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#### ■ Keywords

Ambient temperature; broiler breeder; eggshell thickness; egg production; feeding time.



## Effect of Temperature and Feeding Time on Shell Thickness

### ABSTRACT

The objective of this study was to assess the effect of ambient temperature (T) and feeding time (FT) on eggshell thickness (ST) and egg weight (EW) of broiler breeder hens. Thirty 44-week-old Ross 308 broiler breeder hens were randomly distributed into six environmentally controlled chambers and kept in individual cages. Three thermal treatments were applied: 20 °C (T<sub>1</sub>), cyclic 20-30 °C (30 °C between 1000 h and 1800 h) (T<sub>2</sub>), and 30 °C (T<sub>3</sub>). Birds received 180 g of commercial breeder food at 0730 h (FT<sub>1</sub>) or 1530 h (FT<sub>2</sub>). There were two replications per treatment and egg collection was performed for seven days. The eggs from the birds at T<sub>1</sub> and T<sub>2</sub> fed at 1530 h (T<sub>1</sub>FT<sub>2</sub> and T<sub>2</sub>FT<sub>2</sub> respectively) were significantly heavier than those laid by hens at the same T when fed in the morning (T<sub>1</sub>FT<sub>1</sub> and T<sub>2</sub>FT<sub>1</sub>); eggs from T<sub>1</sub>FT<sub>1</sub> and T<sub>2</sub>FT<sub>1</sub> were heavier than the eggs from T<sub>3</sub>, but there were no significant differences between them. Both T and FT had significant effects on ST, but no significant interactions were found. Birds fed at 1530 h had the highest ST, whilst birds at T<sub>3</sub> showed the lowest. Birds on T<sub>2</sub> produced the thickest shells due to a higher ST in birds fed at 1530 h. Birds fed at 1530 h consumed their food between 1800 h and 2030 h, resulting in a higher dietary Ca<sub>2</sub><sup>+</sup> available during shell mineralization. The effect of FT and biphasic T treatments on ST in feed restricted broiler breeders should be interpreted considering the length of the high T phase to the actual food consumption time.

### INTRODUCTION

The effect of constant and cyclical ambient temperatures (T) on eggshell (ES) quality has been extensively documented in egg-type hens. During periods of high temperature, carbonate sources are diverted from shell formation into maintaining blood pH and preventing acidosis. Several authors reported that exposure to high (constant or cyclical) T resulted in a reduction of 2 g in egg weight (EW) in laying hens and an average of 4 g in broiler breeders. The drop in shell thickness (ST) and EW has been described in hens exposed to high constant and cyclical T.

Broiler breeders are customarily fed at the start of the day so as not to disturb oviposition and mating activities, whilst shifting feeding time (FT) towards the cooler part of the day improves productivity under heat stress conditions. That would ensure that the maximum heat increment due to feeding does not occur during the hottest part of the day. Delay in FT has been reported to improve ST and delay the oviposition time by increasing the inter-ovulation period. Although Harms (1991) described a reduction in the rate of egg production when FT was delayed, Wilson & Keeling (1991) and Samara *et al.* (1996) did not find such an effect. A delay in FT did not significantly affect EW.



The ST and EW are essential parameters in the production of hatching eggs. However, little research has been published on T and FT's combined effect on ST in the past 20 years. Samara *et al.* (1996) studied the productive performance of broiler breeder hens exposed to two different cycling T profiles of equal amplitude and three feeding schemes: single feed at 0700 h, single feed at 1800 h, and the daily ration split between 0700 h and 1800 h. They demonstrated that T increased EW, specific gravity, and ST, but FT did not significantly affect those parameters. Although that study attempted to measure the effects of these treatments on egg production parameters in a more realistic scenario by applying cyclical T, the treatments used might limit the validity of their results. Firstly, continuous variation of T throughout the day may have masked the possible interaction of T and FT, which may complicate the extrapolation of results to situations in which T follows different profiles. Secondly, feeding at 1800 h may be unfeasible in farms with manual feed distribution, common in developing countries. Harms (1991) fed broiler breeders at 0800 h and 1600 h but focussed only on the effect of feeding time without measuring the effect of T.

The present study aimed to investigate the effect of T (stepwise and constant temperature) and FT (early in the morning and mid-afternoon) on ST and EW of broiler breeder hens.

## MATERIALS AND METHODS

### Birds and Experimental Design

Thirty 54-week-old Ross 308 broiler breeder hens were selected based on their egg production and randomly allocated into six environmentally controlled chambers. Each chamber contained five individual cages fitted with nipple drinkers and individual feed troughs. The lighting program was 15L: 9D (lights ON at 0530 h). Birds were subjected to an initial 10-day adaptation period where T was maintained at 20 °C. One hundred and eighty grams of a commercial feed for laying broiler breeders (DM=88.2%, CP =14.6%, AMEn =2770 kcal/kg, Ca =33g/kg, P =5 g/kg) was offered once daily at 0800 h. The same feed was used throughout the study.

The experimental period started when the birds were 54 weeks old and lasted for seven days. There were three thermal treatments with two replications each, namely T<sub>1</sub>: constant 20 °C, T<sub>2</sub>: cyclic between 20 (1800h to 1000h) - 30 °C (1000 h and 1800 h), and T<sub>3</sub>: constant 30 °C. The feeding system consisted of

an allocation of 180g/d at two different times, given the same feeding program within a chamber. For treatment one, feed was given at 0730h (FT<sub>1</sub>), and for treatment two, the feed was given at 1530h (FT<sub>2</sub>). Birds had *ad libitum* access to water. The experiment had a 2x3 factorial design with T and FTs main effects in a completely randomized design. The experiment was approved under the University of KwaZulu-Natal Ethics committee (AREC/19/09).

### Measurements and Estimations

Every morning, before feeding, eggs were collected, identified and individually weighed. Eggs laid between days 3 and 7 of the experiment were selected from each chamber to measure ST (Table 1). Before performing the measurements, ES membranes were removed, and the shells dried overnight at 90 °C. Eggshell thickness was determined in three equatorial shell pieces using a micrometer with a precision of 0.01 mm (Mitutoyo Corporation, Kawasaki, Japan). Feeders were checked every morning to record leftovers. Cracked, soft or misshaped eggs were identified and recorded. Relative humidity was recorded but not controlled. The T in each chamber was monitored daily by T/ RH data loggers (HOBO™ Temp/RH, Mod. H8-003-02, Onset Corporation, Bourne, MA). A weighted average of T was calculated as the average of the upper and lower T multiplied by the proportion of 24 h the birds spend under each T value.

**Table 1** – Average eggshell thickness (ST) of broiler breeder hens exposed to three thermal treatments and fed at 0730 h or at 1530 h.

Factor	ST (µm)		SEM	
Temperature (°C)				
20 (n=40)	325		4.72	
23.3 (n=42)	338		4.63	
30 (n=53)	308		4.12	
Feeding time (h)				
0730 (n=68)	317		3.63	
1530 (n=67)	328		3.66	
Temperature X feeding time				
Feeding time (h)				
Temperature (°C)	0730		1530	
	ST (µm)	SEM	ST (µm)	SEM
20	319bc	6.68	330a	6.68
23.3	326c	8.85	351a	6.23
30	309b	5.55	308b	6.10
ANOVA p-values				
Temperature (T)	<0.001			
Feeding time (FT)	0.018			
TXFT	0.103			

Superscripts on different means within rows differ significantly ( $p \leq 0.05$ ).



## Data Analysis

Egg weight and ST data were subjected to unbalanced analysis of variance to study T and FTs main effects and their interactions. Differences between treatment means were estimated by the least significant difference method. Polynomial regression analysis was performed for ST against T, considering two feeding groups (FT<sub>1</sub> and FT<sub>2</sub>) and assuming T<sub>3</sub> as a weighted average of the proportion of time birds spent at T<sub>1</sub> and T<sub>2</sub> each day. All statistical analyses were performed using GenStat 18<sup>th</sup> Edition (VSN International Limited, 2017).

## RESULTS AND DISCUSSION

In this study, the effects of T and FT were investigated with the aim of improving ES quality of hatching eggs. There was no leftover feed in the troughs in the morning when feed consumption was recorded. Thus, all birds consumed their feed allocation within 24h, as was expected. Feed consumption in this study was not affected by the treatments applied ( $p>0.05$ ).

There was a significant interaction between T and FT for EW (Table 2) ( $p>0.05$ ). Feeding at 1530 h increased EW by 2.9 g for T<sub>1</sub> ( $p<0.05$ ) and by 2.4 g for T<sub>2</sub> ( $p<0.05$ ) compared to morning feeding. No significant differences in EW were found between morning and afternoon-fed birds maintained at a constant 30°C (Table 2).

**Table 2** – Average egg weight (EW) of broiler breeder hens exposed to three thermal treatments (T) and fed at 0730 h or at 1530 h.

Factor	EW (g)	SEM		
Temperature (°C)				
20 (n=40)	62.3 <sup>a</sup>	0.65		
23.3 (n=42)	61.5 <sup>a</sup>	0.59		
30 (n=53)	61.5 <sup>b</sup>	0.53		
Feeding time (h)				
0730 (n=68)	61.1 <sup>b</sup>	0.48		
1530 (n=67)	62.3 <sup>a</sup>	0.47		
Temperature X feeding time				
Temperature (°C)	Feeding time (h)			
	0730		1530	
	EW (g)	SEM	EW (g)	SEM
20	60.8	0.98	63.7	0.85
23.3	60.3	0.87	62.7	0.79
30	61.9	0.71	61.1	0.78
ANOVA <i>p</i> -values				
Temperature (T)	0.589			
Feeding time (FT)	0.062			
T X FT	0.043			

Superscripts on different means within column differ significantly ( $p<0.05$ ).

Both FT and T significantly affected ST ( $p=0.018$  and  $p<0.01$ , respectively), but no interactions were found between the main effects (Table 1). Out of 193 eggs laid during the experimental period, only nine were cracked due to thin shells and were all produced by hens on T<sub>3</sub> fed in the morning. These eggs were distributed throughout the experimental period. The shells of eggs laid by hens on FT<sub>2</sub> were, on average, ten  $\mu\text{m}$  thicker than the ones produced by birds on FT<sub>1</sub> ( $p<0.05$ , Table 1). The thickest shells were found from birds on T<sub>2</sub> and the thinnest from birds on T<sub>3</sub> (Table 1). Despite no significant interaction between FT and T for ST, regression analysis showed that the shells were at thickest from birds on T<sub>2</sub> (weighted average of 23.3 °C) and fed in the afternoon ( $p<0.001$ ) (Table 3, Figure 1). Regression of ST against EW did not show a significant relationship between the variables (results not reported). Feeding in the afternoon resulted in significantly heavier eggs, regardless of T treatment. Although feeding in the afternoon significantly increased EW, the improvement would be too small to affect the management of the egg or the physiology of the embryos during incubation. Nevertheless, the regression analysis showed a significant linear relationship between EW and average weighted temperature (Figure 1). However, the model did not account for more than 5.5 % of the total variance of EW.

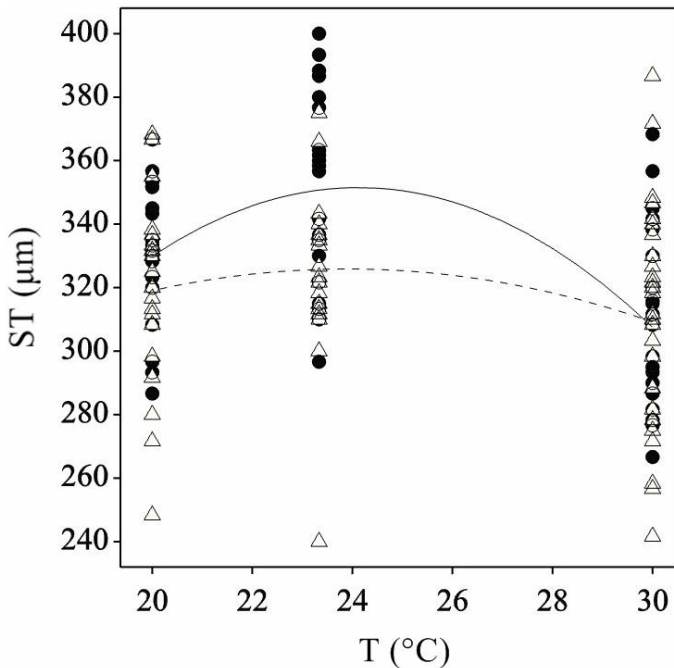
**Table 3** – Estimates of parameters for the second degree polynomial regression of eggshell thickness against ambient temperature with two feeding-time groups (0730 h and 1530 h).

Parameter	Feeding time	
	0730 h	1530 h
n	68	67
Constant term	-179 ( $\pm 163$ )	-168.68 ( $\pm 5.21$ )
Linear regression coefficient	42.60 ( $\pm 13.30$ )	
Quadratic regression coefficient	-0.88 ( $\pm 0.26$ )	
<i>p</i> -value	<0.01	
R <sup>2</sup>	16.20	
s.e. data	30.20	

Without applying thermal treatments, Harms (1991) observed an increase in egg specific gravity when feeding broiler breeder hens at 1600 h, while Backhouse & Gous (2006) found an increase of 3.5  $\mu\text{m}$  in ST per hour delay on feeding. Conversely, Samara *et al.* (1996), in Experiment 1, found no significant increase in ST when feeding at 1800 h (as opposed to feeding at 0700 h) regardless of the thermal treatment. This study showed significantly thicker shells in birds fed at 1600 h when compared to birds fed at 0730 h. In mature hens, the diet provides the majority of Ca<sub>2</sub><sup>+</sup> for ES formation during the early stages of oviposition



(Kerschnitszi *et al.*, 2014). Therefore, feeding closer to the time of ES formation would have increased the availability of dietary  $\text{Ca}_2^+$  to form  $\text{CaCO}_3$ , resulting in thicker shells. The results of this study agree with Experiment 2 reported by Samara *et al.* (1996), even though those birds were fed much later in the afternoon. Thus, the FT determines the extent to which ES formation depends on bone calcium reservoirs.



**Figure 1** – Eggshell thickness (ST,  $\mu\text{m}$ ) of eggs produced by broiler breeder hens when fed at 0730 h ( $\Delta$ ) or at 1530 h ( $\bullet$ ) presented as a function of ambient temperature (T,  $^{\circ}\text{C}$ ).

The results obtained for the effect of T on ST were in contradiction with previous findings. Samara *et al.* (1996) reported a reduction in ST for birds exposed to the higher of two cyclic thermal treatments (Experiment 1). In egg-type hens, de Andrade *et al.* (1977) showed that constant 21 $^{\circ}\text{C}$  were related to higher ST, while exposure to constant 31  $^{\circ}\text{C}$  resulted in thinner shells; cyclical T with a mean of 31 $^{\circ}\text{C}$  resulted in intermediate ST. In this experiment, exposure to T<sub>3</sub> (constant 30 $^{\circ}\text{C}$ ) resulted in significantly thinner shells than the other two thermal treatments. This effect results from low available  $\text{HCO}_3^-$  due to the loss of carbon dioxide through increased panting. Hens under T<sub>2</sub> produced thicker eggshells than birds kept under T<sub>1</sub>, which was in disagreement with the findings by de Andrade *et al.* (1977). The regression analysis showed that the higher ST for T<sub>2</sub> was mainly due to an increase in ST when birds were fed at 1600 h (Table 3 and Figure 1), disagreeing with Samara *et al.* (1996). The greater ST in T<sub>2</sub> may be explained by a possible difference between food administration time and the actual time of feed

ingestion. At lower temperatures, the heat increment of feeding can be easily dissipated; hence birds may have consumed their food immediately after it was offered, while those on T<sub>2</sub> may have taken longer to eat their daily ration. As a result, a shorter overlapping time between the postprandial elevation of  $\text{Ca}_2^+$  and the period of eggshell formation might have occurred. This effect may have been even more pronounced if the delayed ovulation occurred (Backhouse & Gous, 2006).

Feed consumption for birds on the T<sub>2</sub> FT<sub>2</sub> treatment could have shifted later in the day, between T dropping and lights switched off. Shifting feed consumption to cooler parts of the day is a coping strategy in birds under heat stress (de Andrade *et al.*, 1977; Samara *et al.*, 1996). By doing so, hens would have been able to dissipate the heat generated during feeding more easily. Simultaneously, dietary  $\text{Ca}_2^+$  would have been available for a more extended period during ES mineralization. Then, the lower ST in T<sub>1</sub> may be caused by deficient mineralization due to a lack of synchronization between the postprandial increase of  $\text{Ca}_2^+$  and the increase of the  $\text{Ca}_2^+$  demand from the ES gland, rather than the result of an enhancement of the shell deposition process in birds exposed to T<sub>2</sub>.

Previous research on the effect of FT and T has been conducted in egg-type laying hens, which cannot be extrapolated to feed restricted broiler breeder hens. Early feeding and consumption of feed could significantly reduce the integrity of ES, rendering the egg more prone to breakage during transport and manipulation during placing in the incubator trays. Birds respond better to conditions mimicking T variations during day and night. The daily variation in T could have allowed the bird to adjust its thermoregulation mechanisms and feed consumption when it is more beneficial. Shifting of FT to a later time in the day, when the birds could consume feed during a cooler part of the day could increase ES quality, thus significantly enhancing the integrity of hatching eggs and increasing the production of healthy day-old chicks.

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