yolk Sac (\%)} = \frac{\text{Yolk sac Weight (g)}}{\text{Chick Weight (g)}} \times 100$$

$$\text{Heart Weight (\%)} = \frac{\text{Heart Weight (g)}}{\text{Chick Weight (g)}} \times 100$$

$$\text{Liver Weight (\%)} = \frac{\text{Liver Weight (g)}}{\text{Chick Weight (g)}} \times 100$$

$$\text{Gizzard Weight (\%)} = \frac{\text{Gizzard Weight (g)}}{\text{Chick Weight (g)}} \times 100$$

$$\text{Proventriculus Weight (\%)} = \frac{\text{Proventriculus Weight (g)}}{\text{Chick Weight (g)}} \times 100$$

$$\text{Yolk Free Body Weight (YFBW)} = \text{Chick Body Weight (g)} - \text{Yolk Sac Weight (g)}$$

$$\text{Relative growth (\%)} = \frac{7\text{th or 14th day chick weight (g)} - 0 \text{ day chick weight (g)}}{0 \text{ day chick weight}} \times 100$$

$$\text{FPC (g)} = \frac{\text{Total amount of feed consumed by the compartment during the period (g)}}{\text{Average Number of Chicks Living in the Period}}$$

$$\text{FCR} = \frac{\text{Total Amount of Feed Consumed During the Period (g)}}{\text{Total Live Weight at the End of the Period (g)} + \text{Total Weight of Dead Animal (g)}}$$

$$\text{Body Weight Gain (g)} = 7 \text{ d or 14 d Chick Body Weight (g)} - 0 \text{ d or 7 d chick Body Weight (g)}$$

3.4. Statistical Analysis

The variance analysis was used to determine the mean differences between the group of the experimental factors on the internal eggs quality characteristics, the eggshell microbial population, the egg weight loss, hatching results, initial chick body weight, body weight gain, relative growth, residual yolk sac, relative organs weight, and chick live weight for different periods, feed consumption per animal and feed conversion ratio. Attention was given to the preparation of the experiment in a randomized design model (Düzgüneş et al., 1987). Treatment means were separated using Tukey's multiple range tests at a 0.05 significance level. For this purpose, MINITAB 16 computer package program was used and MS (2007) Excel computer package programs were used to organize and store the analysis data.

4. EXPERIMENT I: THE EFFECTS OF SHORT PERIODS OF INCUBATION DURING 28 DAYS EGG STORAGE ON EGGSHELL MICROBIAL POPULATION, HATCHABILITY, CHICK QUALITY, AND TWO (2) WEEKS CHICK PERFORMANCE OF EGGS FROM 59 WEEKS AGE LAYER PARENT STOCK FLOCK

4.1. Materials and Methods

4.1.1. Experimental Setup

In this experiment, the eggs were stored for 28 d and heated at 6, 8, 12, 17 and 18 d of storage

4.1.2. Hatching eggs and study period

A total of 1,750 eggs from a commercial Babcock white parent stock breeder, were used. All eggs were sourced through a commercial hatchery (Anadolu Damızlık Konya/Turkey) and came from a single 59-wk-old flock. The experiment was conducted during March-May 2020.

4.1.3. Egg storage conditions and pre- incubation warming

In this experiment, the eggs were stored in the storage machine (Çımuka model) at an average temperature and relative humidity (RH) of 10.70°C and 81 %, respectively. All the eggs were received on the same day of collection at the breeder farm, transported in a temperature-controlled vehicle to the University of Selcuk. A total of 1,750 eggs were distributed randomly in 5 groups with 350 eggs per treatment and each treatment consists of 5 replications containing each one 70 eggs. The eggs were stored for 28 d and the experimental groups, as follow: Control “(CO) treatment”: the eggs stored 3 days and then incubated; non-SPIDES control eggs “(NS)” indicating the eggs stored for 28 d , without heat treatment; "S1" = eggs were three times treated at 36°C until EST reached 32-34°C, delivered at 6 ,12 d and 18 d over of 28 d of storage; "S2" = eggs were three times treated at 36°C until EST reached 28-30°C, delivered at 6, 12 and 18 d over of 28 d of storage; "S3" = eggs were two times treated at 36°C until EST reached 32-34°C, delivered at 8 and 17 d over of 28 d of storage. In both trials, each heat treatment group after reaching the target EST eggs were immediately returned to the storage machine.

4.1.4. Microbiological analysis

To determine the eggshell total mesophilic aerobic bacteria (TMAB), total coliform bacteria (CF), total yeast and mold YMR, *Salmonella* spp. (SL) on the 18th day of incubation 6 eggs were randomly selected in each treatment group.

4.1.5. Egg weight loss

For each treatment before storage, a total of 45 eggs were selected randomly and then enumerated from 1 to 45. To determine the EWL during storage in each treatment eggs were weighed individually at the (0) day of storage and at the end of day 28 of storage. To determine the weight loss during incubation the same eggs were weighed on the 18th day of incubation.

4.1.6. Incubation and hatching conditions

Following a storage period of exactly 28 d the eggs were moved from the storage machine to the setter and were preheated for 6 hours at a temperature of 30°C. A standard single-stage incubation program was used during the setter and the setpoint temperature was 38°C during the first five (5) days of incubation and was reduced gradually to 37.7°C at E6 of incubation until E18 with a relative humidity of 55%. During incubation, the eggs were turned every hour until day 18 of incubation. At 18th day of incubation, the eggs were transferred to hatching cabinets where the air temperature and relative humidity were kept at 37.2°C and 70% RH, respectively. All the eggs were distributed into 35 hatching baskets with ten eggs per replicate according to their group. Each group had 35 replicates with a total of 350 eggs and unhatched eggs were broken and opened to determine the fertility and embryonic mortality stage.

4.1.7. Chick quality

After 512 h of incubation quality criteria were determined for 40 female chicks randomly selected from each group and weighed and were used to assess Tona score chick quality. After 528 h of incubation to determine the relative organ weight, 20 late hatched chicks randomly selected from each group were weighed and euthanized by cervical dislocation and then necropsied to determine the residual yolk sac (RYS), heart

weight (HW), liver weight (LW), Gizzard weight, proventriculus weight (PrW), and yolk free bodyweight (YFBW).

4.1.8. Body weight, relative growth, feed consumption, and feed conversion ratio

After 528 hours of incubation, 160 female chicks from 4 treatments (40 chicks per treatment) were placed in Çımuka grower battery cage. A total of 40 female chicks selected from each treatment were weighed individually and distributed among 16 (4 pens × 4 treatments) floor pens (120 cm× 120 cm) and each pen contained 2 nipple-type drinkers. Individual CW, CWG, and RG were controlled weekly for up to 14 days of life. Chicks were fed ad libitum with a broiler starter ratio. During the same period, feed consumption and feed conversion ratio (FCR) were determined each week per pen up to the 14th day.

4.2. Statistical Analysis

The effects of SPIDES on eggshell microbial population, egg weight loss, incubation results, chick quality, and performance were analyzed by one-way variance analysis by using the GLM (general linear model) procedure (SAS, 1989). Accordingly, the significant differences in the treatment means were determined by Tukey's multiple range tests at a 0.05 significance level.

4.3. Results

4.3.1. Temperature and relative humidity during storage

Figure 4.1 shows the storage machine's air temperature and humidity during the eggs storage period. The average storage ambient temperature, relative humidity and DewPt during 28 days of storage were 10.70°C, 81% and 7.6°C, respectively.

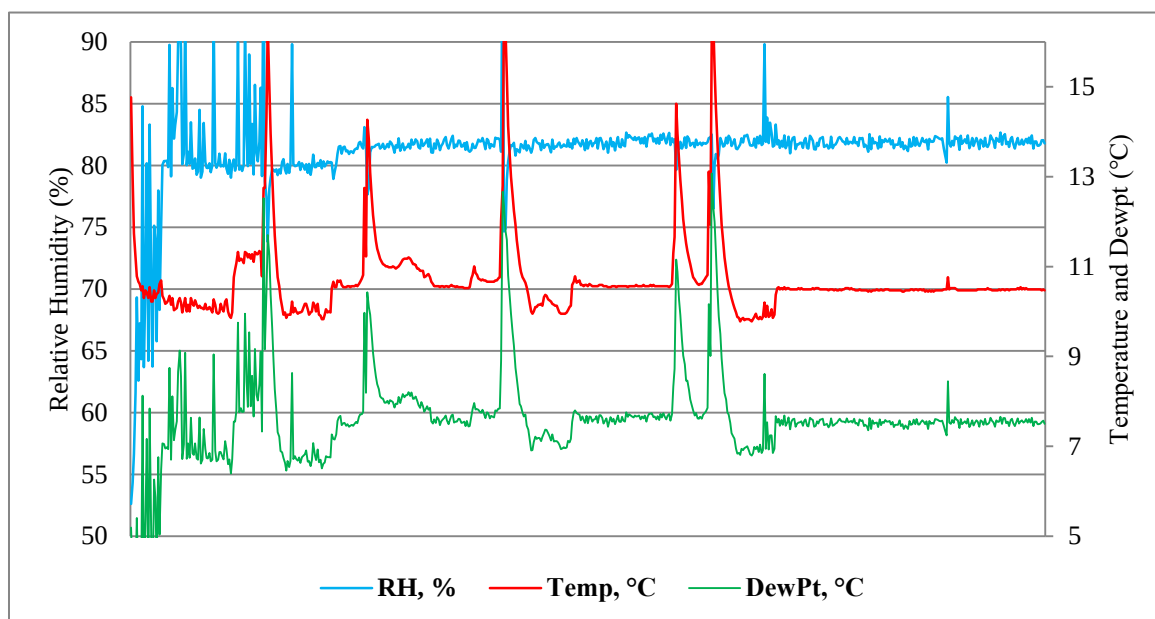


Figure 4.1. Storage machine air condition during 28 days eggs storage

4.3.2. Eggshell Temperature before heating and during SPIDES

The eggshell temperature (EST) before SPIDES (photo A) and after SPIDES in S2 (photo B) and in S1 and S3 (photo C) are shown in photos A, B, and C taken by a thermal camera (Figure 4.2). During the SPIDES the target eggshell temperature in each SPIDES treatment was determined by a thermal camera. As observed on photo A before preheating EST was 10.0°C, for treatment S2 (photo B) was 28.0°C while for treatments S1 and S3 (Photo C) was 33.6°C

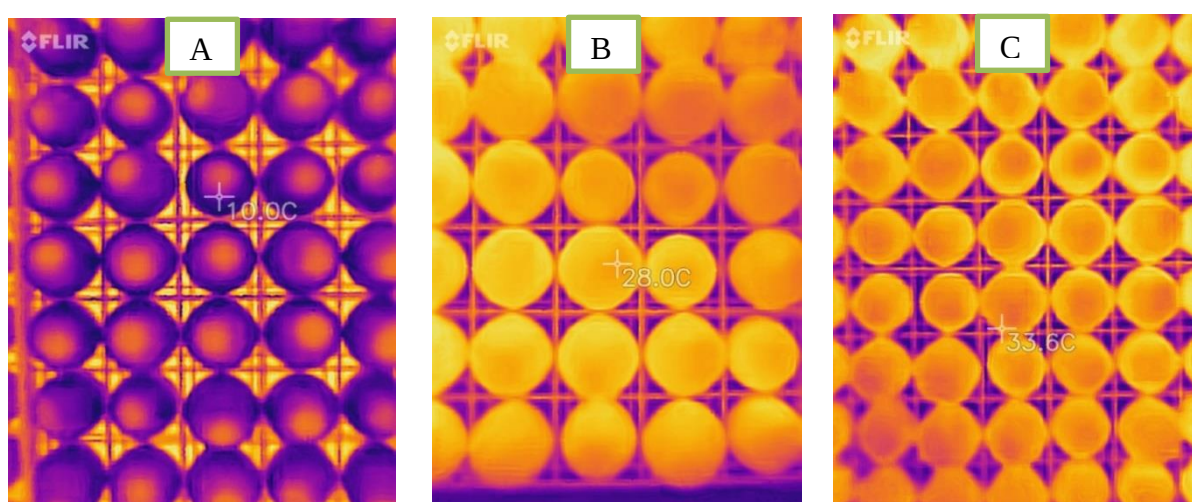


Figure 4.2. Eggshell temperature before short incubation (A), (B) Eggshell temperature during short incubation during storage (S2), (C) Eggshell temperature during short incubation (S1 and S3)

4.3.3. Egg weight loss

Table 4.1 presents the effects of pre-incubation heat treatment on EWL at different periods during storage. The pre-incubation heat treatment had an effect on egg weight loss during storage ($P < 0.05$). Pre-incubation heat treatment increased EWL during storage by 0.14% in S1, 0.06% in S2 and 0.03% in S3 when compared to NS ($P < 0.05$) respectively. However, Egg weight loss during 18 days of incubation and total egg weight loss were not affected ($P > 0.05$).

Table 4.1. Effects of SPIDES on egg weight loss during 28 d of storage and at 18 d of incubation

Group	n ¹	IEW	EWLS	EWLI	TEWL
		(g)		(%)	
NS	45	65.02 ^a	1.47 ^c	12.60	14.07
S1	45	64.47 ^a	1.61 ^a	12.65	14.26
S2	45	63.46 ^b	1.53 ^b	12.43	13.96
S3	45	64.72 ^a	1.50 ^{bc}	12.70	14.20
SEM		0.19	0.08	0.13	0.34
P-Value		0.000	0.000	0.454	0.664

^{a-b-c} Means within a column that do not share a letter are significantly different ($P < 0.05$)

NS: Eggs were stored 28 d without pre-incubation heat treatment during storage period; S1: Eggs were three times short period incubated at 6, 12 d and 18 over of 28 d of storage until the eggshell temperature reached 32-34°C then eggs were immediately cooled down; S2: Eggs were three times short period incubated at 6, 12 d and 18 over of 28 d of storage until the eggshell temperature reached 28-30°C then eggs were immediately cooled down, S3: Eggs were two times short period incubated at 8 d and 17 d over of 28 d of storage until the eggshell temperature reached 32-34°C then eggs were immediately cooled down, n¹: number of eggs used in each group to determine the egg weight loss. IEW: initial egg weight; EWLS: egg weight loss during 28 d storage; EWLI: egg weight loss during 18 d of incubation; TEWL: total egg weight loss.

4.3.4. Eggshell microbial population

Table 4.2 shows the effects of SPIDES during egg storage on the eggshell microbial population on the 18th day of incubation. Pre-incubation heat treatment during storage had an effect on the eggshell microbial population. Compared to the S1, S2, and NS treated eggs which had the highest number in TMAB with 4.18, 3.98, and 3.74 log CFU/g respectively, the S3 treated eggs had the lowest eggshell total mesophilic aerobic bacteria (TMAB) with 2.98 logCFU/g. With regards to the total eggshell yeast and mold (YMR), on the S3 treated eggs, no population was detected while the highest total eggshell YMR population was obtained on S1 and NS-treated eggs with 1.19 and 1.17

logCFU/g, respectively. Though, on the S2 treated eggs, the total eggshell YMR population was 0.48 logCFU/g. In the terms of total coliform bacteria (CF), there was not any microbial population detected on S2, S3, and NS treated eggs. However, the S1 treated eggs exhibited the highest eggshell CF with 0.83 logCFU/g ($P < 0.05$). There was not any microbial population detected in the terms of eggshell *Salmonella spp* in all treatment groups ($P > 0.05$).

Table 4.2. Effects of SPIDES on eggshell microbial population during long eggs storage

Group		18 DOI Microbial population			
	n ¹	TMAB	YMR	CF	SL
-----LogCFU/g-----					
NS	6	3.74 ^a	1.17 ^a	0.00 ^b	nd
S1	6	4.18 ^a	1.19 ^a	0.83 ^a	nd
S2	6	3.98 ^a	0.48 ^b	0.00 ^b	nd
S3	6	2.98 ^b	0.00 ^b	0.00 ^b	nd
SEM		0.15	0.18	0.16	nd
<i>P-Value</i>		0.000	0.000	0.000	

^{a-b} Means within a column that do not share a letter are significantly different ($P < 0.05$), NS: Eggs were stored 28 d without pre-incubation heat treatment during storage period; S1: Eggs were three times short period incubated at 6, 12 d and 18 over of 28 d of storage until the eggshell temperature reached 32-34°C then eggs were immediately cooled down; S2: Eggs were three times short period incubated at 6, 12 d and 18 over of 28 d of storage until the eggshell temperature reached 28-30°C then eggs were immediately cooled down, S3: Eggs were two times short period incubated at 8 d and 17 d over of 28 d of storage until the eggshell temperature reached 32-34°C then eggs were immediately cooled down; n¹: number of eggs used in each group to determine the eggshell microbial population, TMAB: Total mesophilic aerobic bacteria, CF: Total coliform bacteria, YMR: Total yeast and mold; SL: *Salmonella spp*. nd: non detected, DOI: day of incubation.

4.3.5. Hatchability characteristics

Table 4.3 shows the effects of SPIDES during egg storage on HOF and EM stages. Control embryos (CO) were stored only 3 d and then incubated to hatch. These eggs exhibited a HOF of 75.42% and low early (12.71%), middle (4.20%), and late (7.63%) embryonic mortality rates. On the other hand, NS eggs, stored for 28 days, show a decreased hatch rate of 21.69%. This was partly rescued by the SPIDES treatments, improving the HOF to 36.13% in S3 and 30.12% in S1. However, the HOF in S3 was 6.01% higher than in S1. With the exception of S2 treated eggs, the overall effect of pre-incubation heat treatment in S1 and S3 treated eggs on HOF was significant compared to NS treated eggs ($P < 0.05$). The heat treatment during storage until EST reached 28-30°C in the S2 eggs did not exhibit a positive effect on HOF. Embryonic mortalities were increased in NS-treated eggs (60.03%, 9.99%, and 8.29%

of fertile eggs at early, middle, and late-stage EM respectively) compared to CO eggs (12.71% early, middle 4.20%, and 7.63% late); the SPIDES treatments when compared to NS eggs slightly rescued early mortality (EEM), by decreasing it to 42.45% in S3 and 43.47% in S1, whereas EEM was extremely higher in S2-treated eggs, at 56.16%. The middle mortality (MEM) was higher in the S1 group at 18.37% compared to the NS (9.99%), S2 (11.69%), and S3 (10.04%); while in late mortality (LEM) was higher in S2-treated eggs at 11.04% and S3-treated eggs (11.38%) compared to NS (8.29%) and S1 (8.03%) treated eggs.

Table 4.3. Effects of short periods of incubation during egg storage on hatching results

Group	HOF (%)	Embryonic mortality (% of fertile eggs)		
		Early	Middle	Late
CO	75.42 ^a	12.71 ^d	4.20 ^c	7.63 ^b
NS	21.69 ^d	60.03 ^a	9.99 ^b	8.29 ^b
S1	30.12 ^c	43.47 ^c	18.37 ^a	8.03 ^b
S2	21.10 ^d	56.16 ^b	11.69 ^b	11.04 ^a
S3	36.13 ^b	42.45 ^c	10.04 ^b	11.38 ^a
SEM	0.71	0.79	0.47	0.42
P-Value	0.000	0.000	0.000	0.000

^{a-b-c-d} Means within a column that do not share a letter are significantly different ($P < 0.05$)

CO: Eggs were stored 3 d without pre-incubation heat treatment and then incubated

NS: Eggs were stored 21 d without pre-incubation heat treatment during storage period; S1: Eggs were three times short period incubated at 6, 12 d and 18 over of 28 d of storage until the eggshell temperature reached 32-34°C then eggs were immediately cooled down; S2: Eggs were three times short period incubated at 6, 12 d and 18 over of 28 d of storage until the eggshell temperature reached 28-30°C then eggs were immediately cooled down, S3: Eggs were two times short period incubated at 8 d and 17 d over of 28 d of storage until the eggshell temperature reached 32-34°C then eggs were immediately cooled down, HOF: hatching of fertile eggs.

4.3.6. Chick quality and organs weight

The effects of SPIDES on day-old chick weight, chick length, YFBW, RYS, organ weights are shown in Table 4.4. In the present experiment, SPIDES treatments had no influence on chick weight, YFBW, relative HW, RYS and LW ($P > 0.05$). However, the relative GW and proventriculus weights were affected by SPIDES treatments ($P < 0.05$). The GW was higher in chicks from S1 (7.54 g) treated eggs group ($P < 0.05$) compared to those from S2 (6.38 g) treated group, and intermediate to those from NS (6.71 g) and S3 (6.96 g) treated eggs groups while the pro-ventriculus weight was higher in chicks from S1 (1.26 g) and S3 (1.15 g) compared to those from NS (0.97 g) and S2 (0.98 g). The SPIDES treatment had an effect on chick length; chicks hatched

from S1 (18.18 cm) and S3 (17.84 cm) treated eggs were longer ($P<0.05$) than those hatched from S2 (17.26 cm) and NS (17.36 cm) treated eggs.

Table 4.4. Effects of SPIDES on chick length, relative yolk sac, yolk-free body weight, and organs weight

Group	Chick average Weight (g)	Chick length (cm)	YFBW (g)	RYS (%)	HW (%)	LW (%)	GW (%)	PrW (%)
NS	34.29	17.36 ^c	31.41	8.33	0.78	2.64	6.71 ^{ab}	0.97 ^c
S1	34.15	18.18 ^a	31.78	6.86	0.72	2.77	7.54 ^a	1.26 ^a
S2	34.85	17.26 ^c	31.76	8.82	0.80	2.68	6.38 ^b	0.98 ^c
S3	36.05	17.84 ^b	32.86	8.77	0.74	2.64	6.96 ^{ab}	1.15 ^b
SEM	0.82	0.06	0.69	0.89	0.02	0.08	0.27	0.02
<i>P-value</i>	0.357	0.000	0.493	0.375	0.062	0.629	0.032	0.000

^{a-b-c} Means within a column that do not share a letter are significantly different ($P<0.05$)

NS: Eggs were stored 28 d without pre-incubation during storage period; S1: Eggs were three times short period incubated at 6, 12 d and 18 over of 28 d of storage until the eggshell temperature reached 32-34°C then eggs were immediately cooled down; S2: Eggs were three times short period incubated at 6, 12 d and 18 over of 28 d of storage until the eggshell temperature reached 28-30°C then eggs were immediately cooled down, S3: Eggs were two times short period incubated at 8 d and 17 d over of 28 d of storage until the eggshell temperature reached 32-34°C then eggs were immediately cooled down; The residual yolk sac (RYS), yolk free body weight (YFBW) and relative HW (Heart weight), LW (Liver weight), GW (Gizzard weight) PrW (Proventriculus weight) were calculated as percentages of live weight.

In the current study, chicks hatched from CO (85.63) and all SPIDES treatment groups (S1=84.90, S2=84.25, S3=84.29) exhibited better Tona chick quality (Figure 4.3) than those hatched from NS (73.10) treated eggs ($P<0.05$).

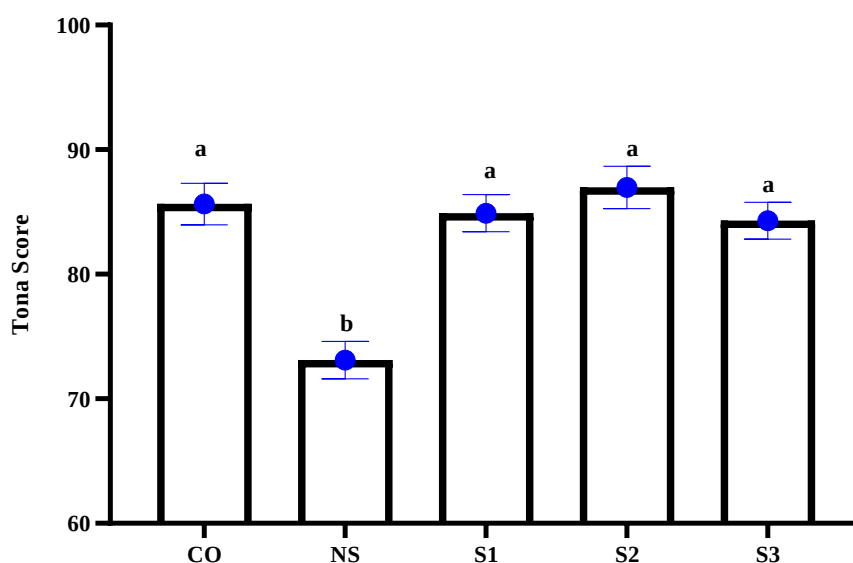


Figure 4.3. Effects of short periods of incubation during egg storage on Tona score chick quality

4.3.7. Chick performance

The effects of SPIDES on chick CW and CWG are shown in Table 4.5. In the present study CW and CWG were statically significant among treatment groups ($P < 0.05$). At (0 d) hatch, CW was higher ($P < 0.05$) for chicks hatched from S2 and S3-treated eggs, lower for chicks hatched from S1 treated eggs and intermediate for NS-treated eggs chicks. At 7 d, chicks from S3-treated eggs (87.82 g) were heavier than S1 (82.45 g) and S2 (80.32 g) chicks ($P < 0.05$), and intermediate for NS-treated eggs chicks (84.38 g). However, at 14 d chicks that hatched from S3-treated eggs (145.30 g) were heavier than those that hatched from S2-treated eggs (136.80 g) ($P < 0.05$), and intermediate from NS (143.01 g) and S1 (138.00 g) treated eggs chicks. At 7 d, chicks that hatched from S3 (49.91g) and NS (47.25 g) treated eggs exhibited the highest CWG ($P < 0.05$), then those that hatched from S2 (42.68 g) treated eggs groups, and intermediate to those hatched from S1 (45.56 g) treated eggs but at 14 d CWG were similar among all treatment groups ($P > 0.05$).

Table 4.5. Effects of SPIDES on chick weight and chick weight gain

Group	Body weight				
	Period				
	ICW	7 d CW	0-7d CWG	14 d CW	7-14 d CWG
			(g)		
NS	37.13 ^{ab}	84.38 ^{ab}	47.25 ^a	143.01 ^{ab}	58.62
S 1	35.90 ^b	82.45 ^b	46.56 ^{ab}	138.00 ^{ab}	55.55
S 2	37.64 ^a	80.32 ^b	42.68 ^b	136.80 ^b	56.51
S 3	37.91 ^a	87.82 ^a	49.91 ^a	145.30 ^a	57.49
SEM	0.40	1.25	1.23	2.20	1.30
<i>P-value</i>	0.001	0.000	0.001	0.018	0.349

^{a-b} Means within a column that do not share a letter are significantly different ($P < 0.05$)

NS: Eggs were stored 28 d without pre-incubation heat treatment during storage period; S1: Eggs were three times short period incubated at 6, 12 d and 18 over of 28 d of storage until the eggshell temperature reached 32-34°C then eggs were immediately cooled down; S2: Eggs were three times short period incubated at 6, 12 d and 18 over of 28 d of storage until the eggshell temperature reached 28-30°C then eggs were immediately cooled down, S3: Eggs were two times short period incubated at 8 d and 17 d over of 28 d of storage until the eggshell temperature reached 32-34°C then eggs were immediately cooled down, CW: chick weight, ICW: initial chick weight; CWG: chick weight gain.

The effects of SPIDES on chick relative growth are shown in figure 4.4. In the present study, the RG was higher in chicks hatched from S1 (130.00%), S3 (132.30%), and NS (128.00 %) compared to those hatched from S2 (114.00%) treated eggs ($P < 0.05$) at 7 d. However, at 14 d, RG was higher in chicks that hatched from NS (289.30%) treated eggs than those from S2 (264.50%) treated eggs, and intermediate to those hatched from S1 (284.80%) and S3 (285.10%) treatment groups.

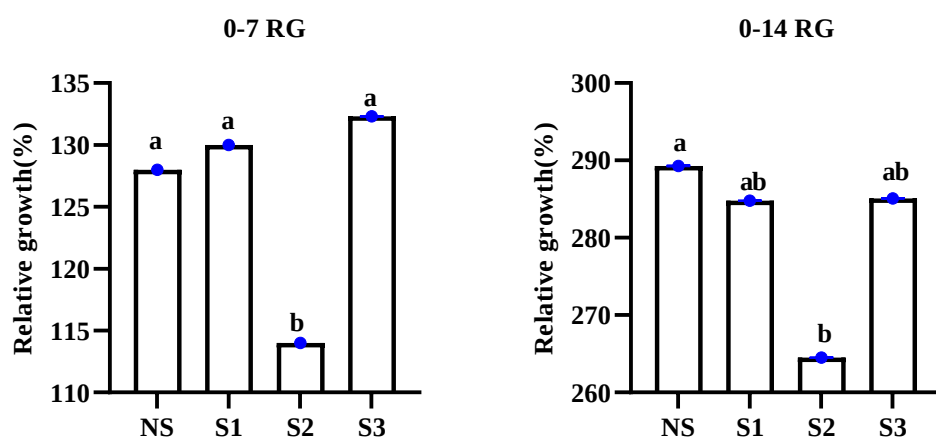


Figure 4.4. Effects of SPIDES on chick relative growth

4.3.8. Feed consumption

Table 4.6 shows the effects of SPIDES on feed intake per chick (FPC) and feed conversion ratio (FCR). At 7 d of age, chicks obtained from NS (86.57 g) and S1 (87.66 g) treated eggs had higher FPC ($P < 0.05$) than from those of S2 (78.80 g) and S3 (79.68 g) treated eggs. However, at 14 d of age, chicks obtained from S1 (250.20 g) treated eggs had the highest FPC ($P < 0.05$) than those of S2 (233.10 g) and S3 (232.30 g) treated eggs, and intermediate for those obtained from NS (245.70 g) treated eggs. However, the results of analysis didn't show any significant difference between the treatments on FCR at the 7 days of age. At 14 days of age, the FCR was lower in S2 (1.571 g) and S3 (1.553 g) treated chicks compared to those of S1 (1.645 g) and NS (1.641 g), which had the highest FPC.

Table 4.6. Effects of SPIDES on feed consumption and feed conversion ratio

Group	Feed Consumption			
	Period			
	0-7 FPC	0-14 FPC	0-7 FCR	0-14 d FCR
	------(g)-----		------(g/g)-----	
NS	86.57 ^a	245.70 ^{ab}	0.955	1.641 ^a
S 1	87.66 ^a	250.20 ^a	0.952	1.654 ^a
S 2	78.80 ^b	233.10 ^b	0.941	1.571 ^b
S 3	79.68 ^b	232.30 ^b	0.936	1.553 ^b
SEM	1.62	3.64	0.01	0.02
<i>P-value</i>	0.000	0.001	0.638	0.000

^{a-b} Means within a column that do not share a letter are significantly different ($P < 0.05$)

NS: Eggs were stored 28 d without pre-incubation during storage period; S1: Eggs were three times short period incubated at 6, 12 d and 18 over of 28 d of storage until the eggshell temperature reached 32-34°C then eggs were immediately cooled down; S2: Eggs were three times short period incubated at 6, 12 d and 18 over of 28 d of storage until the eggshell temperature reached 28-30°C then eggs were immediately cooled down, S3: Eggs were two times short period incubated at 8 d and 17 d over of 28 d of storage until the eggshell temperature reached 32-34°C then eggs were immediately cooled down, FPC: feed intake per chick; FCR: feed conversion ratio.

4.4. Discussion and Conclusion

4.4.1. Eggshell microbial population

In this experiment, SPIDES 2 and SPIDES 3 treated eggs, showed a greater reduction in the numbers of shell bacteria on the 18th day of incubation ($P < 0.05$). Our findings were in agreement with previous studies of Cook et al. (2003) and Cook et al. (2005) that reported that incubation is thought to reduce or limit bacterial growth by altering the microbial populations associated with eggshells, by maintaining the dryness of the shell (D'Alba et al., 2010; Ruiz-de-Castañeda et al., 2011) and by controlling bacterial affluence (Shawkey et al., 2009). Baggott and Graeme-Cook (2002) stated that incubation decreased the abundance of eggshell microbes, influenced the composition of the eggshell microbial population (Grizard et al., 2014; Potter et al., 2013), leading to a less pathogenic microbiota (Brandl et al., 2014; Lee et al., 2014). Bollinger et al. (2018) reported that during oviposition partial incubation can improve egg viability in eastern bluebirds by decreasing eggshell microbial populations. Gentry and Quarles (1972) found that incubation at 38°C showed a higher decrease in the number of shell bacteria after 1 and 4 days and was close to 10% of the original counts after 7 days. In the current study, no microbial population was found in the terms of eggshell salmonella in all treatment groups ($P > 0.05$). Previous studies of Messens et al. (2005)

reported that during storage, the probability of survival *S. enterica* on eggshells was rare due to the rapid death of the pathogen. Salmonella on eggshells can die off during storage at a very low relative humidity (Simmons et al., 1970) or at low temperatures (Baker, 1990; Braun et al., 1999; Radkowski, 2002; Rizk et al., 1966).

4.4.2. Eggs weight loss

The SPIDES treatments increased EWL during storage mainly in S1 treated eggs ($P < 0.05$) in the current study. These findings are in agreement with previous studies of Ebeid et al. (2016) and Ebeid et al. (2017) who found that the SPIDES treatments increased EWL during storage. Similarly, other previous studies reported that prestorage heating eggs for 5 hours (Terčič and Pestotnik, 2016), 6 hours (Abdel-Halim et al., 2015), 9 hours (Gharib, 2013), and 6 or 12 hours (Silva et al., 2008a) increased EWL percentage during storage compared to the non-heated group. Reijrink et al. (2009) and Reijrink et al. (2010a) also found that prestorage incubation and frequent warming increased EWL during storage by 0.18% and by 0.12%. However, the current findings are in contrast to other study that reported SPIDES treatments did not affect the relative EWL (El-Menawey, 2019).

In the current study, SPIDES treatments did not affect EWL during incubation and TEWL ($P > 0.05$). These are in agreement with previous studies of Ebeid et al. (2016) and Reijrink et al. (2009) who found that pre-storage incubation did not affect EWL during incubation and total egg weight loss, whereas in disagreement with (Reijrink et al., 2010a) who stated that frequent warming increased EWL during incubation by 0.28% and TEWL by 0.39%, Silva et al. (2008a) found that eggs stored for 9 and 12 days presented a higher EWL during incubation when heated for 6 hours as compared to non-heated eggs, (Abdel-Halim et al., 2015) who found that heating egg continuously for 6 hours before storage lower EWL after 18 days of incubation compared to those non-heated and (Gharib, 2013) who found that 9 hours pre-storage heated eggs had higher EWL during incubation and the TEWL.

4.4.3. Hatchability characteristics

A positive effect of pre-incubation heat treatments on hatchability was found ($P < 0.05$) in this experiment of the current study. Control eggs (CO) exhibited the

highest hatch rate and the lowest EM while NS-treated eggs exhibited the lowest HOF ($P<0.05$). The HOF was rescued by SPIDES treatments which increased by 8.43% in S1-treated eggs and by 14.44% in S3-treated eggs due to a decrease in EEM ($P<0.05$). Furthermore, the S3-treated eggs exhibited the highest HOF among SPIDES treatments compared to NS group ($P<0.05$). These results agreed with the previous findings of Özlü et al. (2018b), Özlü et al. (2021a) indicated that SPIDES-treatments increased HOF by 3.8%. Additionally, the study findings are similar to those of Özlü et al. (2021b), Damaziak et al. (2018), Tag EL-Din et al. (2017), Al-Samrai and Al-Dhanki (2017) and Gharib (2013) who showed that the pre-incubation heat treatments increases hatchability compared to non-heated groups. Furthermore, in this experiments the HOF was not rescued in S2-treated eggs which exhibited the lowest HOF when compared to the S1 and S3 treatment groups ($P<0.05$) and intermediate with NS-treated eggs ($P>0.05$). In this experiment, the HOF of 28 d stored eggs was mended after SPIDES treatment, HOF was still very low. It can be hypothesized; thus, that SPIDES treatment was insufficient to rescue the HOF of eggs stored long term (28 days).

In the current experiment, EEM was higher in NS-treated eggs compared to the S1, S2, and S3 treatment groups ($P<0.05$). Likewise, Özlü et al. (2021a), El-Menawey (2019) and Abdel-Halim et al. (2015) mentioned that the pre-incubation heat treatments decreased EEM when compared to the non-heated groups. However, the current study findings are contrary to those of Ebeid et al. (2016) and Reijrink et al. (2009) who indicated that the pre-incubation heat treatment did not have effect on EEM.

On the other hand, MME was higher in S1 treated-eggs than in NS, S2, and S3 treated-eggs ($P<0.05$). Previously, Ebeid et al. (2016), Silva et al. (2008a) and Gharib (2013) indicated that the intermediate embryo mortality is not influenced by pre-storage heating treatments as well as Tag EL-Din et al. (2017) and Ebeid et al. (2017) stated that heating eggs during SPIDES has lowered mid embryonic mortalities.

The LEM was higher in S2 and S3 treated eggs compared to NS and S1 treated eggs ($P<0.05$). These findings are contrary to those of Nicholson et al. (2013), Dymond et al. (2013), Gharib (2013) Özlü et al. (2021b), who reported that pre-incubation heating eggs decreased LEM in broiler embryos.

4.4.4. Chick quality and organs weight

The positive effect of pre-incubation heat treatments on chick quality was observed in the current experiment. The hatching chick weight, YFBW, heart and liver, weights were not significantly affected by different pre-incubation treatment groups ($P>0.05$). These results are in agreement with Ebeid et al. (2016) for chick weight and (Silva et al., 2008b) for RYS and Reijrink et al. (2010a) for YFBW. These findings are in contrast to those of El-Menawey (2019) and Silva et al. (2008b) who highlighted that pre-storage heating for six hours increased hatching weight, and chick weight without a yolk sac, and pre-storage heating for six and twelve hours increased LW. However, the relative gizzard and proventriculus weights were affected by SPIDES treatments ($P<0.05$). These findings are contrary to those of Silva et al. (2008b) who found that pre-storage heating for six and 12 hours did not affect relative proventriculus and gizzard weights.

The SPIDES-treatments significantly affected chick length ($P<0.05$). Chicks hatched from S1 and S3 treated eggs were longer compared to NS and S2 treatment groups. Likewise, Reijrink et al. (2009), Gharib (2013), and El-Menawey (2019) reported that pre-storage incubation increased chick length and disproved those of Reijrink et al. (2010a) and Tag EL-Din et al. (2017) who found that pre-storage incubation did not have any effect on chick length. The chicks from all SPIDES treatments had the highest Tona score compared to NS-treated chicks ($P<0.05$). These findings aligned with of the studies of El-Menawey (2019), Damaziak et al. (2018), and (2013) who found that compared to no-SPIDES treated eggs the SPIDES groups had a better Tona score.

4.4.5. Chick performance and feed consumption

In this experiment, CW, CWG, and RG were statically significant among treatment groups ($P<0.05$). Although chick's growth of S1 and S3 treatment groups did not differ significantly from NS, chicks from S3 treated eggs exhibited better chick growth. The chick from S2 treated eggs exhibited worse growth performances. In terms of chick hatching weight, our findings are similar to those of Dymond et al. (2013), Ebeid et al. (2016) and Tag EL-Din et al. (2017) who reported that the pre-storage incubation did not affect chick weight, and contradicted those of Gharib (2013) and El-

Menawey (2019) who found that pre-storage heating increased hatching weight and Dymond et al. (2013) who found that the SPIDES treatment group had a higher RG than non SPIDES group.

In the current study, feed consumption was affected by SPIDES treatment groups ($P<0.05$). According to the obtained results, chicks from S2 and S3 treated eggs had the lowest food consumption compared to those from S1 and NS treated eggs ($P<0.05$). It can be hypothesized; therefore, that pre incubation heat treatment in S2 treated eggs did not an effective method in order to improve the chick performance.

Overall, the results of the current study confirmed that SPIDES at d 28 eggs storage had a positive effect; microbial eggshell population, hatchability of fertile eggs, chick quality, and chick performances. In conclusion, the highest hatchability was observed in twice warming treated eggs, on d 8 and 17 after egg collection during d 28 of storage in old-aged flocks. The results of these studies also emphasized that applying twice pre-incubation heat treatment at 36°C until EST reached an average of 32-34°C, delivered two (2) times at 8 and 17 d over of 28 days of storage can be effective by reducing microbial eggshell population, improving hatchability, chick quality, and performance and can reduce the detrimental effects of long storage of hatching eggs. However, when applying three (3) times pre-incubation heat treatment at 36°C until EST reached an average of 28-30°C, delivered at 6, 12, and 18 d over of 28 d of storage can have unsatisfied impacts on hatchability and increased early mortality. Despite the fact that hatchability was partially rescued by SPIDES treatment, the 28 days' storage period affected negatively hatchability by dramatically lowered hatchability and increased embryonic mortality.

5. EXPERIMENT II: THE EFFECTS OF SHORT PERIODS OF INCUBATION DURING 21 DAYS EGG STORAGE ON INTERNAL EGG QUALITY, HATCHABILITY, CHICK QUALITY, AND TWO (2) WEEKS CHICK PERFORMANCE OF EGGS FROM 72 WEEKS LAYER PARENT STOCK FLOCK

5.1. Materials and Methods

5.1.1. Experimental Setup

In this experiment, the hatching eggs were stored for 21 d and pre-incubation heated at 6, 8, 12, 17 and 18 d of storage

5.1.2. Breeder flocks and study site

A total of 2,000 eggs from a commercial Babcock white parent stock breeder, were used. All eggs were sourced through a commercial hatchery (Anadolu Damızlık Konya/Turkey) and came from a single 72-wk-old flock. The experiment was conducted during July 2020 to September 2020.

5.1.3. Egg storage conditions and pre- incubation warming

The hatching eggs were stored in the storage machine (Çımuka model) at an average temperature and relative humidity (RH) of 12.68°C and 83%, respectively. All the eggs were received on the same day of collection at the breeder farm, transported in a temperature-controlled vehicle to the University of Selcuk. A total of 2,000 eggs were distributed randomly in 5 groups with 400 eggs per treatment. The eggs were stored for 21 d and the experimental groups, as follow: Control “(CO) treatment”: the eggs stored 3 d and then incubated; non-SPIDES control eggs “(NS)” indicating the eggs stored for 21 d , without heat treatment; "S1" = eggs were three times treated at 36°C until EST reached 32-34°C, delivered at 6, 12 d and 18 d over of 21 d of storage; "S2" = eggs were three times treated at 36°C until EST reached 28-30°C, delivered at 6, 12 and 18 d over of 21 d of storage; "S3" = eggs were two times treated at 36°C until EST reached 32-34°C, delivered at 8 and 17 d over of 21 d of storage. In each heat treatment group after reaching the target EST, eggs were immediately returned to the storage machine.

5.1.4. Internal egg characteristics on the day of oviposition and during storage

In the current experiment, on the day of oviposition, before the start of the storage treatment (d 0), 50 eggs were selected randomly, weighed, and broken to measure the albumen height, albumen index, yolk index, albumen pH, and Haugh units (HU). At the end of storage (21 d), the same measures were performed in 50 eggs per treatment. The height of the albumen was measured using an Egg Analyzer (05-UM-001, Version B, Orka Food Tech. Ltd.). The Haugh units were calculated from albumen height and egg weight with the following: $\text{Haugh unit} = \log(H + 7.57 - 1.7W^{0.37}) \times 100$, where H refers to the albumen height (mm) and W to the weight of the egg (g) (Haugh, 1937). The albumen index and yolk index were measured with a micrometer caliper and a digimatic height gauge accurate to 0.01 mm (Funk, 1948). Then the pH of yolk and albumen was measured was performed using a Seven2Go pH meter S2-Light-Kit.

5.1.5. Egg weight loss

For each treatment before storage, a total of 45 eggs were selected randomly and then enumerated from 1 to 45. To determine the EWL during storage in each treatment eggs were weighed individually at the (0) day of storage and at the end of day 21 of storage. To determine the weight loss during incubation the same eggs were weighed on the 18th day of incubation.

5.1.6. Incubation and hatching conditions

Following a storage period of exactly 21 d the eggs were moved from the storage machine to the setter and were preheated for 6 hours at a temperature of 30°C. A standard single-stage incubation program was used during the setter and the setpoint temperature was 38°C during the first five (5) days of incubation and was reduced gradually to 37.7°C at E6 of incubation until E18 with a relative humidity of 55%. During incubation, the eggs were turned every hour until day 18 of incubation. At 18th day of incubation, the eggs were transferred to hatching cabinets where the air temperature and relative humidity were kept at 37.2°C and 70% RH, respectively. All the eggs were distributed into 35 hatching baskets with ten eggs per replicate according to their group. Each group had 35 replicates with a total of 350 eggs and unhatched eggs were broken and opened to determine the fertility and embryonic mortality stage.

5.1.7. Chick quality

After 512 h of incubation quality criteria were determined for 40 female chicks randomly selected from each group and weighed and were used to assess Tona score chick quality. After 528 h of incubation to determine the relative organ weight, 20 late hatched chicks randomly selected from each group were weighed and euthanized by cervical dislocation and then necropsied to determine the residual yolk sac (RYS), heart weight (HW), liver weight (LW), Gizzard weight, proventriculus weight (PrW), and yolk free bodyweight (YFBW).

5.1.8. Body weight, relative growth, feed consumption, and feed conversion ratio

After 528 hours of incubation, 160 female chicks from 4 treatments (40 chicks per treatment) were placed in Çımuka grower battery cage. A total of 40 female chicks selected from each treatment were weighed individually and distributed among 16 (4 pens × 4 treatments) floor pens (120 cm× 120 cm) and each pen contained 2 nipple-type drinkers. Individual CW, CWG, and RG were controlled weekly for up to 14 days of life. Chicks were fed ad libitum with a layer starter feed. During the same period, feed consumption and feed conversion ratio (FCR) were determined each week per pen up to the 14th day.

5.2. Statistical Analysis

The effects of SPIDES on eggshell microbial population, egg weight loss, incubation results, chick quality, and performance were analyzed by one-way variance analysis by using the GLM (general linear model) procedure (SAS, 1989). Accordingly, the significant differences in the treatment means were determined by Tukey's multiple range tests at a 0.05 significance level.

5.2. Results

5.2.1. Temperature and relative humidity during storage

Figure 5.1 shows the storage machine air temperature and humidity during the eggs storage period. The average storage ambient temperature, relative humidity and DewPt during 21 days of storage were 12.68°C, 83% and 9.90°C, respectively.

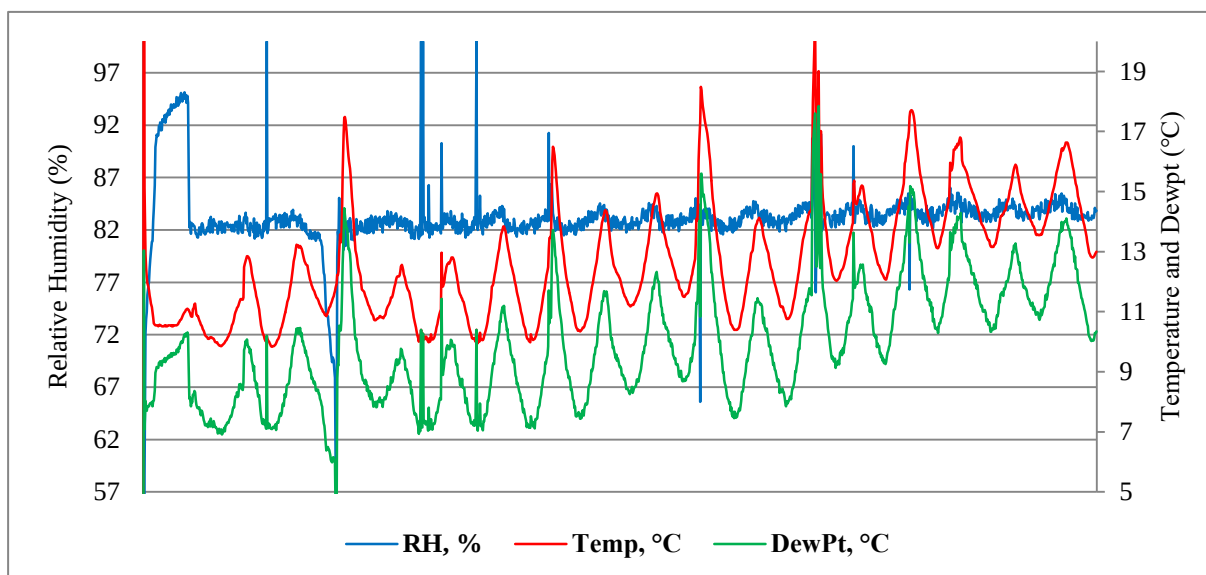


Figure 5.1. Storage machine air condition during 21 days eggs storage

5.2.2. Eggshell Temperature before heating and during SPIDES

The eggshell temperature (EST) before SPIDES (photo A) and after SPIDES in S2 (photo B) and in S1 and S3 (photo C) are shown in photos A, B, and C, taken by a thermal camera (Figure 5.2). During the SPIDES the target EST in each SPIDES treatment was determined by a thermal camera. As observed on photo A before preheating EST was 10.8°C, for treatment S2 (photo B) was 29.4°C while for treatments S1 and S3 (Photo C) was 33.8°C.

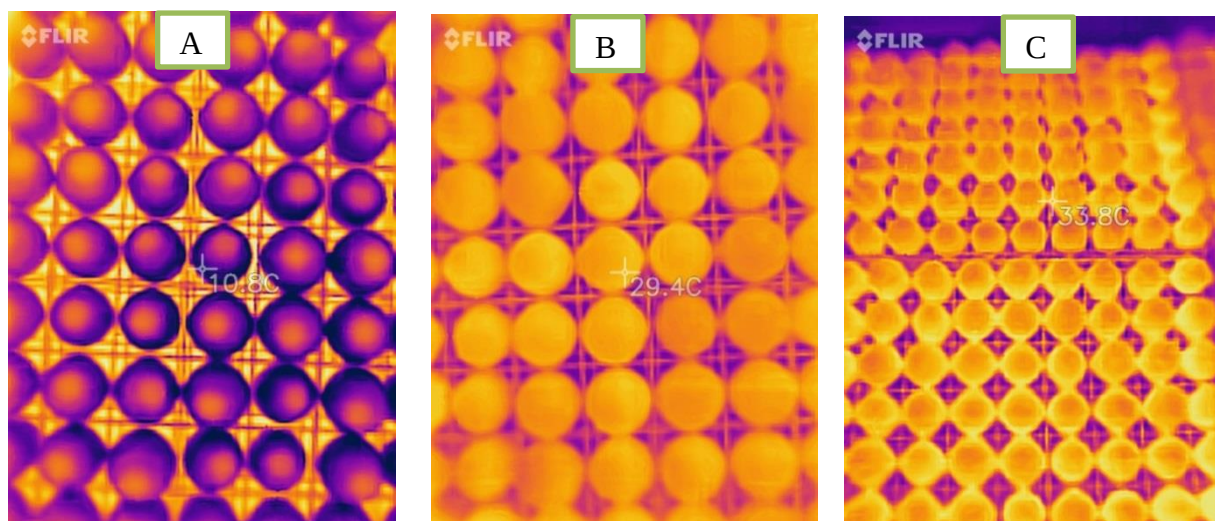


Figure 5.2. Eggshell temperature before short incubation (A), (B) Eggshell temperature during short incubation during storage (S2), (C) Eggshell temperature during short incubation (S1 and S3)

5.2.3. Characteristics of internal egg quality

The effects of pre-incubation heat treatment on internal egg quality traits are shown in Table 5.1. The storage period and pre-incubation heat treatment significantly affected almost all of the parameters of internal egg quality. The albumen height, Haugh unit, albumen index, and yolk index significantly decreased with increased storage period ($P < 0.05$). However, the albumen pH, albumen length, albumen width, yolk diameter increased significantly by increasing the storage period, whereas the yolk height did not affect the storage time and the pre-incubation heat treatment for all treatments ($P > 0.05$). SPIDES treatment did not show any significant effect on the egg internal quality ($P > 0.05$). Despite the difference was not statically different, but the SPIDES 2 (S2) treated eggs were numerically higher in the terms of albumen height, Haugh unit, and albumen index compared to the NS, S1, and S3-treated eggs.

Table 5.1. Internal eggs quality characteristics according to storage time and pre-incubation warming

groups	n ¹	EW (g)	AH (mm)	AL (mm)	AW (mm)	YH (mm)	YD (mm)	HU	A pH	AI	YI
PC	50	63.01	6.97 ^a	81.57 ^b	63.71 ^b	19.12	40.67 ^b	82.35 ^a	8.67 ^b	9.48 ^a	47.13 ^a
NS	50	63.97	5.59 ^b	92.90 ^a	74.96 ^a	19.50	43.18 ^a	72.22 ^b	9.85 ^a	6.72 ^b	45.25 ^{ab}
S1	50	62.13	5.82 ^b	93.49 ^a	73.67 ^a	19.14	44.10 ^a	72.00 ^b	9.83 ^a	6.97 ^b	43.43 ^b
S2	50	63.14	6.15 ^{ab}	95.98 ^a	72.72 ^a	19.02	44.27 ^a	76.28 ^{ab}	9.78 ^a	7.39 ^b	42.99 ^b
S3	50	62.31	5.40 ^b	91.42 ^{ab}	77.21 ^a	19.30	44.58 ^a	72.61 ^b	9.83 ^a	6.70 ^b	43.30 ^b
SEM		1.08	0.27	2.58	1.80	0.26	0.47	2.26	0.05	0.42	0.83
<i>P-value</i>		0.794	0.001	0.000	0.000	0.752	0.000	0.009	0.000	0.000	0.001

^{a-b} Means within a column that do not share a letter are significantly different ($P < 0.05$), PC: Eggs were not stored and broken at arrival day to determine the internal eggs quality, NS: Eggs were stored 21 d without pre-incubation heat treatment during storage period and then broken at d 21; S1: Eggs were three times short period incubated at 6, 12 d and 18 over of 21 d of storage until the eggshell temperature reached 32-34°C then eggs were immediately cooled down and broken at d 21; S2: Eggs were three times short period incubated at 6, 12 d and 18 over of 21 d of storage until the eggshell temperature reached 28-30°C then eggs were immediately cooled down and broken at d 21, S3: Eggs were two times short period incubated at 8 d and 17 d over of 21 d of storage until the eggshell temperature reached 32-34°C then eggs were immediately cooled down and broken at d 21. n¹: number of eggs used in each group to determine the internal egg quality, EW: egg weight, AH: Albumen Height, AL: Albumen Length, AW: Albumen Width, YH: Yolk Height, YD: Yolk Diameter, HU: Haugh Unit, ApH: Albumen pH, AI: Albumen Index, YI: Yolk Index

5.2.4. Egg weight loss

Table 5.2 shows the effect of SPIDES of fertile eggs on egg weight loss. The results of the analysis show that short incubation during egg storage on weight loss was higher in S1-treated eggs ($P < 0.05$) and were intermediate between NS, S2 and S3 groups ($P > 0.05$). In terms of total egg weight loss, although the difference was not significant, the S1 treatment group had a higher total egg weight loss than the other treatment groups (NS, S2, S3) with 14.53%, 15.07%, 14.01%, and 14.28% respectively.

Table 5.2. Effects of SPIDES on egg weight loss during 21 d of storage and at 18 d of incubation

Group	n ¹	IEW	EWLS	EWLI	TEWL
		------(g)-----		------(%)-----	
NS	45	64.18	1.98 ^b	12.55	14.53
S1	45	63.36	2.10 ^a	12.97	15.07
S2	45	62.93	1.99 ^b	12.01	14.01
S3	45	63.77	1.98 ^b	12.30	14.28
SEM		0.78	0.30	0.51	0.59
<i>P-Value</i>		0.694	0.006	0.599	0.626

^{a-b} Means within a column that do not share a letter are significantly different ($P < 0.05$)

NS: Eggs were stored 21 d without pre-incubation heat treatment during storage period; S1: Eggs were three times short period incubated at 6, 12 d and 18 over of 21 d of storage until the eggshell temperature reached 32-34°C then eggs were immediately cooled down; S2: Eggs were three times short period incubated at 6, 12 d and 18 over of 21 d of storage until the eggshell temperature reached 28-30°C then eggs were immediately cooled down, S3: Eggs were two times short period incubated at 8 d and 17 d over of 21 d of storage until the eggshell temperature reached 32-34°C then eggs were immediately cooled down, n¹: number of eggs used in each group to determine the egg weight loss. IEW: initial egg weight; EWLS: egg weight loss during 21 d storage; EWLI: egg weight loss during 18 d of incubation; TEWL: total egg weight loss.

5.2.5. Hatchability characteristics

Table 5.3 shows the effects SPIDES treatments on hatching results. The SPIDES treatments had significant effects on HOF and EM ($P < 0.05$) in the current study. In this trial, CO eggs exhibited a HOF of 73.12% and early (15.18%), middle (4.46%), and late (7.14%) EM. The HOF was increased ($P < 0.05$) in S1 by 7.69% and in S3-treated eggs by 13.38% compared to the NS-treated eggs. Furthermore, the S3-treated eggs exhibited the highest HOF among SPIDES treatments compared to NS group ($P < 0.05$). The S2 eggs did not exhibit a positive effect on HOF and was intermediate to NS-eggs ($P > 0.05$). The average EEM was highest in NS treated eggs compared to the SPIDES treatments and was lowest in S3 treatment group compared to S1 and S2-treated eggs ($P < 0.05$).

In the present study, MEM was higher in S2 and S3-treatment groups compared to NS and S1-treated eggs ($P < 0.05$). The LEM was higher in S1 and S2 heat treatment groups when compared to the NS group ($P < 0.05$) and LEM in S2 was intermediate to S3-treated eggs ($P > 0.05$).

Table 5.3. Effects of short periods of incubation during egg storage on hatching results

Group	HOF (%)	Embryonic Mortality (% of fertile eggs)		
		Early	Middle	Late
CO	73.12 ^a	15.18 ^c	4.46 ^c	7.14 ^d
NS	44.03 ^d	33.27 ^a	12.23 ^b	10.47 ^c
S1	51.72 ^c	16.91 ^c	14.56 ^b	16.82 ^a
S2	44.62 ^d	20.51 ^b	20.59 ^a	14.28 ^{ab}
S3	57.41 ^b	8.80 ^d	22.55 ^a	11.24 ^{bc}
SEM	1.29	1.40	0.79	0.81
<i>P-value</i>	0.000	0.000	0.000	0.000

^{a-b-c-d}: Means within a column that do not share a letter are significantly different ($P < 0.05$)

CO: Eggs were stored 3 d without pre-incubation heat treatment and then incubated, NS: Eggs were stored 21 d without pre-incubation heat treatment during storage period; S1: Eggs were three times short period incubated at 6, 12 d and 18 over of 21 d of storage until the eggshell temperature reached 32-34°C then eggs were immediately cooled down; S2: Eggs were three times short period incubated at 6, 12 d and 18 over of 21 d of storage until the eggshell temperature reached 28-30°C then eggs were immediately cooled down, S3: Eggs were two times short period incubated at 8 d and 17 d over of 21 d of storage until the eggshell temperature reached 32-34°C then eggs were immediately cooled down, HOF: hatching of fertile eggs.

5.2.6. Chick quality and organ weights

The effects of SPIDES on YFBW, RYS, and supply organ weights are shown in table 5.4. In the current study, hatching chick weight, YFBW, heart, liver, and proventriculus weights were not significantly affected by treatment groups ($P>0.05$). However, RYS and GW were affected by pre-incubation heat treatments ($P<0.05$). The RYS was higher in NS and S2 treated eggs when compared to those of S1 and S3 ($P<0.05$). The GW was higher in S1 and S3 treatment groups compared to NS group and were intermediate to S2 ($P<0.05$). With regards to the chick length, S1 and S3 chicks were longer with 18.20 cm and 18.10 respectively than the chicks of the NS and S2 groups with respectively 17.78 cm and 17.63 cm.

Table 5.4. Effects of SPIDES on chick length, relative yolk sac, yolk-free body weight, and organs weight

Group	Chick average Weight (g)	Chick length (cm)	YFBW (g)	RYS (%)	HW (%)	LW (%)	GW (%)	PrW (%)
NS	39.16	17.78 ^b	33.49	14.49 ^a	0.60	2.00	4.32 ^b	0.71
S1	38.35	18.20 ^a	34.10	11.08 ^b	0.58	1.98	4.99 ^a	0.76
S2	39.13	17.63 ^b	33.66	13.87 ^a	0.68	1.97	4.75 ^{ab}	0.75
S3	38.25	18.10 ^a	34.26	11.62 ^b	0.62	2.11	5.03 ^a	0.80
SEM	0.75	0.07	0.41	0.44	0.03	0.05	0.12	0.03
<i>P-value</i>	0.735	0.000	0.515	0.000	0.162	0.105	0.000	0.211

^{a-b} Means within a column that do not share a letter are significantly different ($P < 0.05$)

NS: Eggs were stored 21 d without pre-incubation during storage period; S1: Eggs were three times short period incubated at 6, 12 d and 18 over of 21 d of storage until the eggshell temperature reached 32-34°C then eggs were immediately cooled down; S2: Eggs were three times short period incubated at 6, 12 d and 18 over of 21 d of storage until the eggshell temperature reached 28-30°C then eggs were immediately cooled down, S3: Eggs were two times short period incubated at 8 d and 17 d over of 21 d of storage until the eggshell temperature reached 32-34°C then eggs were immediately cooled down; The residual yolk weight (RYS), yolk free body weight (YFBW) and relative HW (heart weight), LW (liver weight), GW (gizzard weight) and PrW (proventriculus weight) were calculated as percentages of live weight.

In the current study, chicks hatched from CO (91.50) and all SPIDES treatment groups (S1=88.81, S2=, 89.33, S3=91.67) exhibited better Tona chick quality (Figure 5.3) than those hatched from NS (83.13) treated eggs ($P<0.05$).

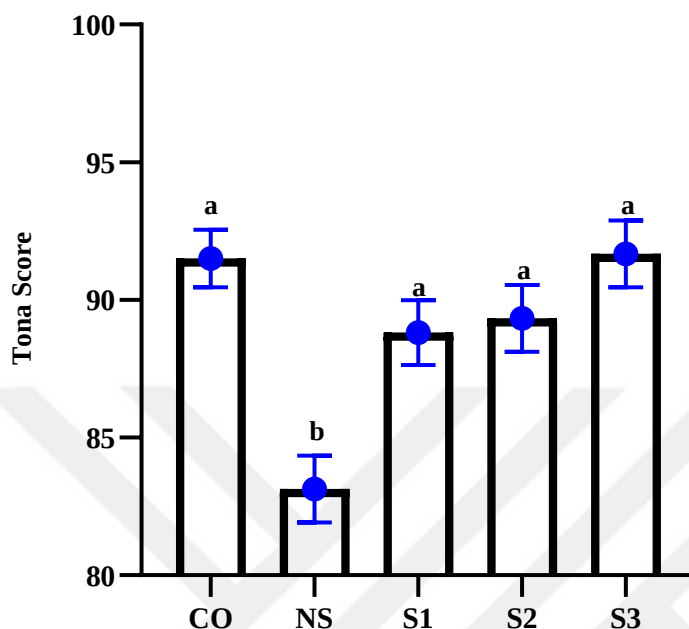


Figure 5.3. Effects of short periods of incubation during egg storage on Tona score chick quality

5.2.7. Chick performance

Table 5.5 shows the effects of short periods of incubation during egg storage on initial chick weight (ICW) and chick weight gain (CWG). Pre-incubation heat treatments had significant effects on average ICW and CWG ($P<0.05$). At wk. 0 (hatch) pre-incubation heat treatment (S1 and S3) groups did not differ significantly from that of NS. However, the S2 group had the lowest initial chick weight ($P<0.05$). Both S1 and S3 groups had higher CW and CWG than NS and S2 groups while, at d 14, CW and CWG did not significantly affect the pre-incubation heat treatment ($P>0.05$).

Table 5.5. Effects of SPIDES on chick weight and chick weight gain

Group	Body weight				
	Period				
	ICW	7 d CW	0-7d CWG	14 d CW	7-14 d CWG
NS	39.04 ^a	58.70 ^b	19.66 ^b	96.37	37.67
S 1	37.10 ^b	61.43 ^a	24.03 ^a	97.38	36.25
S 2	38.28 ^a	58.69 ^b	20.37 ^b	95.44	36.72
S 3	38.25 ^a	61.73 ^a	23.56 ^a	96.80	35.07
SEM	0.25	0.56	0.52	0.90	0.61
<i>P-value</i>	0.000	0.000	0.000	0.489	0.530

^{a-b} Means within a column that do not share a letter are significantly different ($P < 0.05$)

NS: Eggs were stored 21 d without pre-incubation heat treatment during storage period; S1: Eggs were three times short period incubated at 6, 12 d and 18 over of 21 d of storage until the eggshell temperature reached 32-34°C then eggs were immediately cooled down; S2: Eggs were three times short period incubated at 6, 12 d and 18 over of 21 d of storage until the eggshell temperature reached 28-30°C then eggs were immediately cooled down, S3: Eggs were two times short period incubated at 8 d and 17 d over of 21 d of storage until the eggshell temperature reached 32-34°C then eggs were immediately cooled down, CW: chick weight, ICW: Initial chick weight; CWG: Chick weight gain.

The effects of SPIDES on chick relative growth are shown in figure 5.4. At d 7 the RG was higher in the S1 and S3 treatment groups when compared to NS and S2 groups ($P < 0.05$). However, on days 0-14, S1 treatment chicks had a higher growth rate than NS and S2 treatment chicks in terms of RG ($P < 0.05$), but similar growth rate was detected with S3 treatment chicks ($P > 0.05$).

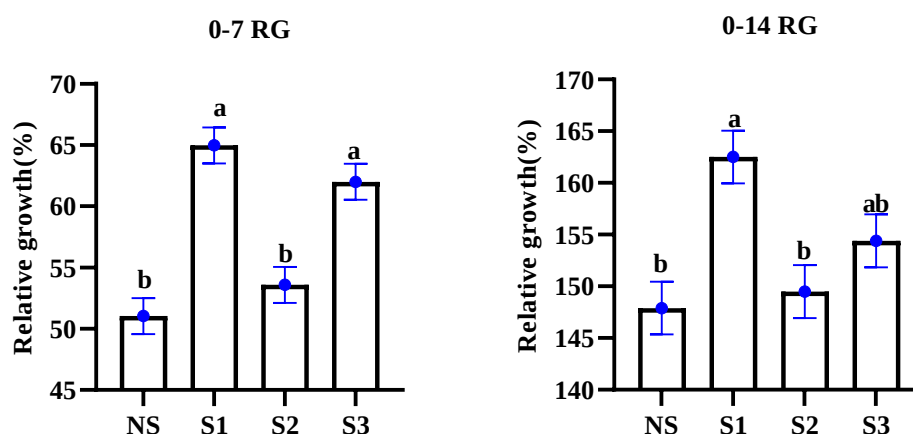


Figure 5.4. Effects of SPIDES on chick relative growth

5.2.8. Feed consumption

Table 5.6 shows the effects of a short period of incubation during egg storage on feed intake (FPC) and feed conversion ratio (FCR). In the current study, at 7 d, no difference in FPC and FCR was observed between treatment groups ($P>0.05$). However, at 14 d, FPC and FCR were higher in chicks from S2, S3 and NS than those from S1 ($P<0.05$). The chicks from S2 group had the highest 14 d FCR compared to other treatment groups ($P<0.05$).

Table 5.6. Effects of SPIDES on feed consumption and feed conversion ratio

Group	Feed Consumption Period			
	0-7 FPC	0-14 FPC	0-7 FCR	0-14 FCR
	----- (g) -----		----- (g/g) -----	
NS	91.46	169.00 ^a	1.559	1.753 ^b
S1	91.56	140.30 ^b	1.482	1.431 ^c
S 2	91.61	173.80 ^a	1.557	1.838 ^a
S 3	91.50	169.80 ^a	1.499	1.730 ^b
SEM	2.91	1.45	0.09	0.01
<i>P-value</i>	1.000	0.000	0.896	0.000

^{a-b-c} Means within a column that do not share a letter are significantly different ($P < 0.05$)

NS: Eggs were stored 21 d without pre-incubation heat treatment during storage period; S1: Eggs were three times short period incubated at 6, 12 d and 18 over of 21 d of storage until the eggshell temperature reached 32-34°C then eggs were immediately cooled down; S2: Eggs were three times short period incubated at 6, 12 d and 18 over of 21 d of storage until the eggshell temperature reached 28-30°C then eggs were immediately cooled down, S3: Eggs were two times short period incubated at 8 d and 17 d over of 21 d of storage until the eggshell temperature reached 32-34°C then eggs were immediately cooled down, FPC: feed intake per chick; FCR: feed conversion ratio.

5.3. Discussion and Conclusion

5.3.1. Characteristics of internal egg quality

The egg albumen plays a key role in the changes and viability of embryos (Brake et al., 1997; Hurnik et al., 1978; Lapao et al., 1999). In this current study, albumen height, Haugh unit, albumen index and yolk index decreased significantly with increasing storage period ($P<0.05$). Besides, the albumen pH, albumen length, albumen width, yolk diameter increased significantly with increasing storage period. These findings aligned with previously reported in terms of albumen height by Lapao et al. (1999) who indicated that the increase of time storage increases the pH of the albumen

whilst the height of the albumen reduces and in terms of yolk diameter by Jones and Musgrove (2005) stated that the elasticity of the yolk sac membrane decreases when increasing in storage period. Furthermore, in terms of Albumen pH, our findings are also aligned with those of Walsh et al. (1995) who reported that on the 4th day of storage the albumen pH approaches 9 and in terms of Haugh unit agreed with Tona et al. (2004) who reported that storage duration decrease the depressed egg albumen Haugh units. However, the yolk height did not affect by both storage time and pre-incubation heat treatment in all groups ($P>0.05$). In the current study, internal eggs quality characteristics were not affected ($P>0.05$) by SPIDES. Yolk index, albumen height, albumen index, Haugh unit, yolk diameter, albumen width, albumen length and albumen pH were statically similar in all preincubation treatment groups. This agreed with a previous report by Özlü et al. (2021b) who found that SPIDES treatment did not affect albumen height and pH and by Reijrink et al. (2010a) which showed that heat eggs for a short time during storage did not negatively affect albumen quality. However, this disagreed with El-Menawey (2019) who found that non-SPIDES treated eggs had the highest yolk index and albumen height as compared to SPIDES groups. In the present study, despite the difference was not statically different ($P>0.05$), the S2 treatment eggs were numerically higher in the terms of albumen height, Haugh unit, and albumen index when compared to the NS and the two other SPIDES treatment eggs. The results of this study have highlighted that pre-incubation heat treatment did not affect negatively the internal eggs characteristics.

5.3.2. Eggs weight loss

The SPIDES treatments increased EWL during storage mainly in S1 treated eggs ($P<0.05$) in the current study. These results are consistent with studies of Ebeid et al. (2016) and Ebeid et al. (2017) who found that the SPIDES treatments increased EWL during storage. Similarly, other previous studies reported that prestorage heating eggs for 5 hours (Terčič and Pestotnik, 2016), 6 hours (Abdel-Halim et al., 2015), 9 hours (Gharib, 2013), and 6 or 12 hours (Silva et al., 2008a) increased EWL percentage during storage compared to the non-heated group. (Reijrink et al., 2009; Reijrink et al., 2010a) also found that prestorage incubation and frequent warming increased EWL during storage by 0.18% and by 0.12%. However, the current findings are in contrast to

other study that reported SPIDES treatments did not affect the relative EWL (El-Menawey, 2019).

In this experiment the SPIDES treatments did not affect EWL during incubation and TEWL ($P>0.05$). These are in agreement with previous studies of Ebeid et al. (2016) and Reijrink et al. (2009) who found that pre-storage incubation did not affect EWL during incubation and total egg weight loss, whereas in disagreement with (Reijrink et al., 2010a) who stated that frequent warming increased EWL during incubation by 0.28% and TEWL by 0.39%, Silva et al. (2008a) found that eggs stored for 9 and 12 days presented a higher EWL during incubation when heated for 6 hours as compared to non-heated eggs, (Abdel-Halim et al., 2015) who found that heating egg continuously for 6 hours before storage lower EWL after 18 days of incubation compared to those non-heated and (Gharib, 2013) who found that 9 hours pre-storage heated eggs had higher EWL during incubation and the TEWL.

5.3.3. Hatchability characteristics

A positive effect of pre-incubation heat treatments on hatchability was found ($P<0.05$) in this experiment of the current study. Control eggs (CO) exhibited the highest hatch rate and the lowest EM while NS-treated eggs exhibited the lowest HOF ($P<0.05$). The HOF was increased ($P<0.05$) in S1 by 7.69% and in S3-treated eggs by 13.38% compared to the NS-treated eggs. Furthermore, S3-treated eggs exhibited the highest HOF among SPIDES treatments compared to NS group ($P<0.05$). These results agreed with the previous findings of Özlü et al. (2021a) indicated that SPIDES-treatments increased HOF by 3.8%. Additionally, the study findings are similar to those of Özlü et al. (2021b), Damaziak et al. (2018), Tag EL-Din et al. (2017), Al-Samrai and Al-Dhanki (2017) and Gharib (2013) who showed that the pre-incubation heat treatments increases hatchability compared to non-heated groups. Furthermore, in this experiments the HOF was not rescued in S2-treated eggs which exhibited the lowest HOF when compared to the S1 and S3 treatment groups ($P<0.05$) and intermediate with NS-treated eggs ($P>0.05$).

In this experiment, the EEM was higher in NS-treated eggs compared to the S1, S2, and S3 treatment groups ($P<0.05$). These results are consistent with studies of Özlü et al. (2021a), El-Menawey (2019) and Abdel-Halim et al. (2015) mentioned that the pre-incubation heat treatments decreased EEM when compared to the non-heated

groups. However, the current study findings are contrary to those of Ebeid et al. (2016) and Reijrink et al. (2009) who indicated that the pre-incubation heat treatment did not have effect on EEM.

On the other hand, in this experiment, MEM was higher in S2 and S3-treatment group compared to NS and S1-treated eggs ($P<0.05$). Previously, Ebeid et al. (2016), Silva et al. (2008a) and Gharib (2013) indicated that the intermediate embryo mortality is not influenced by pre-storage heating treatments as well as Tag EL-Din et al. (2017) and Ebeid et al. (2017) stated that heating eggs during SPIDES has lowered mid embryonic mortalities.

In this experiment, LEM was higher in S1 and S2 heat treatment groups, when compared to the NS group ($P<0.05$) and LEM in S2 was intermediate to S3-treated eggs. These findings are contrary to those of Nicholson et al. (2013), Dymond et al. (2013), Gharib (2013), Özlü et al. (2021a), who reported that pre-incubation heating eggs decreased LEM in broiler embryos.

5.3.4. Chick quality and organs weight

The positive effect of pre-incubation heat treatments on chick quality was observed in this experiment. The hatching chick weight, YFBW, heart, liver, and proventriculus weights were not significantly affected by different pre-incubation treatment groups ($P>0.05$). These results are in agreement with Ebeid et al. (2016) for chick weight and Reijrink et al. (2010a) for YFBW. These findings are in contrast to those of El-Menawey (2019) and Silva et al. (2008b) who highlighted that pre-storage heating for six hours increased hatching weight, and chick weight without a yolk sac, and pre-storage heating for six and twelve hours increased LW. However, in the current experiment, RYS and GW were affected by pre-incubation heat treatments ($P<0.05$). The relative GW was higher and RYS was lower in S1 and S3 treated eggs when compared to those of NS and S2 ($P<0.05$). These findings are contrary to those of (2008b) who found that pre-storage heating for six and 12 hours did not affect relative RYS and GW.

The SPIDES-treatments significantly affected chick length ($P<0.05$). Chicks hatched from S1 and S3 treated eggs were longer compared to NS and S2 treatment groups. Likewise, Reijrink et al. (2009), Gharib (2013), and El-Menawey (2019)

reported that pre-storage incubation increased chick length and disproved those of Reijrink et al. (2010a) and Tag EL-Din et al. (2017) who found that pre-storage incubation did not have any effect on chick length. The chicks from all SPIDES treatments had the highest Tona score compared to NS-treated chicks ($P<0.05$). These findings aligned with of the studies of El-Menawey (2019), Damaziak et al. (2018), and Gharib (2013) who found that compared to non-SPIDES treated eggs the SPIDES groups had a better Tona score.

5.3.5. Chick performance and feed consumption

In this experiment at 0 d (hatch) pre-incubation heat treatment (S1 and S3) groups did not differ significantly from that of NS. However, the S2 group had the lowest ICW. At d 7 both S1 and S3 groups had higher CW and CWG than NS and S2 groups while, at d 14, CW and CWG did not significantly affect the pre-incubation heat treatment ($P>0.05$). The RG was higher in the S1 and S3 treatment groups when compared to NS and S2 groups ($P<0.05$) at d 7. At days 14, S1 treatment chicks had a higher RG than NS and S2 treatment chicks ($P<0.05$), but similar growth rate was detected with S3 treatment chicks. In terms of chick hatching weight, our findings are similar to those of Dymond et al. (2013), Ebeid et al. (2016) and Tag EL-Din et al. (2017) who reported that the pre-storage incubation did not affect chick body weight, and contradicted those of Gharib (2013) and El-Menawey (2019) who found that pre-storage heating increased hatching weight. In terms of RG our results are consistent with study of Dymond et al. (2013) who found that the SPIDES treatment group had a higher RG than non SPIDES group. In the current study, feed consumption was affected by SPIDES treatment groups ($P<0.05$). According to the obtained results, chicks from S1 group exhibited the lowest FPC and FCR compared to NS, S2 and S3 ($P<0.05$).

Overall, the results of the current study confirmed that SPIDES at d 21 eggs storage had a positive effect, hatchability of fertile eggs, chick quality, and chick performances without adversely affecting the internal eggs quality characteristics. In conclusion, the highest hatchability was observed in twice warming treated eggs, on d 8 and 17 after egg collection during 21 d of storage in old-aged flocks. The results of these studies also emphasized that applying twice pre-incubation heat treatment at 36°C until EST reached an average of 32-34°C, delivered two (2) times at 8 and 17 d over of 21 days of storage can be effective by improving hatchability, chick quality, and

performance and can reduce the detrimental effects of long storage of hatching eggs. However, when applying three (3) times pre-incubation heat treatment at 36°C until EST reached an average of 28-30°C, delivered at 6, 12, and 18 d over of 21 d of storage can have unsatisfied impacts on hatchability and increased early mortality.



6. EXPERIMENT III: EFFECTS OF SPIDES DURING 21 DAYS EGG STORAGE ON HATCHING RESULTS, EGGSHELL MICROBIAL POPULATION, CHICK QUALITY, AND TWO (2) WEEKS CHICK PERFORMANCE OF EGGS FROM BROILER BREEDER'S PARENT STOCK FLOCK

6.1. Materials and methods

6.1.1. Experimental setup

In this experiment, the hatching eggs were stored for 21 d and pre-incubation heated at 6, 8, 12, 17 and 18 d of storage.

6.1.2. Hatching eggs and study period

A total of 1,750 eggs from a commercial 308 Broiler parent stock breeder, were used. All eggs were sourced through a commercial hatchery (Beypiliç Bolu/Turkey) and came from a single 40-wk-old flock. The experiment was conducted during May 2020 to July 2020.

6.1.3. Egg storage conditions and preincubation warming

In this experiment, eggs were stored for 21 days in the storage machine (Çimuka model) at an average storage temperature and relative humidity (RH) of 12.45°C and 83%, respectively. All eggs were received on the same day of collection at the breeder farm, transported in a temperature-controlled vehicle to the University of Selcuk, and eggs were randomly divided into 5 experimental groups as follow ; "CO" = eggs were stored for 3 d after collection and then incubated to hatch; "NS" = eggs were stored for 21 d without any heat treatment; "S1"= The eggs were treated three times during the storage at 36°C at the days 6 ,12 and 18 d over the course of 21 d until EST reached 32-34°C (93.2°F); "S2" = The eggs were treated three times during the storage at 36°C at the days 6,12 and 18 d of storage until EST reached 28-30°C (84.2°F); "S3"= eggs were treated twice during the storage at 36°C at the days 8 and 17 over of 21 d of storage until EST reached 32-34°C (93.2°F) during storage to determine the ambient temperature and relative humidity inside the setters and hatchers HOBO MX1101 data loggers (United States) were used as well as machines own data-loggers. A total of 150

eggs were used for each treatment group for measurement of the eggshell temperatures of eggs during pre-heat treatment period using an infrared ThermoScope-HT-F03A (Medicare, Singapore, Malaysia).

6.1.4. Microbiological analysis

To determine the eggshell total mesophilic aerobic bacteria (TMAB), total coliform bacteria (CF), total yeast and mold YMR, *Salmonella spp.* (SL) at the 18th day of incubation in each treatment 6 eggs were randomly selected.

6.1.5. Egg weight loss

For each treatment group after oviposition, a total of 45 eggs were randomly selected and then numbered 1 to 45. To determine the weight loss during storage in each treatment group eggs were weighed individually at the (0) day of storage and at the end of day 21 of storage. To determine the weight loss during incubation the same eggs were weighed on the 18th day of incubation.

6.1.6. Incubation and hatching conditions

A total of 1,750 breeder eggs were used and randomly assigned 350 eggs for each group. Following a storage period of exactly 21 d, eggs were removed from the storage machine and were randomly distributed into the same setter machine (Çımuka Incubator Company, Turkey/Ankara). The setter was programmed to 30°C (86°F) for 6 h to allow all eggs to reach the same temperature prior to incubation. A standard single-stage incubation profile was used in the setter and the set point temperature was constant at 38°C during the first five day of incubation and gradually decreased to 37.7°C at E6 of incubation until E18 with of 55% RH at same setter. From 0 to 18 d eggs were turned every hour. After 18 d of incubation, the eggs were transferred to hatching cabinets where the air temperature and relative humidity were kept as 37.2°C and 70% RH, respectively. All eggs were distributed into 35 hatching baskets with ten eggs per replicate according to their group (A total of 350 eggs). Unhatched eggs were broken open to determine fertility and embryonic mortality stage.

6.1.7. Chick quality

After 512 h of incubation, chick quality scores were determined for 40 chicks (20 males and 20 females) randomly selected from each group, weighed, and were used to assess Tona score chick quality. After 528 h of incubation to determine the relative organ weight, 20 chicks randomly selected from each group were weighed and euthanized by cervical dislocation and then necropsied to determine the residual yolk sac (RYS), heart weight (HW), liver weight (LW), Gizzard weight (GW), proventriculus weight (PrW), yolk free bodyweight (YFBW). The organ's weights were calculated as percentages of live weight.

6.1.8. Body weight, relative growth, feed consumption, and feed conversion ratio

After 512 hours of incubation, a total of 200 chicks from 5 treatments (40 chicks per treatment) were placed in a grower battery cage, where 20 female and 20 male chicks from each treatment were selected, individually weighed, and distributed among 4 compartments for the positive control chicks and 16 compartments (4 pens per treatment) for the preincubation treatments chicks with 120 cm × 120 cm. Each compartment contained 2 nipple-type drinkers. Chicks received 24 h of light (24L: 0D) per d from LED white bulbs. The temperature was applied as 32°C for 1st week (wk.), and then reduced by 3°C each week to a room temperature of 29°C at 14 days. Chicks were fed ad libitum a commercially produced wheat-maize-soya-based diet in a 4-stage system: 0–4 weeks, starter: 12.3 MJ of energy, 20.5% crude protein. Individual CW, CWG, and RG were controlled weekly up to 14 d of life. Feed consumption and feed conversion ratio (FCR) were measured and calculated each week per pen up to the 14th day. The dead birds were collected twice daily and weighed in order to calculate the adjusted FCR.

6.2. Statistical Analysis

The effects of SPIDES on microbial eggshell population, incubation results, chick quality and performance were analyzed by one-way analysis of variance using the GLM procedure (SAS, 1989). Significant differences among treatment means were determined by Tukey's multiple range tests at 0.05 significance level.

6.3. Results

6.3.1. Temperature and relative humidity during storage

The storage machine air temperature and humidity during the eggs storage period are presented in figure 6.1. The average storage ambient temperature, relative humidity and DewPt during 21 days of storage were 12.45°C, 82% and 9°C, respectively.

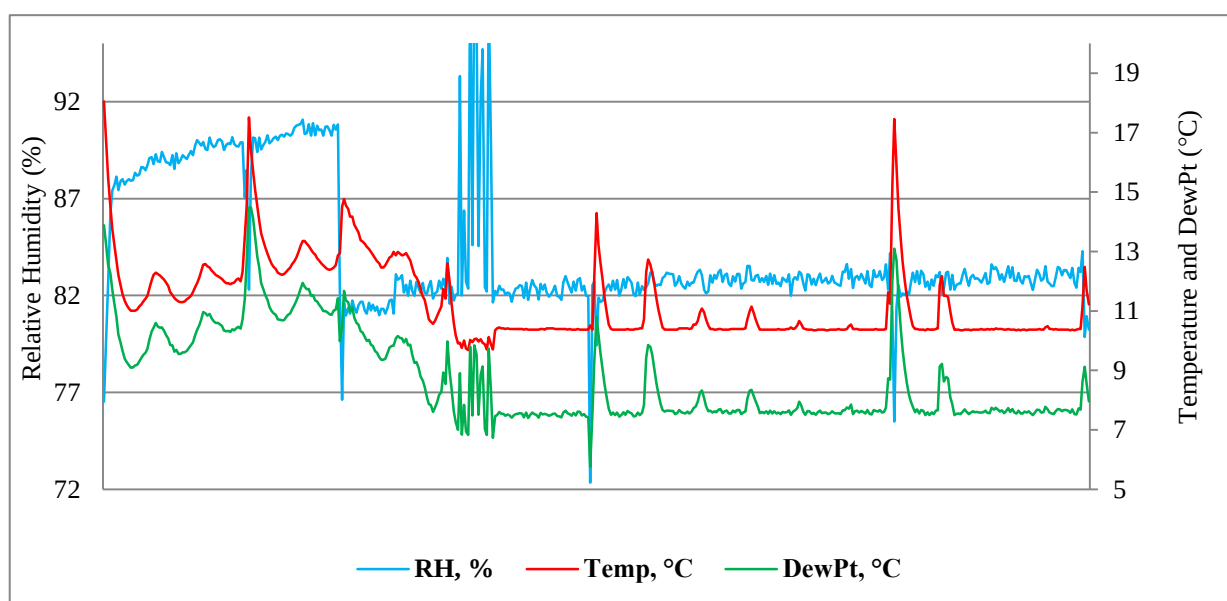


Figure 6.1. Storage machine air condition during 21 days eggs storage

6.3.2. Eggshell temperature before and during SPIDES

The different eggshell temperature (EST) before short period of incubation (A) and after pre-incubation heat treatment in S2 (B) and in S1 and S3 (C) taken by a thermal camera (Figure 6.2). During SPIDES to determine the target EST a thermal camera was used. As shown in photo A before SPIDES the EST is 10.20°C, during SPIDES the EST in SPIDES 2 (B) was 29.20°C, for S1 and S3 (C) treated eggs the EST was 33.60°C.

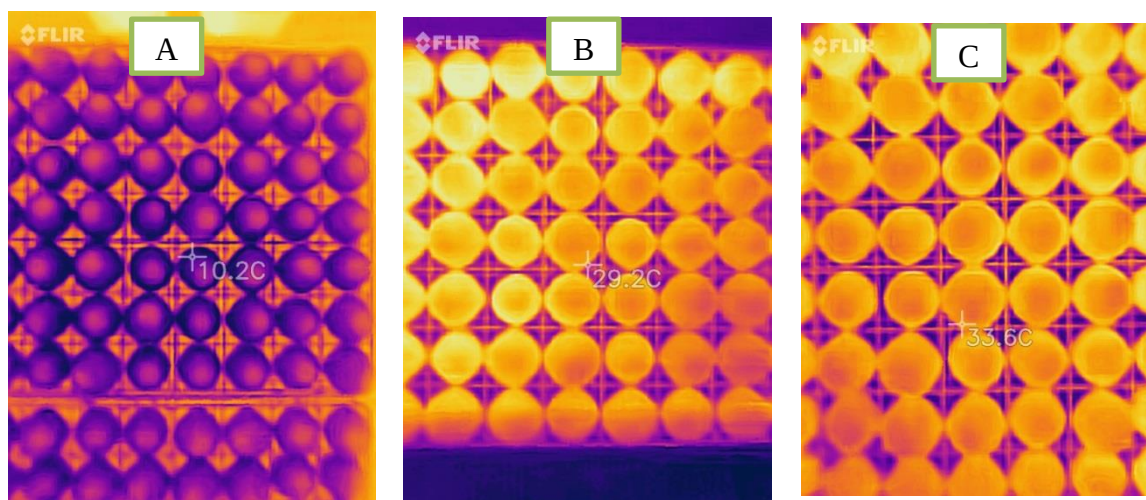


Figure 6.2. Eggshell temperature before short incubation (A); (B) Eggshell Temperature during short incubation during storage (S2), (C) Eggshell Temperature during short incubation during storage (S1 and S3)

6.3.3. Eggs weight loss

Table 6.1 presents the effects of SPIDES on EWL during storage and during incubation. SPIDES treatments had an effect on EWL during storage ($P < 0.05$). The EWL during storage was higher in S1 (1.40%) treated eggs compared to the NS (1.13%) treated eggs ($P < 0.05$), and were intermediate to S2 (1.30%) and S3 (1.30%) treated eggs ($P > 0.05$). The EWL during incubation and the TEWL were not affected by SPIDES treatments ($P > 0.05$).

Table 6.1. Effects of SPIDES on egg weight loss during 21 d of storage and at 18 d of incubation

Group	n ¹	IEW	EWLS	EWLI	TEWL
		(g)	(-%)		
NS	45	63.10	1.13 ^b	11.19	12.32
S1	45	64.72	1.40 ^a	11.30	12.70
S2	45	65.56	1.30 ^{ab}	10.81	12.11
S3	45	65.35	1.30 ^{ab}	11.39	12.69
SEM		0.85	0.05	0.34	0.38
P-Value		0.166	0.004	0.643	0.644

^{a-b} Means within a column that do not share a letter are significantly different ($P < 0.05$)

NS: Eggs were stored 28 d without pre-incubation heat treatment during storage period; S1: Eggs were three times short period incubated at 6, 12 d and 18 over of 21 d of storage until the eggshell temperature reached 32-34°C then eggs were immediately cooled down; S2: Eggs were three times short period incubated at 6, 12 d and 18 over of 21 d of storage until the eggshell temperature reached 28-30°C then eggs were immediately cooled down, S3: Eggs were two times short period incubated at 8 d and 17 d over of 21 d of storage until the eggshell temperature reached 32-34°C then eggs were immediately cooled down, n¹: number of eggs used in each group to determine the egg weight loss. IEW: initial egg weight; EWLS: egg weight loss during 21 d storage; EWLI: egg weight loss during 18 d of incubation; TEWL: total egg weight loss.

6.3.4. Eggshell microbial population

Table 6.2 shows the effects of SPIDES on eggshell microbial population accordingly, on the 18th day of incubation SPIDES treated eggs, showed a greater reduction in the numbers of shell bacteria ($P < 0.05$). The reduction in TMAB numbers was lower in all SPIDES treated eggs ($S1 = 5.83 \log\text{CFU/g}$, $S2 = 6.08 \log\text{CFU/g}$, $S3 = 5.18 \log\text{CFU/g}$) compared to NS ($7.09 \log\text{CFU/g}$) treated eggs which had the highest number of shell bacteria in TMAB ($P < 0.05$) and this decrease was more expressed in S3-treated eggs. The number of shell total YMR and total CF were also higher in NS-treated eggs ($\text{YMR} = 1.33 \log\text{CFU/g}$ and $\text{CF} = 3.14 \log\text{CFU/g}$) compared to SPIDES treated eggs which no shell bacteria in total CF was detected, and the total shell bacteria in YMR was not detected in the S1 treated eggs and was $0.67 \log\text{CFU/g}$ in both S2 and S3 treated eggs. However, no microbial population was detected in the terms of eggshell *salmonella spp* in all treatment groups ($P > 0.05$).

Table 6.2. Effects of SPIDES on eggshell microbial population during long eggs storage

Group		18 DOI Microbial population			
	n ¹	TMAB	YMR	CF	SL
-----LogCFU/g-----					
NS	6	7.09 ^a	1.33 ^a	3.14 ^a	nd
S1	6	5.83 ^b	0.00 ^c	0.00 ^b	nd
S2	6	6.08 ^b	0.67 ^b	0.00 ^b	nd
S3	6	5.18 ^c	0.67 ^b	0.00 ^b	nd
SEM		0.16	0.13	0.18	nd
P-Value		0.000	0.000	0.000	

^{a-b-c} Means within a column that do not share a letter are significantly different ($P < 0.05$)

NS: Eggs were stored 21 d without pre-incubation heat treatment during storage period; S1: Eggs were three times short period incubated at 6, 12 d and 18 over of 21 d of storage until the eggshell temperature reached 32-34°C then eggs were immediately cooled down; S2: Eggs were three times short period incubated at 6, 12 d and 18 over of 21 d of storage until the eggshell temperature reached 28-30°C then eggs were immediately cooled down, S3: Eggs were two times short period incubated at 8 d and 17 d over of 21 d of storage until the eggshell temperature reached 32-34°C then eggs were immediately cooled down; n¹: number of eggs used in each group to determine the eggshell microbial population, TMAB: Total mesophilic aerobic bacteria, CF: Total coliform bacteria, YMR: Total yeast and mold; SL: *Salmonella spp*, nd: non detected, DOI: day of incubation

6.3.5. Hatchability characteristics

Table 6.3 shows the effects of SPIDES on hatching results. Pre-incubation heat treatment affect significantly the HOF and EM ($P < 0.05$). Control embryos (CO) were stored only 3 d, and then incubated to hatch. These eggs showed a HOF of 91.42% and

low early (3.35%), middle (1.87%), and late (3.36%) EM. On the other hand, NS eggs, stored for 21 d, showed a decreased in HOF of 67.88%. However, this was partly rescued by the SPIDES treatments, by restoring the HOF from 67.88% to 78.64% in S3, or an increase of 10.76% and from 67.88% to 71.03% in S1, or an increase of 3.15%. Furthermore, the HOF in S3 was 7.61% higher than in S1. The heating egg during storage until eggs shell reached 28-30°C in the S2 eggs did not show a beneficial effect on HOF (65.61%), greatly increasing EEM in comparison to other treatment groups ($P < 0.05$). Early, middle and late mortality was increased in NS eggs (19.07%, 8.48%, and 5.12% of fertile eggs at early, middle and late-stage respectively) compared to CO eggs (3.35% early, middle 1.87%, and 3.36% late). The early and late EM percentages were significantly influenced by the experimental treatments ($P < 0.05$). The EEM was highest in S2-treated eggs (23.01%) and was lowest in S3-treated eggs (9.08%) compared to NS (19.07%) and S1 (16.18%) treated eggs which were intermediate. However, the LEM was highest in NS-treated eggs (5.12%) compared to S1 (3.67%), S2 (2.76 %) and S3 (3.51%) which were intermediate. The MEM was not influenced by pre-incubation heat treatment groups ($P > 0.05$).

Table 6.3. Effects of short periods of incubation during egg storage on hatching results

Group	HOF (%)	Embryonic mortality (% of fertile eggs)		
		Early	Middle	Late
CO	91.42 ^a	3.35 ^d	1.87 ^b	3.36 ^{bc}
NS	67.88 ^d	19.07 ^b	8.48 ^a	5.12 ^a
S1	71.03 ^c	16.18 ^b	9.12 ^a	3.67 ^{bc}
S2	65.61 ^d	23.01 ^a	8.89 ^a	2.76 ^b
S3	78.64 ^b	9.08 ^c	8.46 ^a	3.51 ^{bc}
SEM	0.72	0.70	0.32	0.22
P-Value	0.000	0.000	0.000	0.000

^{a-b-c-d} Means within a column that do not share a letter are significantly different ($P < 0.05$)

CO: Eggs were stored 3 d without pre-incubation heat treatment and then incubated

NS: Eggs were stored 21 d without pre-incubation heat treatment during storage period; S1: Eggs were three times short period incubated at 6, 12 d and 18 over of 21 d of storage until the eggshell temperature reached 32-34°C then eggs were immediately cooled down; S2: Eggs were three times short period incubated at 6, 12 d and 18 over of 21 d of storage until the eggshell temperature reached 28-30°C then eggs were immediately cooled down, S3: Eggs were two times short period incubated at 8 d and 17 d over of 21 d of storage until the eggshell temperature reached 32-34°C then eggs were immediately cooled down, HOF: hatching of fertile eggs.

6.3.6. Chick quality and organs weight

The effects of SPIDES during storage on chick weight (CW) chick length (CL), yolk-free body weight (YFBW), residual yolk sac (RYS), organ weights, are shown in Table 6.4 The results of the analysis show that the pre-incubation heat treatments at different storage periods had a significant effect on CW, YFBW, CL Tona chick quality ($P < 0.05$). Chicks from all SPIDES treatments had higher ($P < 0.05$) CW, CL, and YFBW compared to those from the NS group. However, the weight or relative weight of the liver, heart, gizzard, pro-ventriculus, and yolk sac were not different ($P > 0.05$) between any of the groups.

Table 6.4. Effects of SPIDES on chick length, relative yolk sac, yolk-free body weight, and organs weight

Group	Chick average Weight (g)	Chick length (cm)	YFBW (g)	RYS (%)	HW (%)	LW (%)	GW (%)	PrW (%)
NS	40.80 ^b	18.90 ^b	37.80 ^b	7.10	0.82	2.64	6.86	1.07
S1	44.60 ^a	19.80 ^a	40.70 ^a	8.70	0.79	2.77	6.54	1.24
S2	43.80 ^a	19.40 ^a	40.10 ^a	8.30	0.80	2.68	6.39	1.04
S3	43.10 ^a	19.80 ^a	39.50 ^a	8.40	0.81	2.64	6.93	1.09
SEM	0.51	0.09	0.40	0.55	0.02	0.08	0.17	0.08
<i>P-value</i>	0.000	0.000	0.000	0.193	0.881	0.630	0.112	0.310

^{a-b} Means within a column that do not share a letter are significantly different ($P < 0.05$)

NS: Eggs were stored 21 d without pre-incubation heat treatment during storage period; S1: Eggs were three times short period incubated at 6, 12 d and 18 over of 21 d of storage until the eggshell temperature reached 32-34°C then eggs were immediately cooled down; S2: Eggs were three times short period incubated at 6, 12 d and 18 over of 21 d of storage until the eggshell temperature reached 28-30°C then eggs were immediately cooled down, S3: Eggs were two times short period incubated at 8 d and 17 d over of 21 d of storage until the eggshell temperature reached 32-34°C then eggs were immediately cooled down; The residual yolk weight (RYS), yolk free body weight (YFBW), HW (heart weight), LW (liver weight), GW (gizzard weight) and PrW (proventriculus weight) were calculated as percentages of live weight.

Figure 6.3 below shows the average Tona chick quality score. In the current study, chicks hatched from CO (95.23) and all SPIDES treatment groups (S1=96.60, S2=, 95.50, S3=97.75) exhibited better Tona chick quality than those hatched from NS (78.90) treated eggs ($P < 0.05$).

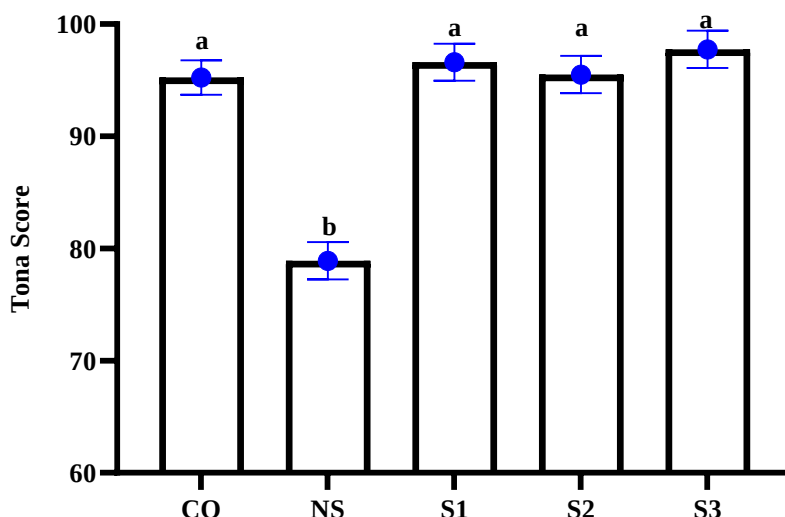


Figure 6.3. Effects of short periods of incubation during egg storage on Tona score chick quality

6.3.7. Chick performance

Table 6.5 presents the effects of SPIDES on chick weight (CW) and CWG. At hatch, chicks from eggs of CO (44.64 g) and S3 (43.10 g) treatment groups were heavier than chicks from NS (41.60 g) group, and chicks from S1 (42.31 g) and S2 (42.34 g) groups were intermediate and not significantly different from both other treatment groups. Chicks from S1 and S3 were the heaviest and had the highest cumulative CWG whereas chicks from CO, NS and S2 were lighter ($P < 0.05$) at 7 days of age. At 14 d, chicks from CO (326.90 g) and S3 (306.70 g) were heavier ($P < 0.05$) than those from NS (294.90 g) and S2 (291.70 g). However, chicks from S3 were intermediate to S1 (301.20 g) chicks ($P > 0.05$). At 14 d, among treatment groups, chicks from CO (192.30 g) had highest cumulative CWG. However, chicks from S3 (150.60 g) and NS (144.40 g) were also heavier than those from, S1 (140.10 g), and S2 (142.60 g) treatment groups ($P < 0.05$).

Figure 6.4 shows the effects of SPIDES on RG. RG to 7 d were lower for chicks from the CO and NS groups than those from SPIDES treatment groups ($P < 0.05$), and among SPIDES treatment chicks from S1 groups exhibited the highest and those from S2 the lowest compared to S3 treatment chicks ($P < 0.05$). With regard to the RG to 14 d chicks from NS chicks exhibited the lowest than those from CO and SPIDES treatment

groups with the highest ($P<0.05$), and among SPIDES treatment groups, the chick from S1 and S3 had the highest 14 d RG than those from S2 treatments groups ($P<0.05$).

Table 6.5. Effects of SPIDES on chick weight and chick weight gain

Group	Body weight				
	Period				
	ICW	7 d CW	0-7d CWG	14 d CW	7-14 d CWG
	(g)				
CO	44.64 ^a	141.50 ^d	97.75 ^d	326.90 ^a	192.30 ^a
NS	41.60 ^c	150.40 ^c	103.64 ^c	294.90 ^c	144.40 ^b
S 1	42.31 ^c	161.10 ^a	118.82 ^a	301.20 ^{bc}	140.10 ^c
S 2	42.34 ^c	149.10 ^c	107.80 ^c	291.70 ^c	142.60 ^c
S 3	43.10 ^b	156.10 ^b	113.07 ^b	306.70 ^b	150.60 ^b
SEM	0.20	1.18	1.27	2.71	2.09
<i>P-value</i>	0.000	0.000	0.000	0.001	0.002

^{a-b-c-d} Means within a column that do not share a letter are significantly different ($P<0.05$), CO: Eggs were stored 3 d and then incubated; NS: Eggs were stored 21 d without pre-incubation heat treatment during storage period; S1: Eggs were three times short period incubated at 6, 12 d and 18 over of 21 d of storage until the eggshell temperature reached 32-34°C then eggs were immediately cooled down; S2: Eggs were three times short period incubated at 6, 12 d and 18 over of 21 d of storage until the eggshell temperature reached 28-30°C then eggs were immediately cooled down, S3: Eggs were two times short period incubated at 8 d and 17 d over of 21 d of storage until the eggshell temperature reached 32-34°C then eggs were immediately cooled down, CW: chick weight ICW: initial chick weight; CWG: chick weight gain.

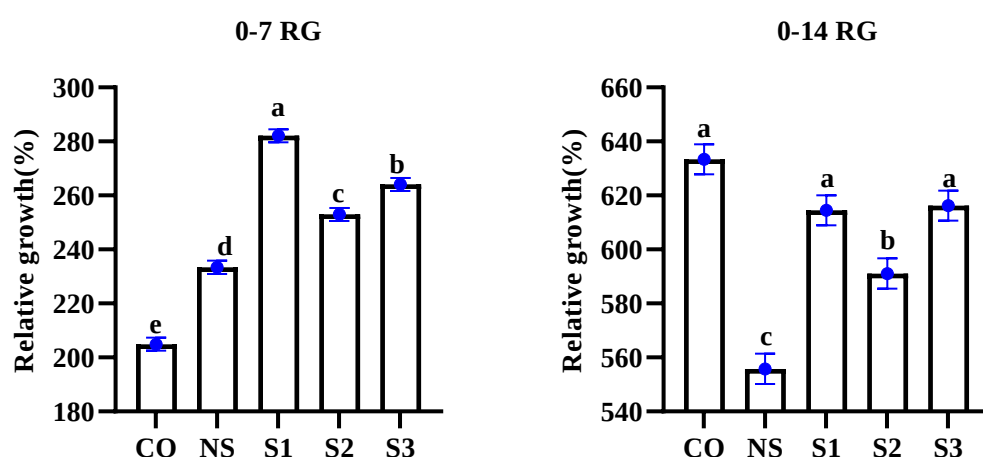


Figure 6. 4. Effects of SPIDES on chick relative growth

6.3.8. Feed consumption

Table 6.6 shows the effects of a short period of incubation during egg storage on feed intake per chick (FPC) and feed conversion ratio (FCR). SPIDES treatments had significant effects on the feed intake and FCR ($P < 0.05$). At 7 d, chicks from S1 group exhibited the highest FPC while chicks from CO, NS, S2 and S3 groups had the lowest. At 14 d, chicks from S3 had the lowest and those from CO had the highest FPC than those from S2 and S1 ($P < 0.05$). At 7 d, the FCR was higher in chicks from the NS group than those from CO and S3 groups ($P < 0.05$) and were intermediate to those from the S2 group ($P > 0.05$). However, chicks from S1 exhibited the lowest FCR. Furthermore, at 14 d, chicks from CO, NS and S2 exhibited the highest FCR than those from S3 which exhibited the lowest FCR ($P < 0.05$) and were intermediate to those from the S1 groups ($P > 0.05$).

Table 6.6. Effects of SPIDES on feed consumption and feed conversion ratio

Group	Feed Consumption			
	0-7 FPC	0-14 FPC	0-7 FCR	0-14 FCR
	----- (g) -----		----- (g/g) -----	
CO	182.10 ^c	497.10 ^a	1.363 ^b	1.540 ^a
NS	202.90 ^b	452.20 ^b	1.397 ^a	1.533 ^a
S1	212.20 ^a	448.60 ^b	1.318 ^c	1.491 ^{ab}
S2	204.80 ^b	461.80 ^b	1.379 ^{ab}	1.531 ^a
S3	205.50 ^b	430.30 ^c	1.364 ^b	1.471 ^b
SEM	0.92	4.43	0.01	0.02
<i>P-value</i>	0.000	0.000	0.000	0.003

^{a-b-c} Means within a column that do not share a letter are significantly different ($P < 0.05$), CO: Eggs were stored 3 d and then incubated, NSP: Eggs were stored 21 d without pre-incubation heat treatment during storage period; S1: Eggs were three times short period incubated at 6, 12 d and 18 over of 21 d of storage until the eggshell temperature reached 32-34°C then eggs were immediately cooled down; S2: Eggs were three times short period incubated at 6, 12 d and 18 over of 21 d of storage until the eggshell temperature reached 28-30°C then eggs were immediately cooled down, S3: Eggs were two times short period incubated at 8 d and 17 d over of 21 d of storage until the eggshell temperature reached 32-34°C then eggs were immediately cooled down, FPC: feed intake per chick; FCR: feed conversion ratio.

6.4. Discussion and Conclusion

6.4.1. Eggs weight loss

During storage the EWL differed among the treatments. EWL was affected by SPIDES treatments and was 0.27% higher in S1 treated eggs when compared to NS-treated eggs ($P < 0.05$). Our findings aligned with those of Abdel-Halim et al. (2015),

Gharib (2013), Silva et al. (2008a), Ebeid et al. (2016), Terčič and Pestotnik (2016) who found that pre-incubation heat treatments increased EWL during storage compared to the non-heated group. Likewise, Reijrink et al. (2010a) found that incubation before storage and repeated warming increased EWL during storage by 0.28% and by 0.12%. However, our results are in opposition to other study that reported SPIDES treatments did not affect the relative EWL (El-Menawey, 2019). The SPIDES treatments did not affect EWL during incubation and TEWL ($P>0.05$). This is in agreement with previous studies of Ebeid et al. (2016) and Reijrink et al. (2010b) who found that pre-storage incubation didn't have any effect on EWL during incubation and TEWL, whereas in disagreement with Reijrink et al. (2010a) who stated that frequent warming increased TEWL by 0.39%, Silva et al. (2008a) found that eggs stored for 9 and 12 days presented a higher EWL during incubation when heated for 6 hours as compared to non-heated eggs, Abdel-Halim et al. (2015) who found that heating egg continuously for 6 hours before storage lower EWL after 18 days of incubation compared to those non-heated and Gharib (2013) who found that 9 hours prestorage heated eggs had higher EWL during incubation and the TEWL.

6.4.2. Eggshell microbial population

All SPIDES treated eggs, showed a greater reduction in the numbers of shell bacteria on the 18th day of incubation ($P<0.05$). Our findings were in agreement with previous studies of Cook et al. (2003) and Cook et al. (2005) that reported that incubation is thought to reduce or limit bacterial growth by altering the microbial populations associated with eggshells, by maintaining the dryness of the shell (D'Alba et al., 2010; Ruiz-de-Castañeda et al., 2011) and by controlling bacterial affluence (Shawkey et al., 2009). Baggott and Graeme-Cook (2002) stated that incubation decreased the abundance of eggshell microbes, influenced the composition of the eggshell microbial population (Grizard et al., 2014; Potter et al., 2013), leading to a less pathogenic microbiota (Brandl et al., 2014; Lee et al., 2014). Bollinger et al. (2018) reported that during oviposition partial incubation can improve egg viability in eastern bluebirds by decreasing eggshell microbial populations. Gentry and Quarles (1972) found that incubation at 38°C showed a higher decrease in the number of shell bacteria after 1 and 4 days and was close to 10% of the original counts after 7 days. In the current study, no microbial population was found in the terms of eggshell salmonella in

all treatment groups ($P>0.05$). This is probably due to the fact that in the present experiment the eggs were stored at a temperature between 10°C and 11°C. Previous studies carried out by Messens et al. (2005) reported that during storage, the probability of survival *S. enterica* on eggshells was rare due to the rapid death of the pathogen, Okamura et al. (2007) concluded that storing eggs at temperatures between 10°C and 20°C can control the development of *Salmonella enterica* serovar Enteritidis (SE) over a period of 6 weeks. *Salmonella* on eggshells can die off during storage at a very low relative humidity (Simmons et al., 1970) or at low temperatures (Baker, 1990; Braun et al., 1999; Radkowski, 2002; Rizk et al., 1966).

6.4.3. Hatchability characteristics

In this present study, significant effects were observed among treatments for embryonic mortality and hatchability ($P<0.05$). The HOF was significantly increased by 10.76% in S3 and by 3.15% in S1-treated eggs compared with that of the NS eggs ($P<0.05$). Furthermore, the HOF in S3-treated eggs was 7.61% higher than that of S1 treated eggs ($P<0.05$). Our findings agreed with previous reported that SPIDES-treatments was increased HOF by 3.8% (Özlü et al., 2021a). Additionally, our results also concur with previous research which showed that pre-incubation heat treatments increased hatchability compared to the non-heated groups (Abdel-Halim et al., 2015; Al-Samrai & Al-Dhanki, 2017; Damaziak et al., 2018; Dymond et al., 2013; Fassenko et al., 2001; Gharib, 2013; Gucbilmez et al., 2013; Özlü et al., 2018b; Özlü et al., 2021b; Seker et al., 2006; Tag EL-Din et al., 2017). Although the HOF was increased in S1 and S3 treated eggs than NS treated eggs, heating egg during storage, until eggs shell reached 28-30°C in the S2 eggs did not show a beneficial effect on HOF (65.61%), greatly increasing early embryonic mortality in comparison to other SPIDES (S1 and S3) and NS groups.

Early embryonic mortality (EEM) did not affect all SPIDES treatments and was lower in the SPIDES 3 (9.08% of fertile eggs) treatment and higher in S2 eggs (23.01% of fertile eggs) compared to the NS and S1 treatments which were 19.07%, 16.18% of fertile eggs respectively ($P<0.05$). The obtained results in S3-treated eggs are in agreement with previous studies of Damaziak et al. (2018) Dymond et al. (2013) Nicholson et al. (2013) and Özlü, et al. (2021a), who found that pre-incubation heat

treatments decrease EEM. However, the current findings disagreed to Ebeid et al. (2016) who found that short incubation during storage did not affect the EEM. Furthermore, the obtained results in S2-treated eggs also agreed with Reijrink et al. (2009) who found that EEM was 2.7% higher in prestorage incubation eggs than in control eggs. In the current study pre-incubation, heat treatment did not affect the middle embryonic mortality (MEM) ($P>0.05$). These results are in agreement with previous studies of Silva et al. (2008a), Ebeid et al. (2016), El-Menawey (2019) and Gharib (2013) who also found no significant effect in the terms of middle embryonic mortality. However, in disagreement with Tag EL-Din et al. (2017) stated that heating eggs for 2.5 hours during SPIDES lowest mid embryonic mortality.

In the current study, LEM was increased in NS eggs compared to SPIDES treatment groups ($P<0.05$). This was in agreement with the results obtained by Özlü et al. (2021a), Nicholson et al. (2013), Dymond et al. (2013), Seker et al. (2006), Gharib (2013) who found that pre-incubation heat treatment decreased LEM. However, in disagreement with Ebeid et al. (2016) who found that SPIDES treatments did not affect the LEM.

6.4.4. Chick quality and organs weight

The ICW, YFBW, CL were significantly affected ($P<0.05$) by the treatment groups, and the ICW, YFBW, CL were higher in chicks from SPIDES treatment groups compared to those from NS group ($P<0.05$). Our findings were in agreement with previous studies, that reported that pre-incubation heat treatments increased CL (El-Menawey, 2019; Gharib, 2013; Reijrink et al., 2009), increased CW, (El-Menawey, 2019; Gharib, 2013), and increased YFBW (El-Menawey, 2019). However, in disagreement with Reijrink et al. (2010a) and Tag EL-Din et al. (2017) who found that pre-storage incubation did not have any effect on CL and Ebeid et al. (2016) who stated that pre-storage heating had no significant effect CW. However, in current study, pre-incubation heat treatments did not effect on organ weights and the RYW ($P>0.05$) among any of the treatment groups. These results were in agreement with the previous studies by Silva et al. (2008b) who found that pre-storage heat treatment did not affect yolk sac weight. Whereas in disagreement with Silva et al., (2008b) who found an increase in liver weight when pre-storage heating for six and twelve hours, and El-Menawey (2019) who found higher relative yolk weight in non-SPIDES group than in

SPIDES treatments. In the current study, the Tona score was significantly affected ($P<0.05$) by SPIDES treatments. Our results were in agreement with previous studies of El-Menawey (2019), Gharib (2013), Damaziak et al. (2018) who reported that hatched in the SPIDES groups had higher Tona score quality compared to the non-heated eggs. Furthermore, the lower Tona score quality observed in chicks from NS treatment group compared to those of CO group was in agreement with Tona et al. (2003) and Tona et al. (2004) who related that long storage eggs lowered Tona score quality.

6.4.5. Chick performance

In the current study, at hatch, chicks from CO treatment group exhibited higher growth performances compared to those from NS group. Tona et al. (2004) reported that hatching chick weight was not affected by storage length. On other hand, S1 and S3 eggs were heavier than chicks from NS group ($P<0.05$) and they remained heavier at d 7 and 14 ($P<0.05$). Similar results were obtained by Gharib (2013) and El-Menawey (2019) who reported that pre-storage heating increased hatching weight. However, in was disagreement with Tag EL-Din et al. (2017), Ebeid et al. (2016) and Reijrink et al. (2009) reported that pre-storage incubation did not affect hatching CW. Dymond et al. (2013) had similar findings that chicks treated with SPIDES exhibit higher CW in wk. 1 to 4 and high relative growth in wk. 1 post-hatch compared to NS-treated chicks. We observed that 7 d cumulative CWG ($P<0.05$) in chicks from S1 and S3 groups was higher than those from NS and S2 groups, but at 14 d this continued to be highest only in chicks from S3 ($P<0.05$) compared to other experiment group chicks.

In the current study, chicks from NS exhibited the lowest RG ($P<0.05$) compared to SPIDES treatments. Our results were in agreement with Dymond et al. (2013) who found that chicks treated with short periods of incubation during egg storage exhibit high 7 d RG. Furthermore, in the current study among SPIDES treatment groups, the chick from S1 and S3 had the highest 14 d RG than those from S2 treatments groups ($P<0.05$).

6.4.6. Feed consumption

In the current study, chicks from S1 and S3 groups exhibited the lowest FCR compared to those from NS and S2 groups ($P<0.05$). The present study shows that SPIDES treatment affects positively feed consumption especially in the S1 and S3 treatment groups.

The findings of this experiment indicate that pre-incubation heat treatments during 21 d period of storage lead to higher hatchability of fertile eggs by minimizing both early and late embryonic mortalities and decrease the eggshell microbial count improve chick quality and increase the weight of broilers during the first 14 d of growth. However, pre-incubation heat treatments had no influence on relative organs weight.

This study also highlighted the differences in pre-incubation heat treatments at various periods during storage. During 21 d storage period applying heat treatment two times on d 8 and 17 d exhibited the highest hatchability and the lowest early embryonic mortality than SPIDES 1 and SPIDES 2 which consist to apply three times heat treatment at 6, 12 and 18 d over 21 day of storage. The results of this study emphasised that applying two times pre-incubation heat treatment at 36°C until eggshell temperature reached 32-34°C, delivered at 8 and 17 d over of 21 d of storage can be effective in improving hatchability, chick quality, and performance and can reduce the detrimental effects of long storage of hatching eggs. However, when applying three times pre-incubation heat treatment at 36°C until eggshell temperature reached 28-30°C, delivered at 6 and 12 d over of 21 d of storage increased early mortality and decreased hatchability of fertile eggs as well as and chick growth performance.

7. CONCLUSION AND RECOMMENDATIONS

7.1. Conclusion

From the present thesis, it can be concluded that extended egg storage until 21 or 28 days decreases hatchability of fertile eggs due to an increase in early embryonic mortality during the first 7 days of incubation. Furthermore, it can also increase the eggshell microbial population. The current experimental findings reveal that applying short incubation during storage can repair the detrimental effects of long-stored eggs. The SPIDES treatment did not negatively affect the internal egg quality in the current study. The highest HOF was obtained when applying heat treatment two times on d 8 and 17 d until the eggshell temperature reached 32-34°C over 21 or 28 d of storage compared to an application of heat three times on 6, 12, and 18 d over 21 or 28 d of storage. This can be explained by the fact that during the storage of eggs longer than 7 days, hatchability begins to drop around 8 days of storage, and then heat treatment has kept the embryo alive. Applying three times pre-incubation heat treatment at 36°C until the eggshell temperature reached 28-30°C, delivered at 6 and 12 d over of 21 d or 28 d of storage increased early mortality and decreased HOF. One possible explication for this detrimental effect is that the temperature which was exposed was not sufficient to develop the embryo at another stage, so we may assume that exposed the eggs until the shell temperature reached 29-30°C could be considered as a temperature at zero physiology. Further experiments are necessary to determine whether 29-30°C can be considered as physiology zero. Even though some of the treatments used in the current project have a positive effect on hatchability of fertile eggs, chick length, and Tona chick quality, neither of the treatments affected the quality of the chicks on the day of hatching in terms of the relative yolk sac and relative organ weight. The SPIDES treatments did not have any effect on the performances of layer chicks. However, it had a positive effect on broiler chicks and had improved 2 weeks' chick performances by increasing body weight and relative growth compared to the NS treatment group. According to the results, it can be concluded that applying two times heat treatment during storage at 36°C until EST reached 32-34°C, delivered at 8 and 17 d over of 21 d of storage (S3) can be an effective method for improving HOF and chick quality by minimizing the detrimental effects of long term stored of hatching eggs.

7.2. Recommendations

The findings from this thesis confirm that during long storage of hatching eggs applying short period incubation is crucial to reduce the early embryonic mortality and to improve the hatchability of fertile eggs. Several solutions have been proposed that pre-incubated eggs during long storage in order to improve the livability of the embryo and which was shown beneficial for hatchability (Dymond et al., 2013; Nicholson et al., 2013). In the present study, the SPIDES 3 treatment group was found to be the effective method during long storage of fertile eggs by lowered the early embryonic mortality and improving the hatchability as well as the chick quality. In addition, our study was carried out under a laboratory scale, application of this method on a large scale as in grandparent broiler hatcheries can help to reduce the detrimental effects of long-term storage eggs. Finally, our results will help to save energy expenses because of applying two repetitions of a short incubation during extended storage of fertile eggs is more economical than doing three during long storage, especially in the high scale hatcheries. Further experiments are necessary to determine the embryos developmental stages. Little research has been conducted till now on the effects of SPIDES on the eggshell microbial population. From the present data, it appears that heating eggs during storage may reduce total mesophilic aerobic bacteria, total coliform bacteria, total yeast and mold. Research should reveal if the eggshell microbial population can be the cause of the decrease in the hatchability of fertile eggs. Furthermore, it may recommend heating the eggs during the long storage period in order to prevent the eggshell bacteria from penetration into the eggs. Further experiments are necessary to determine why heating eggs until eggshell temperature reached 28-30°C did not have an effect on hatchability of fertile eggs. In the present study, despite the fact that hatchability was partially rescued by SPIDES treatment, the 28 days' storage period affected negatively hatchability by dramatically lowered hatchability and increased embryonic mortality. This suggests that fertile eggs should not be stored for longer than 21 days.

8. REFERENCES

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