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I am submitting herewith a dissertation written by Maen Helmi Samara entitled "Interaction of Feeding Time and Temperature and their Relationship to Performance of the Broiler Breeder Hen." I have examined the final copy of this dissertation for form and content and recommended that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Animal Science.

Kelly R. Robbins, Major Professor

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Accepted for the council:

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and Dean of the Graduate School

INTERACTION OF FEEDING TIME AND TEMPERATURE AND THEIR RELATIONSHIP TO PERFORMANCE OF THE BROILER BREEDER HEN

A Dissertation Presented for the

Doctor of Philosophy

Degree

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Maen Helmi Samara

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DEDICATION

This dissertation is dedicated to

My late father

(Helmi Abdalla Samara)

and

My mother

(Bahia Hassan Abdelmatti)

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ABSTRACT

Experiments with broiler breeder hens were undertaken to determine the effect of feeding time and environmental temperature on various production parameters, feed consumption, body weight, blood pH and blood concentrations of calcium, estradiol and androstenedione. Two temperature treatments were used: low cyclic temperature (10-25 C or LO); and high cyclic temperature (21-39 C or HI). Hens were randomly assigned to feeding times of either 0700, 0700 and 1800, or 1800 h. In another experiment, hens were assigned to feeding times of either 0700 or 1800 h.

The results obtained indicated that feeding time and temperature did not markedly affect rate of egg production; however, hens at HI and fed at two times per day produced the fewest eggs. High temperatures caused a significant reduction in egg weight, specific gravity and shell thickness. A numerical improvement occurred in egg specific gravity and shell thickness due to feeding at 1800 h. Significant body weight loss occurred in hens at HI and fed at 0700 h. Time of oviposition, ovulation and the transit time of the egg through the different regions of the oviduct were not significantly changed due to feeding time or temperature despite an increase in number of eggs laid late in the day when feeding time was changed to 1800 h. Different patterns of feed consumption were observed in relation to feeding time, oviposition, and temperature.

High temperatures did not affect blood calcium, plasma total calcium, estradiol, or androstenedione, but increased blood pH in hens fed at 0700 h. Laying hens maintained at LO exhibited a cyclic pattern in blood ionized calcium, and plasma total calcium over the egg cycle, whereas hens at HI exhibited a similar pattern in pH and plasma total calcium but not ionized calcium during this time. Non-laying hens showed no significant changes in blood calcium concentrations throughout the same sampling period.

In conclusion, feeding broiler breeder hens at 1800 h may represent a viable means for improving egg shell quality particularly in hens at high temperatures. The results also indicate that the adverse effects of heat stress on egg shell quality are linked to factors other than a decline in blood ionized calcium.

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INTRODUCTION

Poor egg shell quality is a significant source of loss to the poultry industry worldwide. In the U.S. alone, estimates of losses due to poor eggshell quality were $ 61 million in 1970 and $ 478 million in 1988 (Sunde, 1971; Roland, 1988).

Several physiological and biochemical factors are involved in egg shell formation. These include reproductive hormones, calcium balance, acid-base balance, hormone-induced Ca-binding proteins, and some enzyme activity (Simkiss, 1967; Mongin, 1968; Hughes, 1988).

Environmental temperature is one factor known to affect the overall performance of laying hens (Hamilton et al., 1979). The physiological mechanisms through which heat affects the normal egg cycle (ovulation, egg shell calcification, and oviposition) have not been fully defined; blood flow redistribution, reduced calcium concentrations, reduced feed intake, have each been implicated (Mueller, 1966; Smith, 1974; De Andrade et al., 1977). However, much of the evidence described in temperature stress studies on biochemical criteria contributing to egg shell quality is derived from studies conducted under constant temperatures and not those of a cyclic temperatures.

Diverse techniques have been proposed to improve egg shell quality of laying hens maintained under thermoneutral conditions. These include: source, level, and particle size

of dietary calcium; type of feeding system; feed additives; and dietary modifications. Some of these techniques have been shown to minimize the adverse effects of heat stress on egg production and egg shell quality. Studies have shown that practices such as evaporative cooling, and acclimatization can positively affect the performance of heat stressed hens. However, due to the paucity of information on it's significance, acclimatization remained a largely experimental approach waiting to be defined and analyzed. Unless shown to be officious, feeding dietary supplements would have considerable negative economic consequences since, in most cases, diets formulated for laying hens contain sufficient levels of vitamins, minerals and other nutrients even with a large safety margin. The same thing can be said in regard to evaporative cooling and poultry house design, especially when the means available to alleviate heat stress are either limited or not practiced. Therefore, it may be more economically attractive to use alternative feeding systems as a means of minimizing the adverse effects of heat stress on egg shell quality.

Objectives of the present studies were to determine effects of feeding time and cyclic environmental temperatures on 1) rate of egg production, egg weight, egg shell quality and body weight changes; 2) patterns of feed consumption; 3) time of ovulation, time of oviposition, and the time interval between successive ovipositions; and 4) the levels of blood

ionized calcium, plasma total calcium, estrogens, and androgen during the egg cycle of broiler breeder hens.

PART I

LITERATURE REVIEW

LITERATURE REVIEW

Description of Egg Formation in the Hen

Components of the hens reproductive system and their function have been well described (Warren and Scott, 1935; Romanoff and Romanoff, 1949; Gilbert, 1967). The ovary contains a hierarchy of ovarian follicles that serve as a source of various steroid hormones. The oviduct consists of five distinct regions: infundibulum (to engulf the ovulated ovum); magnum (the albumen secreting region); isthmus (inner and outer shell membrane forming region); uterus or shell gland (shell forming region), and the vagina. Plumping or addition of water and salts to the egg is another function of the shell gland (Wyburn et al., 1973).

The lengths of the various regions of the oviduct, determined mostly in the White Leghorn hen are: infundibulum, 3.2 cm; magnum, 38.9 cm; isthmus, 10.5 cm; uterus, 10.3 cm; vagina, 4.9 cm which gives a total length of 67.8 cm (Warren and Scott, 1935). These same authors reported that the total length of the oviduct may range from 55.5 to 73.0 cm. Romanoff and Romanoff (1949) calculated the average length of the different segments of the oviduct from 9 studies. They estimated the length of the infundibulum as 7; magnum, 33.6; isthmus, 8; shell gland, 8.3; and vagina, 7.9 cm; making a total of 64.8 cm. Taylor and Hertelendy (1960) reported different measurements: infundibulum, 9; magnum, 38.9 cm;

isthmus, 10.5; and uterus and vagina, 21 cm which totals 79.4 cm.

The developing egg spends 0.3 h in the infundibulum, 2.9 h in the magnum, 1.2 h in the isthmus, and 21.7 h in the shell gland (Warren and Scott, 1935). Similar estimates have been reported by Romanoff and Romanoff (1949), although they estimated the length of stay in the shell gland to be 19.2 h. Melek et al. (1973) reported that the developing egg spends 5.15 h in the infundibulum, magnum and the isthmus, and 19.76 h in the uterus, making the total time the egg spends in the hen's oviduct 24.91 h compared to 25.06 h reported by (Warren and Scott, 1935).

Under normal day light conditions (14 L:10 D) the first oviposition in a sequence takes place 9-10 h after the onset of darkness (early morning) (Cunningham, 1987), followed (.4-.5 h) by ovulation of the next egg in this sequence. Therefore, ovulation does not occur every 24 h, consequently eggs are laid later each successive day of the sequence. The delay between the oviposition of successive eggs in a sequence is known as the lag period. The sequence is terminated when an egg is laid about 17-18 h after the onset of darkness occurs. The time interval between two successive ovipositions has been reported to range from 24 to 26 h (Warren and Scott, 1935; Melek et al., 1973). Hens with a longer sequence have shorter resting or pause days (Gilbert, 1967), whereas birds with a shorter sequence have longer intervals between eggs

(Romanoff and Romanoff, 1949), possibly due to delay in ovulation.

Impact of Constant and Cyclic Environmental Temperatures on Performance of Laying Hens

Heat stress is one of several environmental factors that provoke physiological responses in animals (Siegel, 1971). Such physiological responses are intended to remove excess deep body heat. Earlier work on the sum of the bird's physiological responses associated with high environmental temperatures have been well summarized (Ota, 1967; Siegel, 1968; Siegel, 1971). The physiological changes associated with heat stress are: reduced feed intake and physical activity, probably to control the heat production that accompanies feeding and digestion; increased water consumption and panting; vasodilation; increased blood flow to the unfeathered organs such as wattles, comb and shanks; and several behavioral changes such as splashing water over the head and drooping wings to facilitate heat transfer to the environment. Radiation, convection, conduction and evaporation are the four means by which heat is removed from the body of the bird (Siegel, 1971). However, at high environmental temperatures evaporative cooling becomes the predominant and most important means of heat dissipation.

Deep body temperature of the chicken is 42.2 C (Siegel, 1968). Several factors affect the hen's body temperature.

Heavy breeds have lower body temperatures than the lighter ones and laying hens have higher body temperature than non-layers (Hewang, 1938; Siegel, 1968). Physical activity and feed intake (caloric intake) also cause an increase in the body temperature (Siegel, 1968). Body temperature increases as the environmental temperature increases above 26 to 27 C (Lee et al., 1945; Wilson, 1948). Regardless of whether the increase in body temperature is due to activity or to an increase in environmental temperature, there is diurnal variation in body temperature. Hewang (1938) reported that hens have the highest body temperature at 1600 h and the lowest body temperature at mid-night. The range of thermal neutrality for the adult chicken is 18-24 C (Siegel, 1968). At this range the chicken is able to optimally maintain its body temperature. Above 24 C, panting will ensue; and as the environmental temperature exceeds 32 C, the resources of the bird will be directed to survival rather than production (Smith and Oliver, 1971).

It is well known the rate of egg production, egg weight, and egg shell quality are measures most often used to measure the production performance of the laying hen. It is well known that egg specific gravity, shell thickness, breakage, and shape are measures of egg shell quality.

Efficiency of production by table egg and broiler breeder stock is similarly influenced by poor egg shell quality. Estimates of the economic loss to the U. S. poultry industry

increased from 61 in 1971 (Sunde, 1971), and 100 in 1982 (Hamilton, 1982) to 478 million $/ year in 1988 (Roland, 1988). As for broiler breeders, McDaniel et al. (1979) demonstrated that eggs with specific gravity below 1.080 had the greatest weight loss during storage and lowest hatchabilities. Bennet (1992) reported that hatchability of thin-shelled eggs was 3.9% less than thick-shelled eggs in 30-60 week old breeder flocks.

Reduced feed intake, rate of egg production, egg weight and shell quality in hens subjected to high constant or cyclic temperatures have been observed by many researchers (Bennion and Warren, 1933; Mueller, 1959, 1961; Campos et al., 1960; Payne, 1966; De Andrade et al., 1976; Vohra et al., 1979; Cowan and Michie, 1980; Deaton et al., 1981, 1982; Scott and Balnave, 1988). Others have reported a decline in egg weight and various egg shell quality indicators but no effect on rate of egg production at high cyclic temperatures (Squibb et al., 1959; Mobrey and Sykes, 1971; Smith and Oliver, 1972a, 1972b; Wilson et al., 1972; Miller and Sunde, 1975; De Andrade, 1977; Emery et al., 1984; Renden et al., 1984). With regard to the rate of egg production, the variation in the observed responses could be due to the use of high constant or cyclic temperatures, differences in daily temperature cycle, stage of lay at which the responses have been reported, breed and age of the birds used, and the overall conditions of the experiment in which the responses were observed.

Mueller (1961) reported that eggs from hens maintained under high cyclic temperature (13 to 32 C) had shell thickness intermediate to those from hens exposed to constant low (13 C) or constant high (32 C) temperature. El-Boushy et al. (1968) studied the effects of a hot (29 C) and humid (75-80%) environment on egg shell quality in commercial egg-type laying hens. These authors observed that in addition to the reduced egg shell thickness, the structure of various shell sublayers were altered (weakened) due to heat stress. Miller and Sunde (1975) reported that egg-type hens kept under cycling temperatures (10 to 34, 26 to 38, and 26.5 to 35.6 C) with average temperatures of 22, 32, 31 C, respectively, produced more eggs and had better feed efficiency than the hens kept in constant environments with the corresponding average temperature. They observed that gradual as well as abrupt elevation in temperature resulted in a direct (immediate) drop in shell strength, whereas gradual and abrupt decrease in temperature resulted in a direct recovery of shell strength. Deaton et al. (1981) reported that egg-type laying hens exposed to cycling temperatures (21.1 to 35, and 15.6 to 35 C) had a significantly higher ($P<.05$) rate of egg production, daily feed intake, egg weight, and less body weight change than hens maintained under cyclic temperature which ranged from 26.7 to 35 C. Deaton et al. (1982) compared the performance of hens maintained under constant temperature of 25 C to that of hens maintained under cycling temperatures of

15.6 to 35 C. No differences were found in the rate of egg production, egg shell quality, feed consumption and body weight change between both temperature treatments. The effect of cyclic temperatures on the performance of egg-type laying hens has also been studied by Emery et al. (1984). These authors found that hens maintained under cyclic temperatures (15.6 to 37.7 and 21.1 to 37.7 C) with an average of 26.7 and 29.4 C, respectively, had relatively similar percentage of hen-day production, gram of feed per gram of egg mass, and change in body weight compared to hens maintained under constant temperature of 23.9 C. However, daily feed intake, egg weight, and shell thickness were significantly depressed ($P<.05$) by both cycling temperatures. It was stated that the reduction observed in egg weight and egg shell thickness at cyclic temperatures was not merely a consequence of reduced feed intake but as a direct effect of heat stress *per se*, confirming earlier data reported by Smith and Oliver (1972a). A significant ($P<.05$) reduction in egg shell thickness was observed when temperatures peaked during the dark period. These authors have also found that feed efficiency was reduced at high cyclic temperatures which contradicts earlier reports (Mueller, 1961; Wilson et al., 1972; De Andrade, 1977). These researchers found an improvement in feed efficiency of hens maintained under high constant or cyclic temperatures.

Many factors have been implicated in the detrimental effects of heat stress on egg weight and egg shell quality.

Reduced blood flow to the internal organs, especially parts of the reproductive system, has been suggested to be partly responsible for the decline in egg weight and egg shell quality (Payne, 1966; De Andrade, 1977). However, in a recent study, Arad et al. (1993) reported that blood flow to the reproductive tract of the laying hen was not compromised during exposure to a temperature of 35 to 45 C for 1.5 h.

Payne (1966b, 1967) indicated that adverse effects of high constant temperatures (32 C) could be minimized by proper dietary modifications (high levels of methionine and other essential nutrients). Smith and Oliver (1972a) reported that egg-type pullets maintained under constant temperature of 26.5, 29.5 or 32 C produced heavy eggs and were able to increase the average weight of the eggs laid over a 26-wk period. However, pullets kept under a constant temperature of 35 C produced small eggs, and the mean weight of these eggs remained constant throughout the same period. Using paired feeding methodology, Smith and Oliver (1972b) concluded that most of the loss in egg weight was due to factors other than inadequate feed intake. The authors estimated that even at an environmental temperature of 38 C only 20% of the reduction in egg weight was due to inadequate energy intake, while 80% was due to other factors.

Mather et al. (1976) found that the energy content of the diet is not a limiting factor in achieving an adequate level of egg production during heat stress. De Andrade et al.

(1976, 1977) reported that increasing nutrient density (25% of all nutrients and 10% of dietary energy above the recommended requirements) improved rate of egg production but not egg weight or egg shell quality in hens exposed to constant (32 C) or cyclic temperatures (26.7-35.6 C). Tanor et al. (1984) reported a partial improvement in egg production rate when the energy and calcium contents of diet were increased above the recommended levels. They suggested that the diet change, by itself, caused additional stress on laying hens maintained under high temperature conditions. Scott and Balnave (1988) found that hens exposed to cyclic temperatures of 25 to 35 C produced less egg mass (g/hen-d) even when fed a nutrient-dense diet compared to hens at cyclic temperatures of 10 to 24 or 6 to 16.6 C and fed nutrient-sparse diets.

Nordstrom (1971) hypothesized that reduction in egg weight and shell thickness may be caused by accelerated transit time of the egg through the oviduct in heat-stressed hens in an attempt to lower metabolic heat production caused by the egg-forming process. In a subsequent study, Nordstrom (1973) reported that the developing egg may spend 1-2 h longer in the shell gland of laying hens exposed to a constant temperature of 32 C: however, the accuracy of these findings have been questioned by Miller and Sunde (1975). These researchers found that the rate of egg production was higher (64 *vs.* 60%) for birds subjected to high cyclic temperatures than those exposed to a constant temperature of 32, suggesting

that high cyclic temperatures may not affect the length of stay of the egg in the shell gland. More recently, Novero et al. (1991) found that eggs from heat-stressed (35 C) hens were more often laid earlier (<24 h) than eggs from unstressed (17-27 C) hens, but unstressed hens more often laid eggs in a normal oviposition interval (24-26 h) compared with heat stressed hens.

There is an indication that shell calcification is affected by the high temperature and the time during the shell calcification cycle that the peak temperature occurs (Miller and Sunde, 1975). Wolfenson et al. (1979) made an attempt to differentiate between a direct and indirect effect (through a reduction in feed intake) of high temperature on egg formation. To achieve this, the authors subjected hens to high temperatures (34 to 40 C) during either the day (1000 to 1600 h) or night (2000 to 0300 h). Imposing high temperature during the night caused a significant ($P<.05$) reduction in rate of egg production. The authors speculated that this reduction was probably due to a direct effect of heat stress on the reproductive hormones associated with ovulation. Egg shell quality was reduced more when the high temperature was imposed during the day than when it was imposed during the night. This lead the authors to conclude that the early stage of egg shell formation was more prone to heat stress than the later stages. By contrast, Emery et al. (1984) observed that shell thickness was significantly reduced ($P<.05$) when high

temperatures peaked (15.6-37.7 C and 21.1-37.7 C) during the dark period, and that cyclical temperatures did not reduce egg shell thickness when peak high temperature occurred during the day.

Heavy and light breeds of laying hens seem to differ in their response to high environmental temperatures, probably due to their genetic background and body weight. Hutt (1938) and Fox (1951) reported that Rhode Island Reds or Barred Plymouth Rocks were less tolerant to severe increases in temperature than White Leghorn hens.

The effect of heat stress on egg shell quality becomes more severe as the laying hen gets older (Mueller, 1956; Mueller et al., 1960; Pope et al., 1960). Arima et al. (1976) for example, found that rate of egg production and egg shell quality of older laying hens (18 month of age) were more adversely affected than that of younger laying hens.

Reduced feed intake, reduced blood flow to the reproductive tract, breed and age of birds, and prolonged oviductal time, each have been implicated in the detrimental effects of heat stress on egg weight and shell quality. There is lack of evidence supporting the putative association of reduced egg weight and shell quality with the reduced feed intake during high temperatures.

Feed and Calcium Consumption in Relation to Egg Formation

Effects of the egg cycle on feed and calcium consumption

have been examined by several researchers. Feed consumption of birds maintained under continuous and/or a 14 h photoperiod declined for two to three h just before oviposition and then increased for an hour or two following oviposition (Fraps et al., 1947; Wood-Gush and Horne, 1970; Mongin and Sauveur, 1974; Duncan and Hughes, 1975; Nys et al., 1976; Savory, 1977). Some researchers observed an increase in feed intake following ovulation of an ovum and concluded it to be associated with the physiological need for nutrients for albumin synthesis (Warren and Scott, 1935; Morris and Taylor, 1967). It was also suggested that changes in calcium and feed intake during the egg cycle are controlled hormonally or neurally (Hughes, 1972). For this reason, Savory (1977), using egg-type laying hens maintained under continuous lighting, found that feed intake was low during the period of the peak release of luteinizing hormone, increased soon after ovulation, and increased further after the developing egg entered the shell gland.

Stage of egg production has also been reported to affect patterns of feed intake of the laying hen. *Ad libitum* fed laying pullets tend to differ in their feeding patterns when compared to older hens. Pullets consumed more feed on days when an egg was formed than on days when no egg was formed (Fraps et al., 1947; Snetsinger et al., 1971; Roland et al., 1972). Savory (1980) concluded that egg-type laying hens have a tendency to eat more at the end of the day compared to non-

layers. For non-layers, feed consumption late in the day seems to be in response to anticipated onset of darkness. For laying hens, timing of egg formation seems to play a major role in regulating feed consumption patterns. Since timing of the first event in the egg cycle is regulated by the light dark cycle, it was assumed that patterns of feed intake in the laying hen is indirectly regulated, through stage of egg formation, by the light-dark cycle (Roland et al., 1972; Mongin and Sauveur, 1974).

Evidence exists for a specific calcium appetite involved in patterns of feed intake during the ovulatory cycle. Hurwitz and Bornstein (1966) and Hurwitz et al. (1969) observed a decline in feed consumption of laying hens fed a high calcium diet (4.5% Ca/kg). Morris and Taylor (1967) reported that feed consumption was 25% greater in the period between 1400 to 0930 h on egg forming days. Wood-Gush and Horne (1970) reported that there were two peaks of feed intake in resting hens; one in the morning and one just before darkness. However, on egg-forming days when no oviposition occurred and egg-forming days when an egg was laid, there was an afternoon rise in feed consumption. When hens were maintained under regular light-dark cycle and had access to a low-calcium diet and to calcium grit, hens gradually increased their calcium intake after ovulation, reaching a peak intake just before dusk, a time which precedes the beginning of egg shell calcification (Hughes, 1972). Calcium appetite of this

kind has also been observed even when the oviduct was ligated to prevent shell calcification. Nys et al. (1976) reported that the rise in feed consumption after the entry of the egg into the uterus was a consequence of a specific need for calcium. Taylor (1972) reported that hens fed a diet which contained 3.2% Ca consumed more feed on days on which ovulation took place than did hens fed a diet which contained .2% Ca with calcareous grit separately offered as a calcium source.

Mongin and Sauveur (1974) fed egg-type laying hens either 120 g of a diet which contained 3.25% calcium or 111 g which contained 0.6% calcium plus 8.7 g oyster shell. The oyster shell was given for a 3.25 h period at 0900, 1400, or 1715 h. The hens were maintained under a regular light-dark cycle (14L:10D). The authors observed that hens did not consume oyster shell in the morning and that hens which had access to calcium source either in the morning or mid-day produced eggs with thinner shells. Calcium intake was higher on ovulation-days when oyster shell was offered after 1400 h and so was the egg shell thickness. When calcium was offered at all times, the hens consumed more calcium on ovulation-days than on days in which only oviposition occurred. Millam et al. (1986) reached similar conclusions; however, these authors commented that when the hens had access to oyster shell only in the morning, calcium consumption occurred in response to a calcium deficiency persisting from the preceding period of shell

calcification, suggesting even more involvement of calcium in controlling feed consumption.

The above studies indicate that the light-dark cycle, laying status, and stage of egg production are the major factors that influence feed consumption of *ad libitum* fed egg-type laying hens. Little, if any, information is available on the effects of temperature in pattern of feed consumption in broiler breeder hens.

Effect of Feed Management in Egg production and Egg Shell Quality

In addition to changing time of feed intake, which will be discussed in a later section of this review, diverse techniques have been used to improve egg production and egg shell quality under thermoneutral conditions.

Hurwitz et al. (1969) concluded that feed intake of White Leghorn hens was depressed when levels of calcium were increased from 3 to 4.5%. Others have concluded that increasing (to 5.5%) dietary calcium levels above 3.75 g/ hen-d improves egg shell quality both in young and old laying hens (Reichman and Conner, 1977; Ousterhout, 1980; Keshavarz, 1986; Clunies et al., 1992). Recently, Keshavarz and Nakajima (1993) found that Ca retention and plasma and bone Ca levels were not affected by increasing dietary Ca to 5.4%. They concluded that increasing dietary levels of calcium did not have any beneficial effects on egg shell quality and that 3.75

g Ca per hen per day is adequate for optimum shell formation. Abdallah et al. (1993) reported that increasing dietary calcium level from 3.9 to 4.4% and total phosphorus from .55 to .90% had no significant effect on egg production, egg shell weight or feed intake. They also reported that bone ash and bone breaking strength were not affected by these dietary treatments and that hens producing eggs with low egg shell weights had higher plasma calcium and phosphorus levels that hens producing eggs with high egg shell weights.

Replacement of limestone with oyster shell has been shown to reduce calcium turnover because of its low rate of solubilization in the hen's digestive system which in turn leads to prolonged metering of calcium during periods of egg shell formation (Scott et al., 1971; Charles et al., 1972). However, Muir et al. (1976) and Roland et al. (1981) demonstrated that as long as the diet contained sufficient dietary calcium, particle size had no effect on egg shell quality. Van De Velde et al. (1986) reported that addition of oyster shell to the diet of laying hens did not affect the concentration or the pattern of the plasma calcium and phosphorus, and did not reduce calcium mobilization from the medullary bone during shell formation. Roland (1986) suggested, but did not justify, that larger dietary calcium particles are beneficial during unfavorable conditions such as high environmental temperatures. Makled and Charles (1986) reported that egg specific gravity was improved when laying

hens were fed oyster shell in combination with limestone. In a more recent study, Rao et al. (1992) indicated that limestone particles of 1 mm diameter are retained in the gizzard as well as larger particles. Generally, these studies indicate that particle size and rate of solubilization of the calcium source may affect the rate at which it is digested, absorbed and consequently used for egg shell formation.

Similar conflicting results have been reported concerning the effect of sodium bicarbonate on egg shell quality. Addition of increasing levels of $NaHCO_3$ (1 to 10 g/kg) to the diet or drinking water was reported to improve egg shell thickness (Frank and Burger, 1965; Howes, 1966; Mongin, 1968). On the other hand, no effect on egg shell quality (egg specific gravity) was observed when similar levels of $NaHCO_3$ were added (Cox and Balloun, 1968; Latif and Qusenberry, 1968; Grizzle et al., 1992). No explanation for these conflicting results was apparent. Hamilton and Thompson (1980) down-played the importance of acid-base balance on egg shell quality (shell strength, egg specific gravity) and concluded that changes in (Na+K)/Cl ratios affected parameters relating to the hen to a much greater extent than to those relating to the egg. Davison and Wideman(1992) fed a diet with a high concentration of $NaHCO_3$ (44 g/kg diet) and found no effect on egg production or mortality, but hens did increase water consumption. They also stated that high levels of sodium bicarbonate caused metabolic alkalosis and that the continuous

presence of drinking water may have minimized any adverse effects of acid-base imbalance on egg shell quality.

Carbonated drinking water has been reported to improve egg shell quality under heat stress temperatures (Odom et al.,1985; Koelkebeck et al., 1992). However, Koelkebeck et al. (1993) reported that egg specific gravity was depressed in heat-stressed hens provided with carbonated water compared to the hens that received tap water. They were unable to explain these findings; however, they did report that hens that received carbonated drinking water had better bone integrity.

Sodium zeolite (Roland et al., 1985; Rabon et al., 1991; Hagedorn et al., 1991), zinc methionine (Moreng et al., 1991), zinc bacitracin (Manner and Wang, 1991) and acetylsalicylic acid (McDaniel, 1993) have all been reported to improve egg shell quality with varying degrees of success. Mechanisms of action of these compounds are speculative. Zinc bacitracin is believed to prevent alkalosis or have a sparing effect on buffer systems involved with acid-base balance (Manner and Wang, 1991). In other studies, zinc compounds have been suggested to affect activity of the enzyme carbonic anhydrase for which zinc is a cofactor. Balnave and Zhang (1993), using zinc-methionine (.5 g/kg diet), zinc-sulfate (.46 g/kg diet), or chelated zinc-EDTA (.54 g/kg diet), found that egg shell quality was improved and attributed this to improvement in the activity of the enzyme carbonic anhydrase. The relationship of acetylsalicylic acid to egg production and egg shell

quality has also been examined by several researchers. Thomas et al. (1966) found that feeding graded levels of acetylsalicylic acid (.05 to .2% of the diet) to egg-type laying hens for 32 wk improved hen-day production. They also found that .05% dietary acetylsalicylic acid improved egg shell quality. Theoretically, the explanation for such findings was that the egg spends a longer amount of time in the shell gland due to the slower synthesis of the hormone prostaglandin which is involved in normal egg laying. McDaniel et al. (1993) reported that short-term use of acetylsalicylic acid (0, .025, .05, .1, .2, .4%) as a dietary supplement had no effect on egg production or feed efficiency, whereas long-term use was detrimental (reduced feed intake and excessive internal and external bleeding) to the early breeder's liveability and egg shell quality. They suggested that this discrepancy in the effect of acetylsalicylic acid may, in part, reflect differences in management, environments, breeds and strain of birds used.

Vitamin D metabolites have also been shown to possibly aid in improving egg shell quality (Charles and Ernest, 1974; Abdulrahim et al., 1979; Soares, 1979; Hamilton, 1980; Grunder et al., 1990; Tsang et al., 1990a,b). However, vitamin D metabolites (i.e. calcitriol) are highly toxic (Soares et al., 1983) which limits their use as feed supplements. In addition, Sunde et al. (1978) reported that replacement of D_3 with 1 to 8 ug 1,25 $(OH)_2$ D_3 per kg diet maintained normal egg

shell quality but, interestingly, reduced hatchability which also limits their use as feed supplements especially for broiler breeders.

The ability of the hen to synthesis vitamin C makes its addition to the diet unnecessary (Roy and Guha, 1958). However, at temperatures as high as 35 C, it has been found that addition of vitamin C to the diet can limit the increase in body temperature of the laying hen (Ahmad et al., 1967; Lyle and Moreng, 1968). Moreover, supplementing the laying hen diet with vitamin C has been reported to improve egg shell thickness especially under high temperature conditions (Thornton and Moreng, 1959; Thornton, 1960). It is not known if vitamin C interacts with other nutrients or if it acts by preventing an increase in body temperature, positively affects egg shell quality.

Tadiyanant et al. (1991) reported that egg weight and egg shell quality were not improved when heat-stressed hens (29.4 and 33.3 C) were fed wet feed (50% moisture) despite an increase in DM and water intake. They had suggested that lack of water consumption during the dark period might be one factor that contributes to the decline in egg production and egg weight. In support of this, Grizzle et al. (1992) observed that egg shell quality was improved in heat-stressed laying hens that received midnight lighting (intermittent light programs). Egg shell quality was also improved in hens kept under extended period of light (24 h) (Makled and

Charles, 1987). Therefore, it is likely that the effect of water and /or feed intake on egg production and egg shell quality are inseparable. Damron (1991) observed a general improvement in egg shell quality but not in the rate of egg production when the drinking water of heat-stressed hens was cooled from 28 to 21.1 C but not to 10 C.

Although some of these studies demonstrate some improvement in egg shell quality especially under heat stress conditions, the fact that the layer diet is usually formulated to provide adequate levels of all vitamins and minerals made the supplementation of the diet with these nutrients uneconomical. Since high temperatures reduce feed intake, it is likely that intake of vitamins and minerals is below requirement levels. Therefore, the reponse to dietary supplements is likely to be related to the degree of reduction in feed consumption, and any practice directed at increasing feed intake may result in significant economic savings.

Performance of Broiler Breeder Hens as Influenced by Feeding Time

The relationship between oviposition time and egg weight and egg shell quality has been the subject of several reports. Eggs laid in the morning (before 1100 h) weighed more than eggs laid in the afternoon (after 1400 h) (Roland and Harms, 1974; Roland et al., 1977). Arafa et al. (1982) reported that egg-type laying hens laid the heaviest eggs between 0600 and

0800 h. They also reported that shell weights, thickness and percentage-shell were higher in eggs laid in the early morning, then criteria dropped around mid-day, and increased in eggs laid in the afternoon. Brake (1985), using limited-fed broiler breeders (0800 or 1300 h), concluded that time feeding was responsible for the differences in egg weight, specific gravity and shell weight between eggs laid in the morning and those laid in the afternoon. They indicated that part of the differences in egg specific gravity was due to the differences in egg weight. Similar findings have been reported by Harms (1991) who reported that egg weight, specific gravity and shell weight were high in the morning, declined until 1245 to 1545 h, and then increased thereafter in commercial layers and broiler breeders.

It is common practice for broiler breeder hens to be provided with a limited amount of feed each morning. Under these conditions, birds produce egg shells of variable quality, and this becomes more pronounced in birds exposed to higher temperatures. Changing time of feeding (from morning to afternoon) was among many alternatives used to improve egg shell quality of broiler breeder hens maintained under optimal environmental conditions (Wilson and Keeling, 1991). Effects of such practice on the rate of egg production were varied. Balnave (1977) reported that egg production by broiler breeders fed in the afternoon was higher that those fed in the morning. The author also observed that heavier breeds

consumed more feed and had better egg production when fed in the evening compared to lighter hens. When full-fed commercial layers were restricted to meal-feeding regimes (4 h in the morning, 4 h in the evening, or 2 h in the morning plus 2 h in the evening) egg production of those fed twice daily was marginally reduced compared to those fed *ad libitum* (Daniel and Balnave, 1981). These authors also reported that hens fed twice daily ate more feed and laid heavier eggs. Cave et al. (1981) reported that increasing the frequency of feeding from one to three times daily increased egg weight, decreased body weight, and increased hen-day production. Bootwalla et al. (1983) found no significant ($P<.05$) effect of feeding time on the rate of egg production. Lack of feeding time effect on egg production could be due to the fact that, in the above study, the feeding time was changed on a gradual basis (Harms, 1991). Bootwalla et al. (1983) reported that hens fed at 1600 h laid eggs with higher specific gravity than hens fed at 0800, 1300, or *ad libitum*. Improvement in egg shell thickness has also been reported by Farmer et al. (1983b). Brake and Peebles (1986) observed an increase in egg weight and egg specific gravity but not in egg production due to feeding at 1300 *vs*. 0800 h. Wilson and Keeling (1991) found that feeding broiler breeders (34 wk old) at 0800, 1300, or 1430 h had no significant ($P<.05$) effect on egg shell thickness or egg production. They observed that afternoon feeding increased the proportion of eggs laid in the afternoon

in Arbor Acre broiler breeders. It was not clear, however, whether this shift in time of oviposition was due to feeding time or to bird age since these birds were either 34 or 64 wk old. Arbor Acre broiler breeder hens (52 to 58 wk old) experienced a significant ($P<.05$) body weight loss and decreased rate of egg production when feeding time was changed from 0800 to 1600 h (Harms, 1991). No explanation for these conflicting results was apparent. However, one possible explanation for the improved egg shell quality when broiler breeder hens are fed later in the day is enhanced availability of dietary calcium for eggshell calcification (Roland et al.,1973; Lennards and Roland, 1981). Farmer and Roland (1981) reported that force feeding calcium (at 1600 h) to caged broiler breeder hens enhanced their shell quality. Farmer et al. (1983a, b, c) concluded that broiler breeder hens fed in the afternoon have more calcium available during the stage of the egg shell calcification, and excreted less calcium via feces, suggesting more efficient calcium absorption during this period (Hurwitz and Bar, 1965). Moreover, hens restricted to morning feeding did not maintain a uniform flow of dry matter and calcium from the crop into the digestive tract throughout the rest of the day. Farmer et al. (1986) reported that supplying calcium during the period of shell formation reduced skeletal calcium utilization for shell deposition to 15% compared to 30 to 40% reported by others (Driggers and Comar, 1949; Mueller et al., 1964).

Based on the above studies, hens fed in the afternoon perform better than hens fed in the early morning. More information is needed to better determine the effects of feeding time on egg production of hens maintained under different environmental conditions.

Description of Calcium Metabolism in the Laying Hen

Nutritional factors which affect egg shell quality have been reviewed by Wolford and Tanaka (1970). Calcium is especially important in that a period of apparent calcium challenge occurs during egg shell formation.

The hormones controlling calcium metabolism have been reviewed by Hurwitz (1978). He stated that at least three hormonal systems are directly involved in the control of plasma calcium. These are: parathyroid hormone secreted by the parathyroid gland, calcitonin secreted by the ultimobranchial gland, and 1,25-dihydroxycholecalciferol secreted by the kidney. Estrogen and androgen also play a critical role in the regulation of calcium prior to and during egg laying (Figure 1) (Simikiss, 1967, Mueller et al., 1969).

The role of the above hormones in calcium absorption, transport, deposition and mobilization in and out of the bone, and transport to and within the shell gland has been reviewed by several workers (Simkiss, 1967; Copp, 1969; Norman, 1987). Briefly, parathyroid hormone (PTH) induces osteoclastic bone reabsorption, increases Ca reabsorption in the kidney,

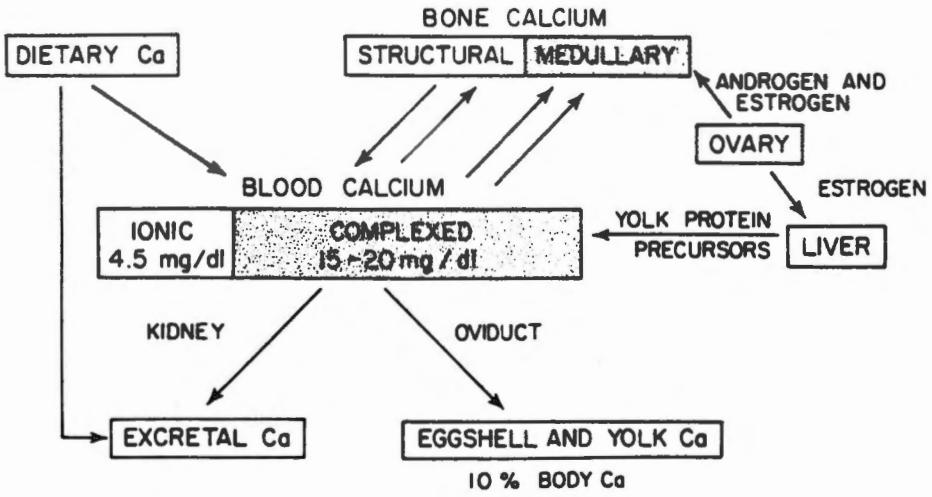

Figure 1. Calcium metabolism in egg-laying birds. Yolk protein precursors complex plasma calcium so that the total calcium can rise to very high levels leaving the plasma ionic calcium at normal levels. Under the influence of both estrogen and androgen, medullary bone is formed in the cavities of the long bones. Calcitonin has no overt effects on avian calcium metabolism. (Adopted from A.D. Kenny, 1986. Avian Physiology. P.D. Sturkie, ed., page 467. New York, Springer-Verlag.).

inhibits PO_4^- reabsorption in the kidney and activates vitamin D. Calcitonin (CT) decreases osteoclastic activity. 1,25-dihydroxycholecalciferol increases Ca absorption, acts together with PTH to mobilize Ca from the bone and facilitates calcium transport (via vitamin D-dependent calcium binding proteins) in the shell gland. Estrogens cause an increase in protein bound calcium (Urist et al., 1958), stimulates Ca transport in the shell gland (Navikis et al, 1979).Estrogens may stimulate Ca absorption from the digestive tract and estrogen together with androgen increase Ca turnover in bone of the laying hen (Eastin and Spaziani, 1978).

Calcium Metabolism and the Egg Cycle

The average body weight of the egg-type laying hen is approximately 2 kg. The hen's skeleton contains around 20 g of calcium which was estimated to be 97.2 to 98.7% of all the calcium in the hen's body (Common, 1938). The average egg contains 2 g of calcium which is equal to 10 % of total body calcium (Figure 1). Since that the egg cycle is 24-26 h, Soares (1984) estimated that a hen which lays on a regular basis will utilize 1000 ug Ca/ kg BW per day for shell formation. In a more recent study, Soares (1987) estimated that an egg-type laying hen will absorb (from the diet) and mobilize (form the bone) about 1500 ug Ca/ d to produce an egg shell, assuming a 67% hen-d production rate.

Calcium in the egg comes partly from the feed and partly

from the medullary bone. Driggers and Comar(1949) reported that 60-70% of the shell calcium comes from the diet and the remainder is derived from the bone.

Winget and Smith (1958) reported that the bound form of calcium (total calcium) declines during egg shell calcification. This decline in total calcium resulted in the apparent increase in plasma ionized:total calcium ratio. By contrast, Luck and Scanes (1978) measured ionized calcium in plasma of laying hens during the ovulatory cycle and found that blood ionized calcium concentration (Ca^{++}) peaked 3-6 h after oviposition, and fell slowly as calcification proceeded, reaching a minimum 3-6 h before oviposition. They also reported that total calcium did not change in the same manner as ionized calcium, whereas plasma phosphorus increased during this time period. Eastin and Spaziani (1978a) reported that rate of calcium secretion in the shell gland began to rise 5-7 h following oviposition, increased to a maximum rate as the egg arrived in the shell gland, decreased by 20 h and returned to the basal level 2 h before oviposition. Parson and Combs (1981) measured the changes in plasma total calcium and blood Ca^{++} in 18-month-old laying hens. They observed that plasma total calcium did not vary significantly ($P>.05$) during the ovulatory cycle (remained about 10.08 to 12.83 mEq/ L), whereas blood Ca^{++} showed similar patterns to that reported by Luck and Scanes (1978). In the study conducted by Parson and Combs (1981) ionic calcium reached a maximum of level 2.83

mEq/ L following an oviposition and a minimum level of 2.46 mEq/ L shortly before the next oviposition. Moreover, they reported that blood phosphorus was lowest at oviposition and gradually increased to a maximum value around 16 h later. Similar data on phosphorus levels during the egg cycle have been reported by Mongin and Sauveur (1979).

Effect of Calcium Deprivation on Egg Production and Shell Quality

Taylor et al. (1962) reported that egg production was brought to an end after six days of feeding a calcium deficient diet (0.2%); however, if injected with crude pituitary preparations, hens continued to lay eggs for 5 more days. It was speculated that inhibition of ovulation was mediated through the hypothalamus in response to the severe decline in blood ionized calcium concentration (Taylor, 1972). The author proposed a "pituitary-cut-off mechanism". Theoretically, if the concentration of the blood Ca^{++} drops below a fixed threshold level, the "cut-off mechanism" will cause a reduction in the secretion of gonadotrophins (FSH and LH) and a delay in follicular growth and ovulation. Ligating (By placing a surgical thread in the shell gland) the oviduct has been reported to cause laying of shelless eggs (premature oviposition) due to the occurrence of multi-ovulations which deprive the hen's calcium stores. It was assumed that this calcium effect on ovulation was mediated at the hypothalamus-

pituitary level. This assumption was investigated by Luck and Scanes (1979). They challenged calcium-deficient and calcium-replete hens with injections of gonadotropin releasing hormone (GnRH) and progesterone and found that the pituitary of calcium-deficient hens was capable of secreting a larger amount of luteinizing hormone (LH), but for a shorter period of time, compared to the calcium-replete hens. They concluded that the hypothalamic activity was regulated by blood Ca^{++} and suggested that ovulation had stopped to avoid calcium stress. It is of importance to note that egg laying in the calcium-deficient hens ceased when blood Ca^{++} dropped to 1 mM (2.04 mEq/ L) which was about 70% of the concentration of blood Ca^{++} in normal hens.

The Relationship between Calcium and Acid-base Balance and their Effect on Egg Shell Quality

It is well known that a constant physiological pH is essential for all metabolic processes. There are several buffer systems such as hemoglobin, and to a lesser extent plasma proteins, and inorganic phosphate; however, the bicarbonate/CO_2 ($CO_2 + H_2o = HCO_3^- + H^+$) is the main buffer system. The concentration of CO_2 can be changed during normal metabolism or through pulmonary ventilation, whereas the concentration of HCO_3^- can be altered via kidney excretion. An elevation or a decline in the CO_2 concentration can result in elevation or a decline in pH. The opposite is true for

bicarbonate. Earlier work on the relationship between acid-base balance and calcium metabolism has been summarized by Mongin (1968) and Hughs (1988). Evidently, feed metabolism, egg shell formation, and heat stress are considered primary causes of acid-base disturbance. Renal as well as respiratory compensation are involved in regulation of acid-base balance. The respiratory system regulates the concentration of CO_2 in all fluid compartments of the body via exchange of CO_2 or HCO_3^- with the blood. In the kidney, acid-base equilibrium is achieved by total absorption of filtered bicarbonate, the regeneration of bicarbonate consumed by acidosis via excretion of titratable acidity, and the excretion of ammonia (NH_4^+).

The changes in shell gland function during the process of calcification are illustrated in Figure 2. Mongin (1968) estimated that the egg shell contains 100 mEq of carbonate ions which come from 50 mEq of bicarbonate. The author concluded that the bicarbonate pool in the extracellular fluid (13.5 mEq) is renewed 3.7 times during the 15-h shell formation, suggesting a close relation between acid-base balance and Ca metabolism.

Investigation of the origin of carbonate ions for egg shell synthesis has received little attention. It is not known whether shell carbonate comes from the blood bicarbonate ion pool or from carbon dioxide produced during the normal metabolic activity of the oviduct. Locher and Hodges (1969) injected $^{45}CaCl_2$ and $^{14}NaHCO_3$ into the blood of either egg-

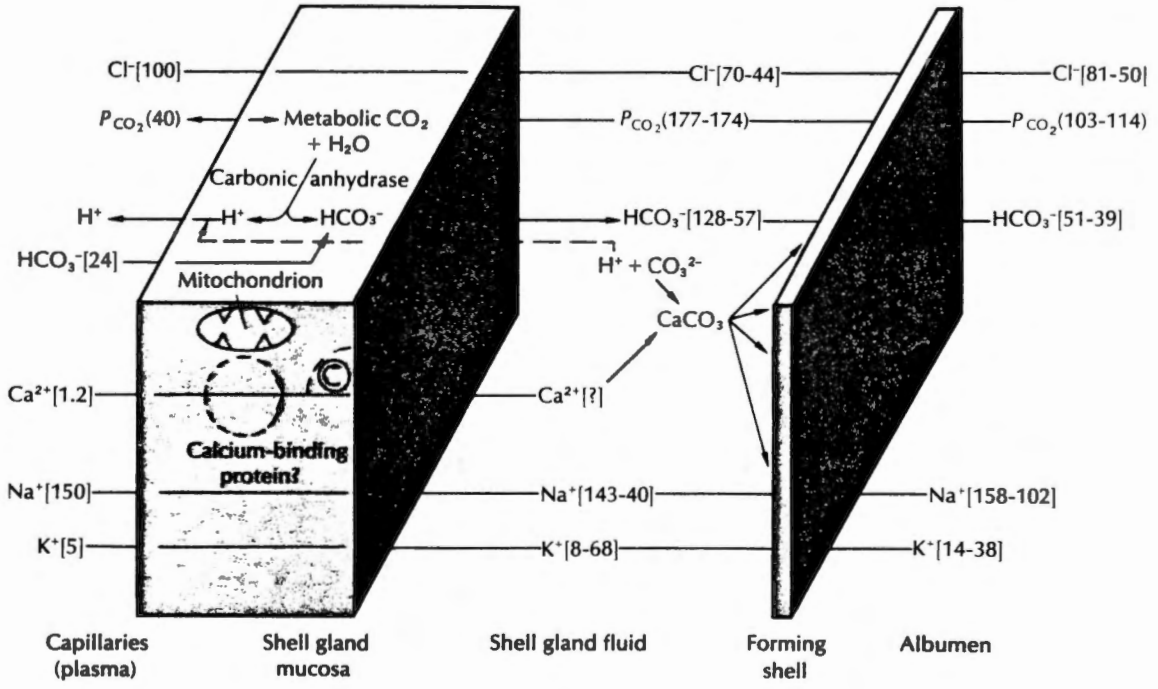

Figure 2. A tentative model of ion transfer during shell formation in the hen. Figures in square brackets indicate approximate concentrations in millimole per liter; the values for pCO_2 are in mmHg. If two concentrations are given, the first refers to early and the second to late shell formation. Throughout shell formation, Ca^{2+} is actively transported against a concentration gradient from serum into the shell gland fluid. Metabolic CO_2 is converted by carbonic anhydrase in the shell gland mucosa to H^+ and HCO_3^-; H^+ is exported to the circulatory system and HCO_3^- accumulates in the shell gland fluid. The equilibrium between HCO_3^- and CO_3^{2-} provides the latter anion during eggshell formation and raises the concentration of CO_2 in the shell gland fluid. The changes in the concentrations of Cl^-, Na^+, and K^+ may be associated with active transport of Ca^{2+} or with the movement of H_2O into albumen during calcification. (Adopted from R.J. Etches, 1993. Reproduction In Domesticated Animals. G.J. King, ed., page 503. New York, Elesvier Science Publisher B.V.).

shell forming or non-forming hens. They observed a difference (arterio-venous) in the radioactivity of blood samples taken from egg-shell forming hens. They concluded that a significant proportion of the egg shell carbonate ions came from the carbon dioxide produced metabolically in the oviduct.

Sturkie and Mueller (1976) demonstrated that a total of 2 H^+ ions are released for every calcium carbonate molecule generated during egg shell formation. The first comes from CO_2 hydrolysis and the second results from the conversion of HCO_3^- to CO_3^-. Since H^+ ions are produced during egg shell formation, a metabolic acidosis is believed to occur. This is followed by respiratory as well as renal compensation. Simkiss (1968) reported a progressive drop in blood bicarbonate concentration, pH, and CO_2 and a rise in the ventilation rate. These returned to normal when shell formation was completed. Anderson (1967) observed a fall in the urine pH (from 8 to 5.5) during egg shell formation, and found little or no bicarbonate in the urine during egg shell formation, suggesting that renal compensation was occurring. Perashad and Edwards (1973) observed an increase in the rate of phosphate clearance during egg shell calcification; however, the authors pointed out that increased phosphate excretion was primarily intended to eliminate excess phosphate rather than to buffer the released H^+ ions during shell calcification. Mongin and Sauveur (1979) confirmed that the beginning of shell secretion (from 10 to 22 h after

oviposition of the previous egg) was always accompanied by an increase in inorganic phosphorus (P_i) excretion. Mongin and Lacassagne (in Sturkie and Mueller, 1976) demonstrated that before an egg entered the shell gland, venous blood pH was 7.53, then declined to 7.41 by 17 h after the entry of the egg into the shell gland. During the same time period, serum bicarbonate level decreased from 31.3 to 20.7 mEq/ L. Buss et al. (1980) measured urinary calcium levels in White Leghorn hens before and during egg shell formation. They observed a decline (from 44 mg% to 6 mg%) in calcium excretion in urine during shell formation. Widman (1987) reviewed endocrine control of Ca and phosphorus excretion in the laying hen, and stated that the quantities of Ca and inorganic phosphorus (P_i) in urine are determined by cumulative rate of three processes: glomerular filtration, tubular reabsorption, and tubular secretion. Plasma P_i is generally considered to be completely ultrafilterable, although a small fraction may be protein bound, whereas ultrafilterability of plasma calcium depends on nutrition, blood pH, PTH and the reproductive status. The author pointed out that the patterns of Ca and P excretion are more correlated with the stage of egg shell calcification than with time of day or with time of feeding.

Winget and Smith (1962) indicated that relative concentration of ionic calcium seems to be minimal in plasma of normal pH, increasing as the pH changes in either direction. The authors also demonstrated (*in vitro*) that an

increase in [H^+] favors the dissociation of protein-bound calcium. Therefore, production of H^+ ions in the shell gland during calcification facilitates the dissociation of the calcium-protein complex (Hodges, 1969). Earlier, Taylor and Hertelendy (1961) reported that the equilibrium between the ionized and protein-bound calcium is sensitive to plasma pH. These authors observed that the plasma pH from laying hens increased by 0.3 units when it was exposed to the air for 3 h during which ionized calcium levels decreased by 1-2 mg/ 100 mL.

There has been evidence for at least 30 years that chemical compounds which interfere with acid-base balance depress egg shell quality when given to laying hens. Helbacka and Hall (1958) and Hunt and Aitken (1962) observed a decline in egg shell thickness and specific gravity when ammonium chloride and hydrochloric acid were given to laying hens at levels of 1.5 to 2.0%. Frank and Burger (1965) gradually increased atmospheric pCO_2 from 0.3 to 19 mm Hg for a period of 21 d and found that blood pH of laying hens declined from 7.48 to 7.40. More recently, Miles and Harms (1982) and Junqueira et al. (1984) reported that increasing dietary phosphorus caused a decline in HCO_3^- and pCO_2 which in turn caused a reduction in egg shell quality. Supplementing the diet with sodium bicarbonate reversed this effect. The authors assumed that addition of sodium bicarbonate may have acted as a buffer to the acidity caused by feeding dietary

phosphate.

It is well known that hyperventilation causes respiratory alkalosis. It is evoked during oxygen deficiency, accompanied by a decline in pCO_2. For HCO_3^-/CO_2 to remain in equilibrium, a decline in HCO_3^- concentration is necessary. On the other hand, H^+ ions are being provided by non-bicarbonate buffers (i.e. hemoglobin). Therefore, the concentration of bicarbonate drops further in respiratory compensation of metabolic acidosis. For the pH to be restored, a further drop in the concentration of the bicarbonate ions is inevitable, which is achieved through renal compensation (an increase in bicarbonate excretion and a decrease in H^+ secretion or secretion of bicarbonate and reabsorption of H^+) (Mongin, 1968; Hughs, 1988).

Conrad (1939) reported that increasing environmental temperature from 21 to 32 C resulted in a decline in plasma total calcium by 25%. Warren and Schnepel (1940) found that the decline in blood total calcium was accompanied by a decline in egg shell thickness. Mueller (1959) reported that blood total calcium declined from 23.4 mg/ 100 mL at a temperature of 13 C to 21.2 mg/ 100 mL at 29 C. The author proposed that differences in total calcium could be explained by differences in calcium intake. Mueller (1959),in contrast to the conclusion drawn by Warren and Schnepel (1940), found a negative correlation between serum calcium level and egg shell thickness.

Respiratory alkalosis caused by hyperthermic temperatures may coincide with egg shell formation causing a competition between the shell gland, which is using bicarbonate for $CaCO_3$ formation, and the kidney which is excreting bicarbonate from the body. For this reason, when temperature was increased from 13 to 34 C, egg shell thickness in White Leghorn hens declined immediately (within one day) as a result of the change of blood pH and pCO_2 (Mueller, 1966). Bragg et al. (1971) reported that high temperatures decreased both shell quality and the amount of ^{45}Ca activity secreted into the shell gland. Odom et al. (1986) determined blood ionized calcium (Ca^{++}) in egg-type laying hens exposed to acute heat stress. Blood samples were obtained every 30 min. when the chamber temperature increased from 23 to 35 C (for 3 h), and then decreased to 23 C. They found that blood Ca^{++} was reduced (from 7.27 to 5.90 mg/ 100 mL) during the 3 h exposure to the high temperature. Blood Ca^{++} increased to 6.90 mg/ 100 mL 13 h after the temperature was reduced to 23 C. It is noteworthy that the authors did not consider blood sampling time relative to the egg cycle stage which by itself alters blood Ca^{++} levels independent of temperature effects. The justification for the decline in blood Ca^{++} during heat stress was that elevated blood pH favors the association of Ca^{++} to proteins and organic anions produced during hyperventilation. Fanconi and Rose (1958) reported that hyperventilation (non-thermic origin) in normal subjects was accompanied by a decline in

plasma ionized calcium. The authors observed an increase in the amount of calcium complexed with citrate. In chickens, Frankel and Frascella (1968), found that increasing environmental temperature to 43, 44, and 45 C caused a decline in pCo_2, whereas pO_2, pH, lactate and pyruvate concentrations were increased. An interesting observation reported by Odom et al. (1986) was that levels of lactate and pyruvate dropped back immediately after the temperature was lowered to 23 C. It could be argued that a decline in the blood pH, pyruvate, and lactate should be accompanied by a recovery in the levels of Ca^{++}, which was not observed in the study conducted by Odom et al. (1986).

There are several reports suggesting that acclimation of laying hens to high environmental temperatures aided in the maintenance of some physiological processes including thermoregulation, acid-base balance and egg shell quality. Arad et al. (1981) concluded that temperature-induced decreases in total plasma calcium concentration were less in heat acclimated fowl and resulted in maintenance of egg shell weight, shell quality and rate of egg production in White Leghorn hens. Similar results have been reported by Arad and Marder (1982) and Arad and Marder (1983). However, to date, there are no data available on the effects of heat stress on blood ionized calcium levels of hens acclimated to high temperatures. Arad (1983) studied thermoregulation activities and acid-base balance in dehydrated and heat-stressed laying

hens (20 mo old) acclimated to increasing temperatures. Despite the decline in pCO_2 due to a compensatory drop in HCO_3^- concentration, acid-base balance during heat stress was not adversely affected. The author attributed this to the closely regulated respiration rate, and tidal and minute volumes which provides a means to avoid acid-base disturbances.

Effects of Heat Stress on Biochemical Variables of Calcium Metabolism

Two calcium binding protein (Ca-BP's) are present in the blood of the laying hen (Guyer et al., 1980): albumin and vitellogenin. Both are involved in calcium transport, however; vitellogenin (an estrogen induced Ca-BP) is the major Ca-BP in the blood of the laying hen. Grunder et al. (1980) observed a positive correlation between levels of vitellogenin and egg shell thickness.

These Ca-BP's are different from other calcium binding proteins present in the small intestine or in the shell gland (Wasserman and Tayler, 1966). Hurwitz and Bar (1973) reported that the molecular weight of the Ca-BP present the shell gland was higher than that of intestinal mucosa. Both of these Ca-BP's are vitamin D-dependent.

Corradino et al. (1968) administered vitamin D to vitamin D-deficient hens. The authors observed a sustained increase in the number of eggs laid which began after 3-4 days, whereas administration of estrogen was without effect. They concluded

that vitamin D had a direct effect on the shell gland. The authors speculated that this effect was due to enhanced calcium transport of calcium across the shell gland, which was later confirmed by Nys et al. (1986). A different views on the relative role of vitamin D in maintaining egg shell quality has been reported by Bar et al. (1984) who suggested that vitamin D does not appear to regulate either level of Ca-BP in the egg shell gland or calcium transport.

The presence of a Ca^{2+}-Mg^{2+}-adenosine triphosphate (ATPase) has been reported in the microsomal fraction of the shell gland of Japanese quail (Pike and Alvarado, 1975). These authors concluded that Ca^{++} or Mg^{++} ATPase activity is higher during shell formation. *In situ* perfusion of different segments of the oviduct indicated that movement of calcium from the shell gland mucosa in the lumen is an active process. More recently, several reports have demonstrated a positive relationship between egg shell quality and the Ca-BP present in the shell gland (Bar et al., 1984, Castaldo and Maurice, 1990), and adenosine triphosphate (ATPase) activity (Watanabe et al., 1989).

Carbonic anhydrase is present in the mucosa of the shell gland (Common, 1941) as well as other tissues such as the red blood cells and the kidney (Yoshihara et al., 1987). Carbonic anhydrase is required for the hydration of CO_2 to carbonic acid (CO_2+ H_2O= H_2CO_3) which serves as a source of carbonate ions for egg shell formation (Figure 2). Gutowaska and

Mitchel (1945) demonstrated a relationship between the concentration of carbonic anhydrase in the shell gland and the hen's ability to produce strong and defectless eggs. By contrast, Heald et al. (1967) did not find any special relationship between shell strength (measured by deformability) and carbonic anhydrase activity as had been reported earlier by Mueller (1962).

There is little, if any, information on the direct effect of heat stress on the above biochemical parameters. On the other hand, there is a significant body of evidence that alteration of acid-base balance may impair several metabolic pathways related to calcium metabolism. Mongin (1968) reviewed data from studies in which carbonic anhydrase inhibitors (i.e. sulfonamide compounds) have been used to evaluate the effects of carbonic anhydrase in the regulation of calcium metabolism and acid-base balance. The author suggested several possibilities for the effect of carbonic anhydrase inhibitors in egg shell formation. These possibilities are: a drop in the rate of bicarbonate ion formation in red blood cells; increased excretion of bicarbonate ions due to a decrease of H^+ ion secretion by the nephron; failure of carbonic anhydrase to mobilize bone calcium due to local secretion of H^+; and inhibition of blood bicarbonate flow towards the shell gland lumen. Pearson and Goldener (1973) studied the flux of calcium across isolated quail shell gland. They suggested that the net flux of

calcium from the serosa to mucosa and mucosa to serosa surfaces may be dependent on bicarbonate concentration. Pearson et al. (1977) demonstrated that activity of the shell gland carbonic anhydrase was greater in laying than in non-laying quail and suggested that its activity may be related to calcium secretion in the shell gland. In a more recent study, activity of carbonic anhydrase has been reported to influence the active transport of Ca^{++} across intestinal and the shell gland mucosa (Nys and De Laage 1984a, b). These authors attributed this effect to the relation between Mg^{++}-HCo_3^--ATPase and carbonic anhydrase. Odom and Harrison (1985) similarly observed an 80% increase in the unidirectional flux of ^{45}Ca from serosal to mucosal surfaces when the pCO_2 of the buffer bathing the serosal side of isolated shell gland tissue was increased. Silverton (1991) presented other cases in which acid-base balance played an important role in calcium metabolism. The author reported that in marine organisms (i.e. oyster), uptake of Ca^{++} from sea water relies on an adequate supply of bicarbonate. Carbonic anhydrase inhibitors were able to inhibit this process. Also, in the rat Ca^{++} resorbed from bones for calcium homeostasis was dependent on carbonic anhydrase activity. Moreover, inhibition of carbonic anhydrase decreased calcium transport in chick chorioallantoic membrane.

High temperatures (32 C) have been reported to increase the level of uterine carbonic anhydrase activity in eggshell-

forming hens (Goto et al., 1982); however, it not clear why high temperatures (28-35.9 C) did cause a reduction in levels of this enzyme when measurements were taken within 1, 4, and 6 h after oviposition (Gill and Gangwar, 1990).

Estrogen may influence egg shell quality by increasing plasma concentration of protein bound calcium (Urist et al., 1958; Guyer et al., 1980). Navikis et al. (1979) reported that estrogen enhanced calcium transport in the shell gland via modulation of CA-BP concentration. Estrogen had also been proposed to enhance vitamin D activation which in turn induces Ca-BP in the shell gland. Soares et al. (1980), by using two lines of hens (7 mo old) known to produce distinctively different egg shell quality, found a positive correlation between percent-shell of the egg and plasma 1,25-$(OH)_2$ D_3 concentration but not between percent-shell and calcium concentration. A similar relationship was proposed between estrogen and percent-shell. The authors also proposed that shell quality is closely related to the ability of the hen to metabolize calcium. Grunder et al. (1983) reported that plasma estrogen and calcium concentrations were significantly ($P<.05$) higher in hens (in their second laying cycle) producing eggs of high specific gravity compared to hens that produce eggs of low specific gravity.

Two opposing views have been expressed about the effect of high temperature on progesterone levels in the laying hen. Novero et al. (1991), using a group of laying hens acclimated

(for two wk) to a cyclic temperature of 17 to 27 C and a second group of hens acclimated to the same temperature but moved to a constant temperature of 35 C, found that progesterone levels from hens of the second group were significantly lower ($P<.05$) from 6 h to predicted ovulation. The authors suggested that heat stress may have a direct adverse effect on the ovarian tissues since granulosa cells from the heat stressed hens failed to respond (increase basal progesterone production) to challenge by a LH injection, whereas granulosa cells from the unstressed hens responded to LH challenge by increasing their basal progesterone production. By contrast, Grizzle and Scott (1993) reported that exposure of laying hens acclimated to cycling temperatures (22-35 or 23-37) did not significantly ($P>.05$) affect plasma progesterone levels. Nasser et al. (1992) measured the level of estrogen in plasma of broiler breeder hens in relative to the temperature cycle (22-40, 22-42, and 19-25 C), but not relative to the egg cycle. They observed that estradiol increased at high temperatures, but decreased at 1900 h compared to that of hens at low temperatures.

In conclusion, hormones related to calcium metabolism (parathyroid hormone, calcitonin, $1,25\text{-}(OH)_2\text{-}D_3$, estrogens and androgen), acid-base balance, and some enzyme activity are the major factors that influence egg shell quality. Further data are required to fully understanding the functioning of these factors in hens at different environmental temperatures.

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PART II

EFFECTS OF TEMPERATURE AND FEEDING TIME ON PERFORMANCE OF BROILER BREEDER HENS

INTRODUCTION

It is a common commercial practice for broiler breeder hens to be limit fed, usually by providing feed once in the morning of each day. Under these conditions, birds produce egg shells of variable quality which is more pronounced in hens exposed to high temperatures (Fox, 1951; Renden et al., 1984; Nasser et al., 1992).

Changing time of feeding is one possible means of reducing incidence of poor shell quality; however, a review of the literature provides conflicting evidence regarding the relationship between feeding time and egg production. Some workers have found that afternoon feeding resulted in a higher rate of egg production (Balnave, 1977; Bootwalla et al., 1983), whereas others have reported no effect (Wilson and Keeling, 1991). Most reports indicate that late day feeding negatively affects production. Brake and Peebles (1986) observed a decline in egg production when feeding occurred at 1300 h *versus* 0800 h. Harms (1991) similarly reported reduced egg production when feeding time of broiler breeder hens was changed from 0800 to 1600 h. In all these studies, although some disagreement exists, there is evidence that feeding late in the day increases egg weight and improves the egg shell quality.

There is no explanation for the decline in the rate of egg production when broiler breeder hens are fed late in the

day. Wilson and Keeling (1991) speculated that a change in ovulation time, or increased oviductal time may be responsible for the delay in time of oviposition when broiler breeder hens were fed in the afternoon.

It is assumed that a single-morning meal given to hens is completely consumed within the first 3 to 4 h (Farmer et al., 1983b). It is not known, however, how feed intake is regulated, especially when broiler breeder hens are maintained under high cyclic temperatures. In addition, it is not known whether similar patterns of feed consumption exist for hens fed late in the day.

The effect of feeding time on body temperature has been recently investigated by two researchers. Wilson et al. (1989), using four groups of 70-wk-old broiler breeder hens fed a single meal at either 0600, 1000, 1400 or 1800 h, observed an increase in body temperature (.5 C at 1 h post-feeding in all groups and 5 h post feeding in the 0600 h feed group). In another experiment, using 1-year-old broiler breeder males kept under cyclic temperature (22.2-33.3 C), they observed a rise in body temperature at 5, 4, 3 and 2 h post-feeding at feeding times of 0600, 1000, 1400 and 1800 h respectively. The authors observed that birds fed in the afternoon were able to maintain their body temperature even when the room temperature was increasing. Li et al. (1992), using two open-circuit respiratory chambers and four 28-wk-old laying hens, found that at temperatures below 28 C, feed

intake had no effect on abdominal temperature, whereas abdominal temperature increased at temperature of 32 and 36 C with increasing quantity of feed (0, 30, 60, 90 gm/d), suggesting that heat production resulting from feed intake further increased the heat burden at high temperatures. In earlier studies, body temperature increased when the ambient temperature was increased from 21 to 29.4 and 35 C (Ahmad et al., 1976). Heywang (1938) reported that body temperatures were highest at 4 p.m. and lowest at midnight. Body temperatures were the highest during the hottest part of the day but not lowest during the coolest part of the night. De Andrade et al. (1977) observed a definite diurnal rhythm of body temperature with the highest values occurring at noon. Hens maintained under cyclic temperature had higher body temperatures compared to the hens maintained at a constant temperature of 21 C, but body temperatures were similar at night.

In view of these studies, feeding late in the day, theoretically, could prove advantageous for the following reasons: 1) under natural light-dark cycle, laying hens eat more feed at the end of the day compared to the morning (Roland et al., 1972; Mongin and Sauveur, 1974); 2) under cyclic temperatures, heat production associated with morning feeding and oviposition (Cain and Wilson, 1971; Wilson et al., 1989) will coincide with the increasing environmental temperature, whereas feeding late in the day will not; 3) hens

fed late in the day are expected to maintain a lower body temperature through the mid-day period compared with hens fed in the morning.

The objectives of this study were to determine the effects of time of feeding and cyclic temperatures and their interactions on the rate of egg production, egg shell quality indicators, patterns of egg laying, body weight change, calcium balance and feed consumption patterns of broiler breeder hens.

MATERIALS AND METHODS

Experiment 1

Thirty-eight-wk-old Arbor Acre broiler breeder hens were moved to individual laying cages (60x40x45 cm) in two environmentally controlled chambers and during a two wk adjustment period were fed 178 g/d of a complete feed (Table 1) at 0700 h. Hens had free access to water from cup drinkers connected to water containers that were placed above the cage. Hens received 17 h of constant light per day (0700 to 2400). Treatments were factorially arranged and consisted of 2 temperatures and 3 feeding regimens. Hens were randomly assigned to each treatment. Within treatment, 10 hens were randomly assigned to each of two replicate groups. Feeding treatments were: group 1, fed one meal at 0700 h; group 2, fed half of the meal at 0700 and the other half at 1800 h; and group 3, fed at 1800 h. On a typical summer day, temperature starts low in the morning, then gradually increases to a peak value by midday, and decreases to the over-night low. The same pattern exists in other seasons, but temperatures are usually lower. Therefore, two temperature treatments were imposed: treatment 1 (LO) and 2 (HI) were cycled daily from a low of either 10 or 21 C at 0300 to a peak of either 25 or 39 C, respectively, at 1600 h. Figure 1 shows the daily temperature cycle in both chambers. No control of humidity was attempted, but it was recorded throughout the 24 h period.

TABLE 1. Composition of diet

Composition of diet	%
Yellow corn	64.06
Soybean meal	18.00
Alfalfa meal	6.50
Menhaden fishmeal	2.50
Defluorinated rock phosphate	1.62
Limeston	6.20
Vitamin mix[1]	0.65
Salt	0.35
DL-methionine	0.01
Manganese sulfate	0.03
Calculated analyses	
Crude protein	17.00
Metabolizable energy, kcal/kg	2748.00
Calcium	3.16
Available phosphorus	0.48
Arginine	1.13
Isoleucine	0.82
Lysine	0.89
Methionine + cystine	0.67
Threonine	0.70
Tryptophan	0.21
Valine	0.96
Chemical analyses	
Calcium (%)	3.21
Phosphorus (%)	0.74

[1]Contained per kilogram: cholecalciferol, 600,000 ICU; vitamin A, 500,000 ICU; riboflavin, 910 mg; vitamin B_{12}, 1.31 mg; niacin, 5g; D-calcium-pantothenate, 875 mg; choline, 76.6g.

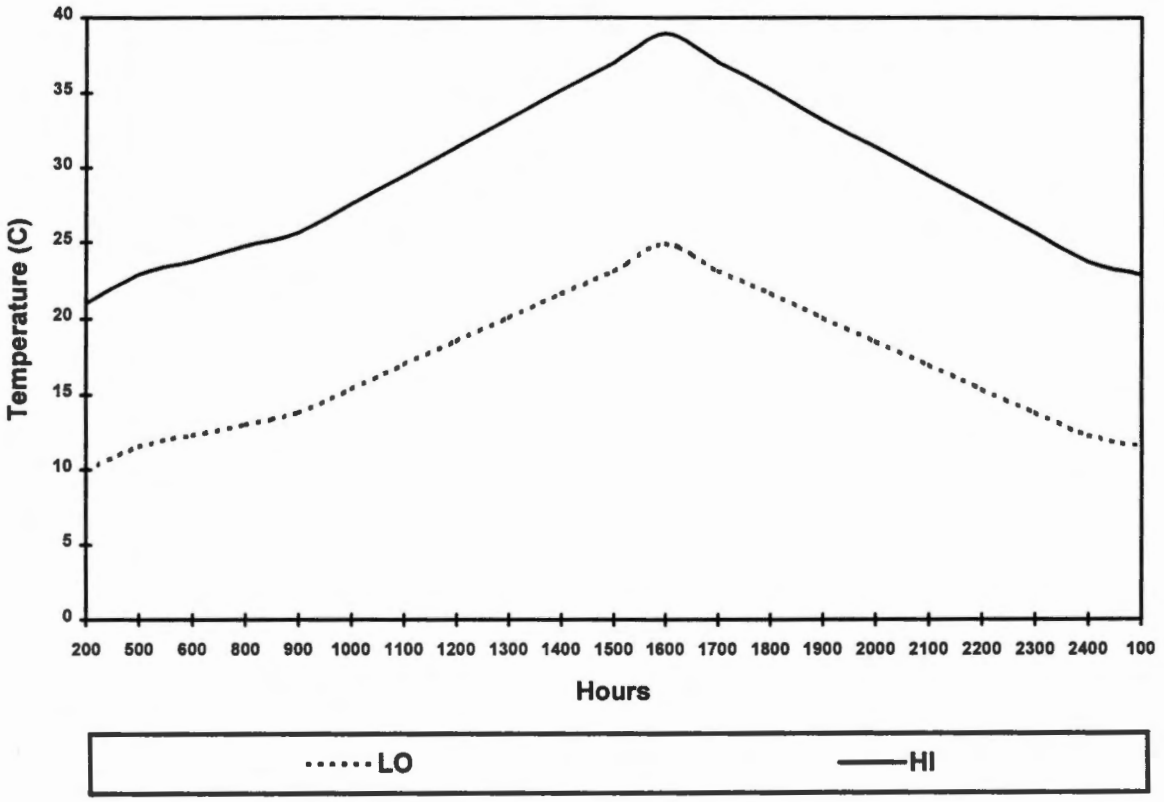

Figure 1. The daily temperature cycle (Experiment 1).

Egg production was recorded daily for a 2-wk adjustment period and 4-wk experimental period. Egg weight, specific gravity and shell thickness were obtained from eggs collected during the last three days of every week. These eggs were marked with the hen's number and time of oviposition. Eggs laid were classified into 12 different classes based on time of oviposition which was observed from 0700 to 1800 h during the last three days of each week. Egg weight was recorded at the end of day. Egg specific gravity was determined the following morning by using the floatation method (Voisey and Hamilton, 1977), taking measurements in increments of .004 (from 1.062 to 1.102). The eggs were washed and allowed to dry at room temperature for 24 h; a piece of (membrane-free) shell was obtained from the egg equator and shell thickness was measured with a micrometer.

On day 24, time of oviposition was recorded every 15 min. Hens identified as regular layers (laying a sequence of 3 eggs or more) and who had laid the first egg in the sequence between 0900 and 1030 h were used for blood sampling. Heparinized syringes were used to collect 1 ml blood from the wing vein of 5 birds in each group. Samples were immediately put in heparinized test tubes and stored in ice until analyzed for blood ionized calcium (Ca^{++}). Measurements of blood Ca^{++} were obtained within 4 h after collection using an ion selective electrode (Beckman, Lablyt™ System 820 Electrolyte Analyzer, Beckman Instruments, Inc., Brea, California, USA).

Feed consumption patterns were determined on day 13 and 27 of the experiment. Feed intake was measured for each group (all hens), starting at the time the feed was served and every 30 min for 3 h, then every hour for 5 h and every 2 h thereafter until the feed was gone.

On day 36 of the experiment, individual body weight was recorded and body weight change was determined. Body weight change was measured by the difference between initial and final individual weights.

Data for egg production, egg weight, specific gravity, shell thickness, interval between two successive ovipositions (op-op interval), body weight change, daily feed and water consumption were subjected to the analysis of variance (ANOVA) using the General Linear Models Procedure of statistical analysis system (SAS) (SAS Institute, 1985). The main effects were feeding time and temperature. All data were analyzed for main effects and their interactions. Repeated measures analysis of variance of SAS was used to test differences in blood Ca^{++} and pH. Differences between means were tested by the least square difference method. A series of Chi-square tests were used to assess the effect of feeding time and temperature exposure on time of oviposition. A non-linear regression model fitting SAS procedure was applied to the data of feed consumption patterns. The model used was $Y= A(1-e^{-Bt})$, where A represent the asymptotic level which feed consumption (Y) approaches as time (t) increases, and B represents the

rate of approach. The model relates cumulative feed intake, expressed as percent of the total feed consumption, with time.

Experiment 2

Sixty broiler breeder hens, 45 wk of age, were randomly assigned to one of three temperature treatments (Figure 2) which differed in time at which peak temperature occurred: 1600 h (treatment 1), 2400 h (treatment 2), and 0800 h (treatment 3). The arrangement of the three treatments in this manner was necessary to insure that in each treatment peak temperature occurs at a different stage of shell calcification. Water was continuously available. Hens were subjected to a 17 h light (0700 to 2400 h) and 7 h dark photoschedule. Relative humidity was not controlled.

The experiment was conducted during three consecutive weeks. High cyclic temperature (21-39 C) was allowed to peak at 1600 h during week 1, 2400 h in week 2, and 0800 h in week 3. Egg production, egg specific gravity and shell thickness were recorded as in experiment 1.

On day 5 of each week, all hens were observed at 15 min intervals from 0700 until 1200 h. Eight hens were cannulated (brachial vein) after the oviposition of the first egg in the sequence (0900 to 1000 h). On day 6, blood samples (1 ml each) were taken starting at the time of oviposition of the second egg in the sequence (1100 to 1300 h) and every 4 h

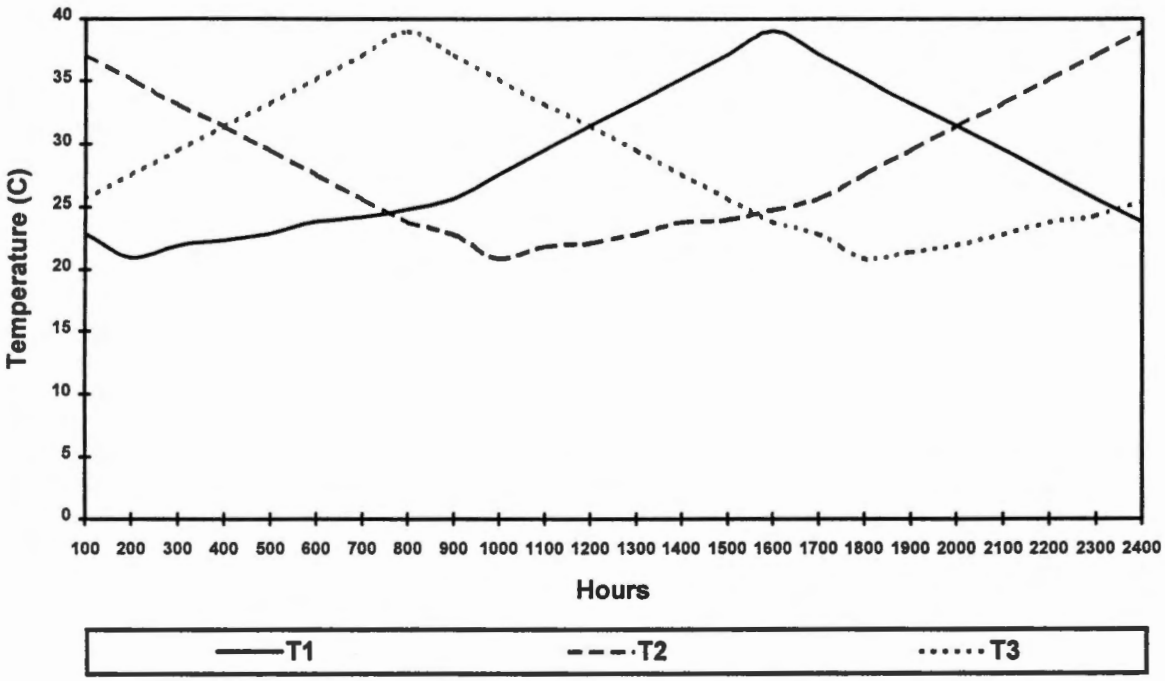

Figure 2. The daily temperature cycle (Experiment 2). Temperature peaked at 1600, 2400, and 0800 h in treatment 1 (T1), treatment 2 (T2) and treatment 3 (T3) respectively.

until the next oviposition (1500 to 1700 h). Hens selected for blood sampling were those that had a regular sequence of at least 3 eggs.

Prior to surgery, all equipment required was put into a 70% ETOH solution. The cannula was prepared from the items shown in Figure 3. A heparin solution (100 unit/ml) was prepared by diluting a stock solution of 1000 unit/ml into physiological saline solution. Penicillin G Sodium (5 million units) was diluted in 23 ml of sterilized distilled water.

To surgically insert cannulae, a hen was placed on its left side on a surgical table and secured. The incision area was cleaned with 70% ethyl alcohol (70% isopropyl alcohol) and feathers around this area were plucked. A small incision was made through the skin, mid-way along the humerus, over the brachial vein. A 0.5% procaine hydrochloride solution was applied as a localized anesthetic. The incision was enlarged to approximately 2 to 2.5 cm long. The connective tissue surrounding the vein was removed using a blunt-tipped forceps. The vein was carefully cleaned. Sutures were placed around the vein. A laterally placed suture was stretched gently to stop the blood flow. Then, using sharp scissors, a cut was made half-way through the vein .5 cm from the stretched suture and the beveled end of the cannula tubing (Figure 3) was gently inserted into the vein for a distance of 6 to 8 cm. Once the cannula was in place, the lateral suture was tied to completely stop the blood flow. The cannula was then tested

1. 70% ETOH
2. Heparin
3. Sterilized distilled water
4. Rubber straps
5. 0.05% procaine.HCl
6. Sutures
7. Blunt-end needles
8. Furox
9. Blunt-tipped forceps
10. Microdissection scissors

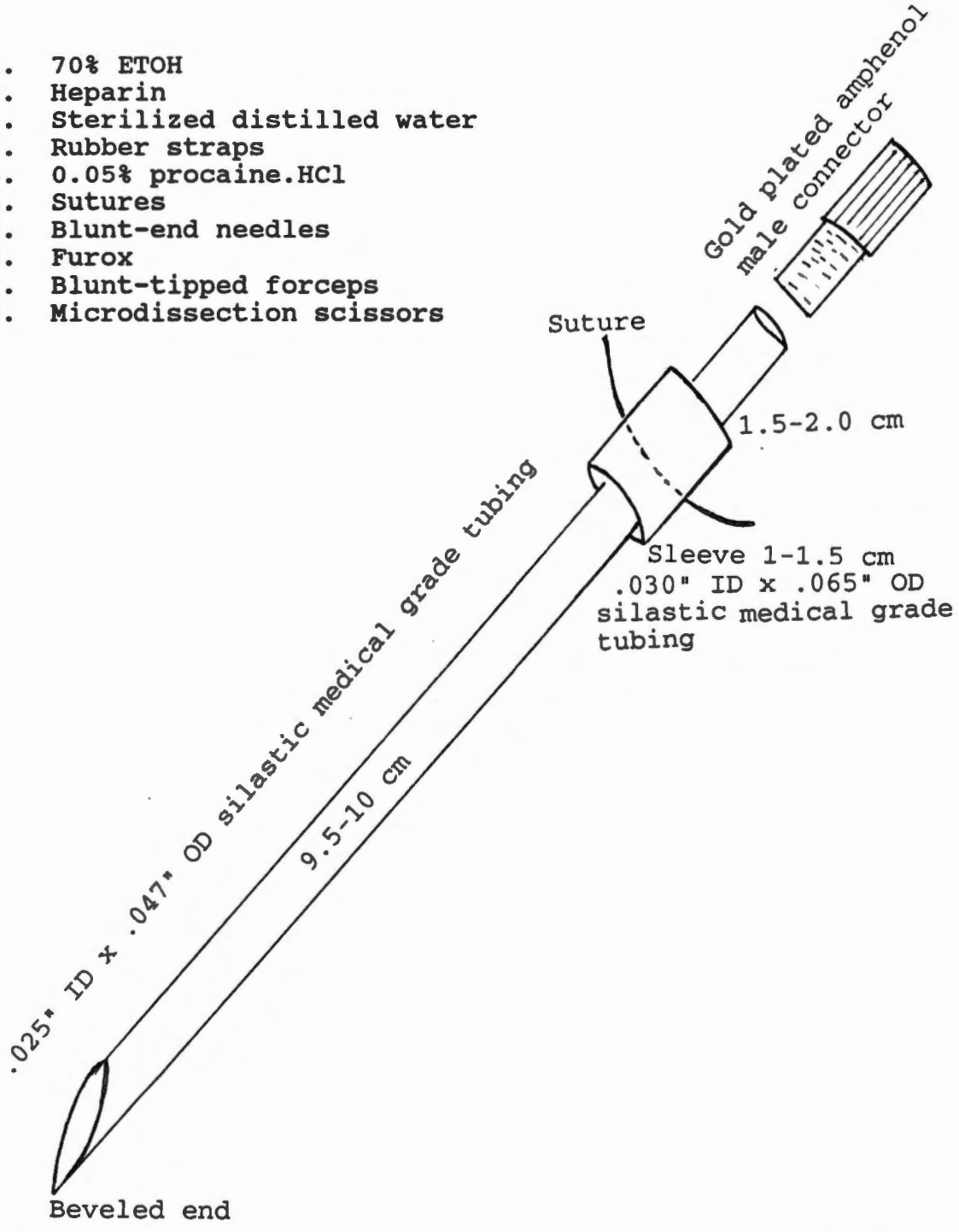

Figure 3. Drawing of equipment used for brachial wing vein cannulation.

for blood flow by using a syringe supplied with blunt-end 23 gauge needle. The medial suture was tied carefully around the vein and the cannula. Next, the hen was given 50000 units (2/10 cc.) of penicillin via the cannula. The cannula then was filled with heparin and closed completely with a gold-plated amphenol male connector. The sleeve was stitched to the skin and the incision was sutured. Furox was applied to the incision area, and the gold plug-end of the cannula (the part outside the body) was firmly taped to the bottom of the wing.

All hens were checked at 2000 h to insure that the cannulae were intact and to replace the old tape. Cannulated hens were also palpated to verify the presence or absence of an egg in the oviduct. On the day of blood sampling, the hen was removed from the cage, placed on the right side on a surgery board and secured using rubber straps. The tape was removed and the gold plug was replaced by blunt-end needle which was attached to a syringe. Then the heparin which was already in the cannula was drawn out along with a very small amount of blood. The syringe was replaced by a new one and 1 ml blood sample was collected. The blood sample was put into a heparinized tube that was closed immediately and immersed in ice. At the same time, another syringe which was filled with heparin was attached to the blunt-end needle. The cannula was filled with heparin. The amount required to fill the cannula was determined previously before the cannula was planted.

Then the incision area was sprayed with furox, and the part of the cannula outside the incision was taped firmly. This procedure was repeated 8 times during the 24 h sampling period. Blood samples were assayed for ionized calcium within 4 h using an ion selective electrode. Data for egg production, egg weight, specific gravity and shell thickness were examined using the GLM procedures of SAS (SAS Institute, 1985). Blood Ca^{++} and pH data were examined using repeated measure analysis of variance of SAS.

RESULTS

Experiment 1

Effects of cyclic temperatures and feeding time on egg production and other performance variables of broiler breeder hens are presented in Table 2. Hens housed under high *vs*. low temperature consumed more ($P<.05$) water and produced lighter ($P<.05$) eggs that had lower ($P<.05$) specific gravity and thinner shells. When compared to hens fed once per day in either the morning or evening, hens fed their feed allowance in two meals produced fewer ($P<.05$) eggs and had shorter op-op intervals only when housed under the high temperature. Both high temperature and meal feeding were observed to reduce feed intake but the effects were not additive. There were no significant ($P>.05$) effects of feeding time nor temperature on blood ionized calcium concentration or pH (Table 3), however, blood pH was higher in the afternoon.

Time of oviposition for hens exposed to low temperature was delayed ($P=.08$) due to feeding at 1800 h (Figures 4 and 5); however, there was a non-significant change ($P>.05$) in time of oviposition due to the feeding time for hens maintained at high temperature. No significant differences ($P>.05$) in time of oviposition of first-of-sequence eggs were observed (Figures 6 and 7). However, hens at LO and fed at 0700 h laid 90% of these eggs before 1200 h compared to 76% for hens fed at 1800 h. A similar trend was also observed for

TABLE 2. Performance characteristics of broiler breeder hens fed at 0700h, 1800h, and twice daily and maintained under low (LO) or high (HI) temperature

Characteristics	Temperature	Time of feeding		
		0700h	Split diet[1]	1800h
Eggs per hen[2]	LO	21.1±.69[a]	22.0±.76[a]	21.2±.67[a]
	HI	22.0±.69[a]	18.0±.69[b]	20.0±.67[a]
Egg weight, g	LO	69.9±.69[a]	71.8±.91[a]	71.6±.91[a]
	HI	65.6±.91[b]	66.3±.91[b]	65.7±.91[b]
Egg specific gravity	LO	1.085±.0009[a]	1.0854±.0009[a]	1.0863±.0009[a]
	HI	1.0808±.0009[b]	1.0788±.0009[b]	1.0824±.0009[b]
Egg shell thickness, mm	LO	0.3542±.006[a]	0.3598±.006[a]	0.3681±.006[a]
	HI	0.3293±.006[b]	0.3265±.006[b]	0.3390±.006[b]
Op-op interval[3], h	LO	26.5±.27[ab]	26.1±.27[ab]	26.7±.27[a]
	HI	26.2±.27[ab]	25.9±.27[b]	26.3±.27[ab]
Body weight change, g	LO	101.1±39.9[a]	102.0±37.9[a]	90.0±37.9[a]
	HI	-37.9±38.9[b]	-8.8±37.9[ab]	19.5±37.9[ab]
Feed consumption, g/d	LO	170.9±4.6[a]	154.8±4.6[b]	164.4±4.6[a]
	HI	151.7±4.7[b]	148.3±4.7[b]	142.6±4.6[b]
Water consumption, ml/d	LO	385.7±37[b]	329.8±37[b]	318.7±37[b]
	HI	756.0±37[a]	652.3±37[a]	660.8±37[a]

[ab]Means + SEM with no common superscript within a variable differ signifcantly ($P<0.05$). n = 20 hens per temperature-feeding time combination.
[1]Birds were fed half their daily allowance at 0700 h and the other half at 1800 h.
[2]Number of eggs produced/hen/28-d period.
[3]Based on data collected on 3-d period every wk.

TABLE 3. Effects of feeding time and cyclic temperature on pH[1] and blood ionized calcium[1] in broiler breeder hens[2]

Variable	Sampling time (h)	Temp.	Time of feeding: 0700h	Split-diet	1800h
Blood Ca (mEq/L)	1100	LO	2.84±.14	2.76±.14	2.80±.14
		HI	2.76±.14	2.66±.15	2.65±.14
	1600	LO	2.76±.13	2.60±.15	2.45±.15
		HI	2.68±.13	2.71±.17	2.51±.13
Blood pH	1100	LO	7.28±.04^{b}	7.29±.03^{b}	7.28±.03^{b}
		HI	7.30±.03^{b}	7.30±.03^{b}	7.39±.03^{a}
	1600	LO	7.29±.04^{b}	7.35±.04^{ab}	7.34±.04^{ab}
		HI	7.37±.04^{ab}	7.34±.04^{ab}	7.42±.04^{a}

^{ab}Means ± SEM with no common superscript within a variable differ significantly (P<.05).
[1]Means of 5 observations + SEM/sampling time.
[2]Blood samples were obtained from the same bird at 1100 and 1600 h.

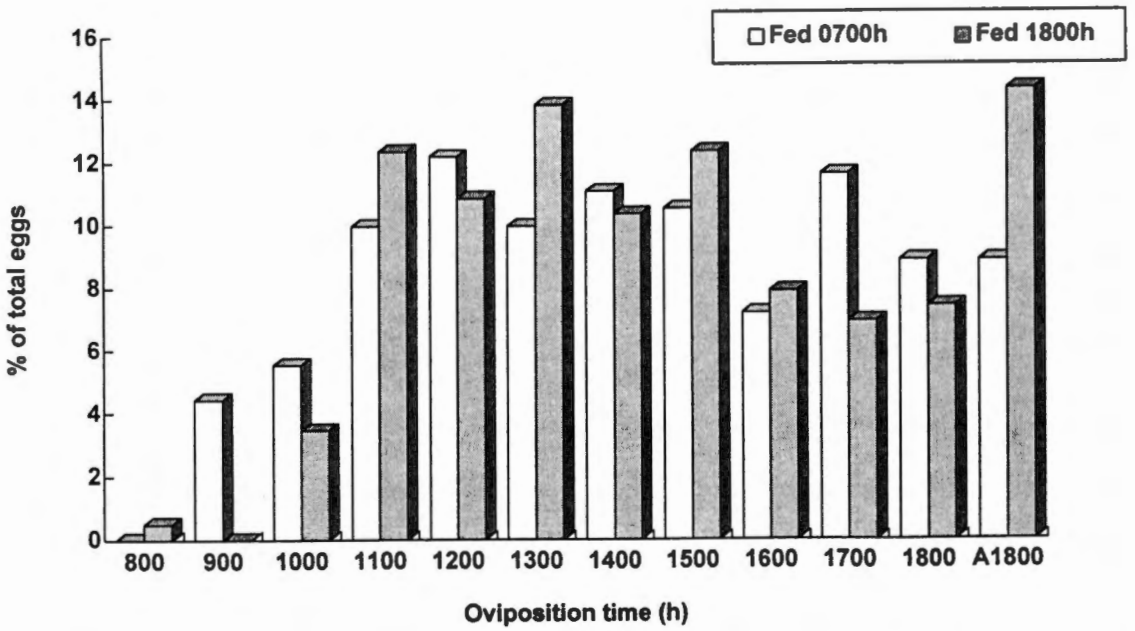

Figure 4. Distribution of oviposition times for broiler breeder hens maintained under low cyclic temperatures and fed at 0700 (n=181 eggs) and 1800 h (n=203 eggs).

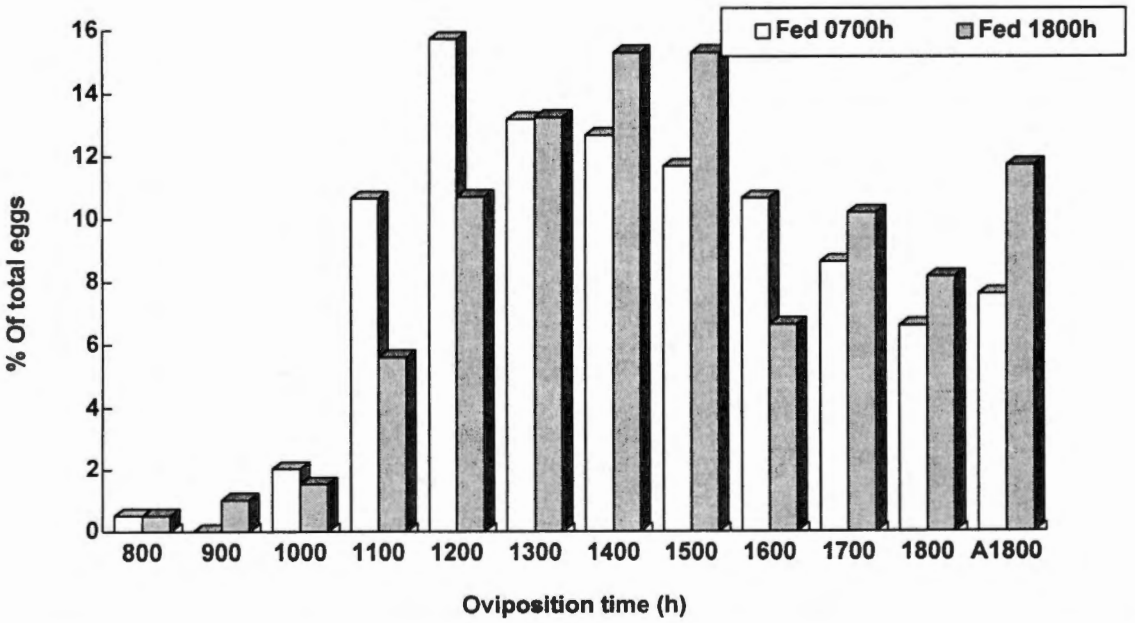

Figure 5. Distribution of oviposition times for broiler breeder hens maintained under high cyclic temperatures and fed at 0700 (n=197 eggs) and 1800 h (n=196 eggs).

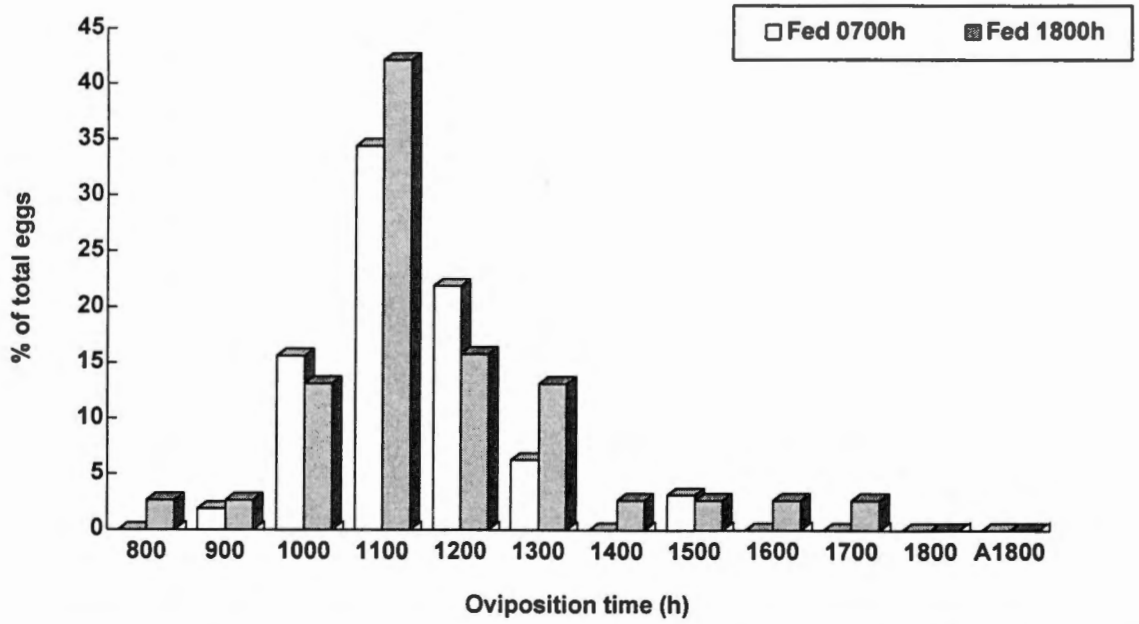

Figure 6. Distribution of oviposition times of first-of-sequence eggs in broiler breeder hens maintained under low cyclic temperatures and fed at 0700 (n=32 eggs) and 1800 h (n=38 eggs).

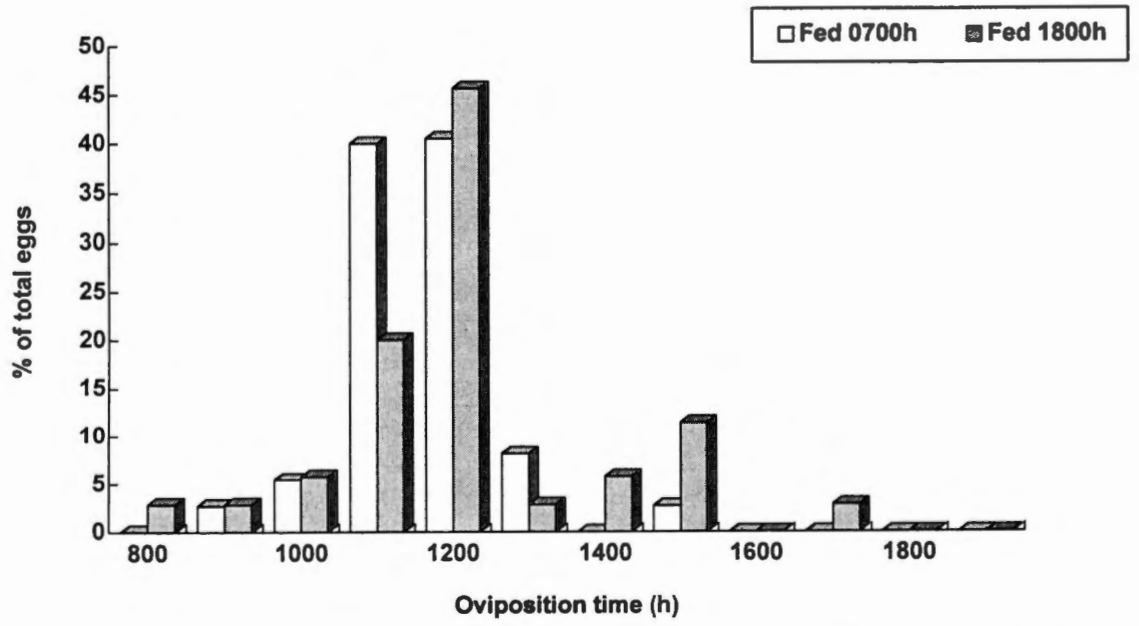

Figure 7. Distribution of oviposition times of first-of-sequence eggs in broiler breeder hens maintained under high cyclic temperatures and fed at 0700 (n=37 eggs) and 1800 h (n=35 eggs).

hens maintained at HI (86% *versus* 77%).

Daily feed and water consumption are given in Table 2. Individual feed intake was based on two-day observation period, whereas water consumption was based on actual daily measurements. Exposure to the high cyclic temperature resulted in a significant ($P<.05$) reduction in feed intake of all the feeding groups. At low cyclic temperature, hens fed the split diet ate significantly less ($P<.05$) feed than hens fed at 0700 h or those fed at 1800 h. At high cyclic temperature, water consumption increased ($P<.05$) in all the feeding groups.

The results of the trial concerning feed consumption revealed different patterns in relation to cyclic temperatures (Figures 8 to 10). Parameters of the model, ($Y= A(1-e^{-Bt})$, for each feeding group were estimated. Hens in the different feeding groups were further divided into subgroups based on whether or not an egg was laid on the day the measurements were taken. The coeffecient of determination (R^2) was calculated in order to assess the reliability of the fit between the estimated curve and the data. The results indicated: 1) high cyclic temperature resulted in significantly slower ($P<.05$) rate of feed consumption by all hens in the different feeding groups regardless of whether the hen laid or did not lay an egg (an exception was the hens fed the split-diet whose rate of feed consumption was not significantly different ($P>.05$) from that of their

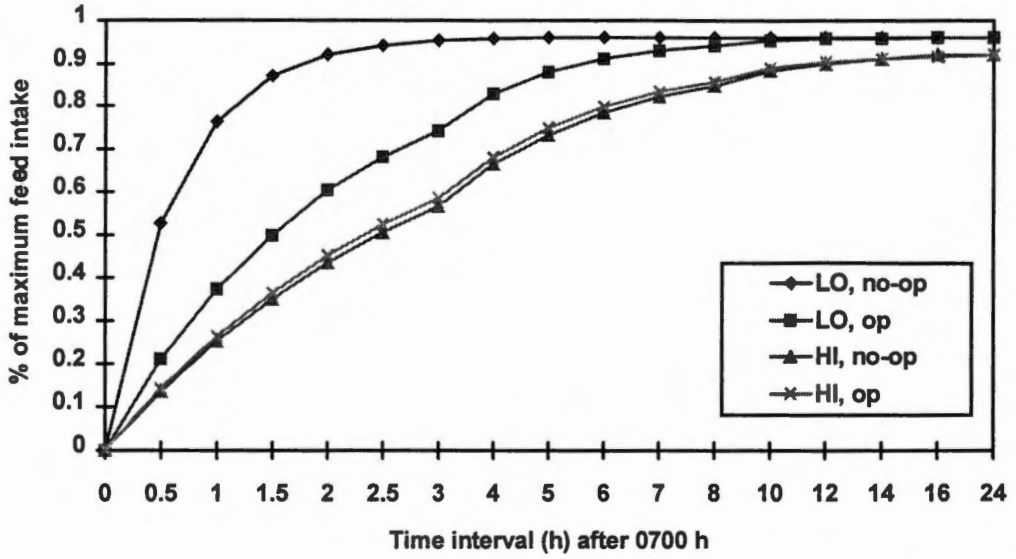

Figure 8. Effect of temperature and laying status of broiler breeder hens (fed at 0700 h) on rate of feed consumption (expressed as percentage of mean daily feed in take). LO and HI indicate low and high cyclic temperatures, respectively. No-op indicates a non-oviposition day and op indicates an oviposition day. Parameters (A and B) of the equation ($Y=A(1-e^{-BT})$) for hens at LO are: .96 and 1.59 (no-op) and .96 and .496 (op). At HI, A and B are: .92 and .32 (no-op) and .92 and .338 (op). Number of hens observed was: LO, no-op 1; LO, op 19; HI, no-op 6; and HI, op 14.

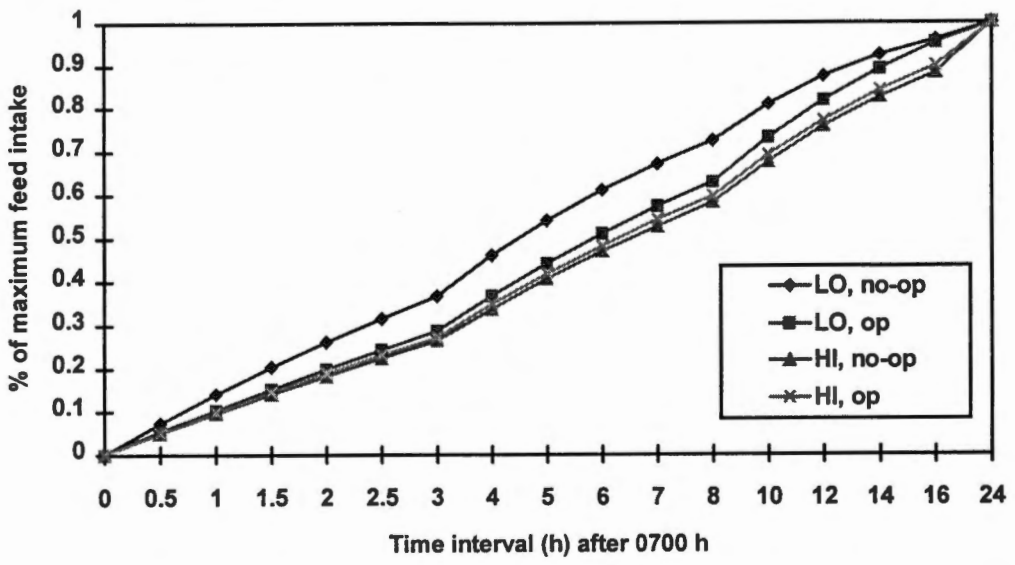

Figure 9. Effect of temperature and laying status of broiler breeder hens (fed at 0700 and 1800 h) on rate of feed consumption (expressed as percentage of mean daily feed intake). LO and HI indicate low and high cyclic temperatures, respectively. No-op indicates a non-oviposition day and op indicates an oviposition day. Parameters (A and B) of the equation ($Y=A\ (1-e^{-BT})$) for hens at LO are: 1.08 and .139 (no-op) and 1.29 and .084 (op). At HI, A and B are: 1.21 and .082 (no-op) and .085 (op). Number of hens observed was: LO, no-op 6; LO, op 14; HI, no-op 13; and HI, op 6.

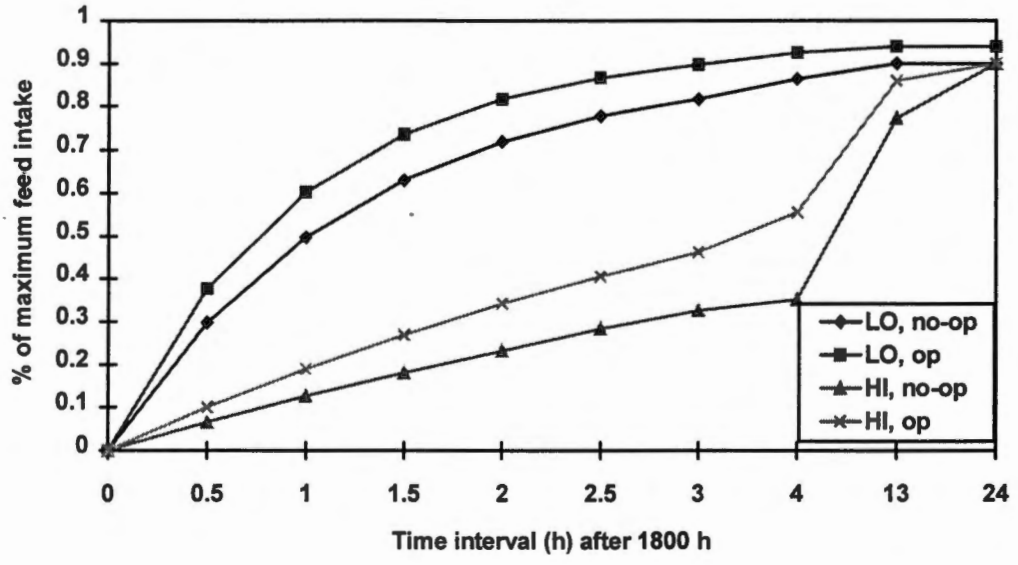

Figure 10. Effect of temperature and laying status of broiler breeder hens (fed at 1800 h) on rate of feed consumption (expressed as percentage of mean daily feed intake). LO and HI indicate low and high cyclic temperatures, respectively. No-op indicates a no-oviposition day and op indicates an oviposition day. Parameters (A and B) of the equation ($Y=A(1-e^{-BT})$) for hens at LO are: .9 and .8 (no-op) and .94 and 1.02 (op). At HI, A and B are: .9 and .15 (no-op) and .9 and .24 (op). Number of hens observed was: LO, no-op 1; LO, op 19; HI, no-op 7; and HI, op 13.

counterparts maintained at low temperature); 2) by 5 h after feed was served, hens at low temperature consumed more of their feed allowance than at high temperature; 3) at LO, hens that did not lay an egg and were fed at 0700 h and those fed the split-diet tended to eat more and had higher rates of feed consumption than hens that laid an egg. By contrast, hens fed at 1800 h that had laid an egg ate more feed more quickly than hens that did not lay an egg the same day; 4) at HI, rates of feed consumption of hens that did not lay an egg and of those that laid an egg did not differ (P>.05) regardless of the hen's feeding group.

Experiment 2

Effects of the three temperature treatments on egg production, egg weight, specific gravity and shell thickness are presented in Table 4. Number of eggs produced was highest for hens subjected to high temperature that peaked at 1600 h and lowest for hens at temperature that peaked at 2400 h. Eggs from hens exposed to high temperature that peaked at 0800 h were significantly lighter than those of hens at temperature that peaked at 1600 h but they were not lighter than those of hens at temperature that peaked at 2400 h. Specific gravity of eggs from hens exposed to high temperature that peaked at either 2400 or 0800 h was significantly reduced compared to those from hens at temperature that peaked at 1600 h. There were no differences in egg shell thickness among the three

TABLE 4. Egg production, egg weight, egg specific gravity and shell thickness of broiler breeder hens exposed to different daily periods of high temperature[1]

Treatment[1]	Eggs per hen[2]	Egg weight (g)	Egg specific gravity	Egg shell thickness (mm)
T1	4.88[a]	65.97[a]	1.0791[a]	.3269[a]
T2	3.30[c]	65.06[ab]	1.0754[b]	.3217[a]
T3	3.92[b]	63.58[b]	1.0757[b]	.3208[a]

[abc]Means within a column with no common superscript differ significantly (P<.05). n = 60 birds per temperature treatment.

[1]High temperature peaked at 1600 h (T1), 2400 h (T2), and 0800 h (T3).

[2]Based on number of eggs produced per hen per week.

treatments.

There were no significant treatment effects on blood ionized calcium concentrations (Figure 11) or blood pH (Figure 12). Values for blood pH of hens maintained under HI that peaked at 2400 h were lower than those of hens maintained at HI that peaked at either 1600 h or 0800 h.

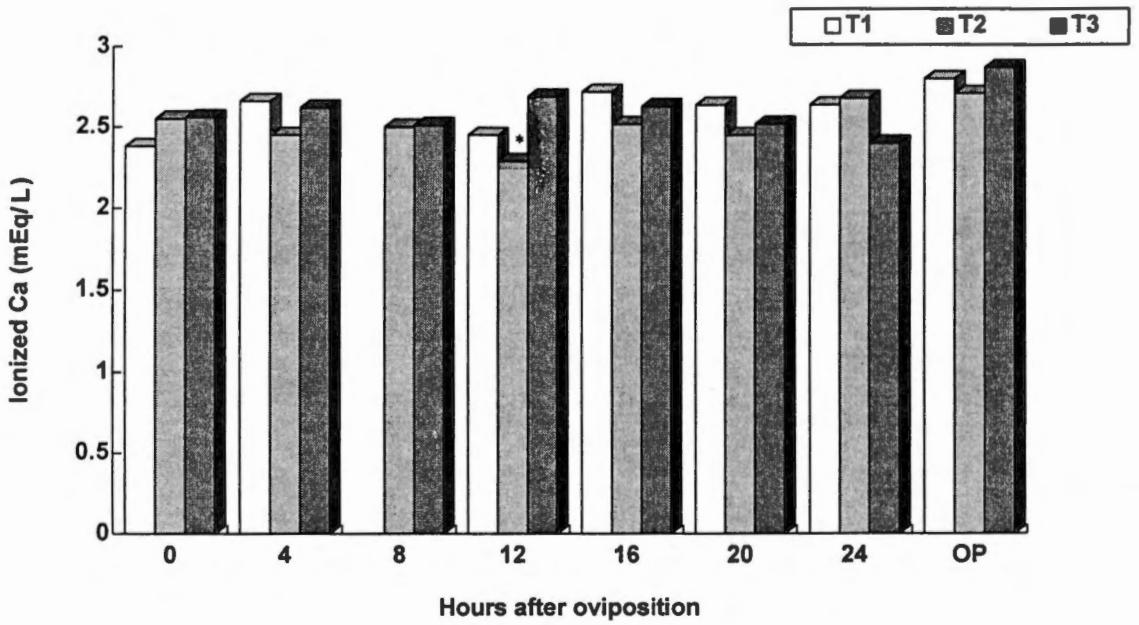

Figure 11. Concentrations of ionized calcium in blood of broiler breeder hens exposed to high temperatures that peaked at 1600 h (T1), 2400 h (T2), or 0800 h (T3). T1, SEM= .1 and n= 4; T2, SEM= .12 and n= 3; and T3, SEM= .15 and n= 2. [x,y]Means within the same T with no common letter are significantly different ($P< 0.05$). Asterisks denotes significant difference between treatment ($P< 0.05$) at the same sampling period.

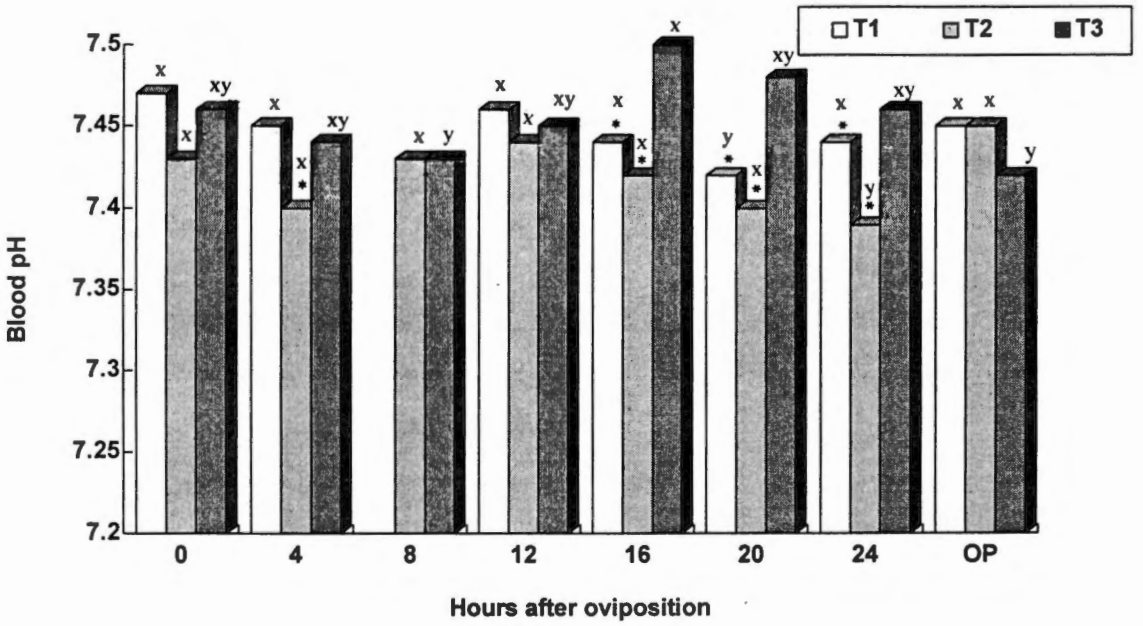

Figure 12. Blood pH during the egg cycle of broiler breeder hens exposed to high temperatures that peaked at 1600 h (T1), 2400 h (T2), or 0800 h (T3). T1, SEM= .016 and n=4; T2, SEM= .018 and n=3; and T3, SEM= .02 and n=2. ^{x,y}Means within the same T with no common letter are significantly different (P<.05). Asterisks denotes significant difference (P<.05) between treatments at the same sampling period.

DISCUSSION

Experiment 1

We did not observe a significant (P>.05) temperature treatment effect on number of eggs produced. These results are consistent with those of Miller and Sunde (1975), Deaton et al. (1981, 1982) and Emery et al. (1984). These authors showed some beneficial effects of cyclic temperature. However, our results contradict those of De Andrade et al. (1977) who reported that high cyclic temperature (26.7 to 35.6 C) reduced the rate of egg production. We did observe that egg weight, specific gravity and shell thickness were reduced in hens exposed to HI. Similar effects of high temperatures on egg shell quality have been previously reported (Smith and Oliver, 1972a, b; Wilson, 1972; Miller and Sunde, 1975; De Andrade et al., 1977; Emery et al., 1984).

The effect of time of feeding on egg production and egg shell quality are not clear. Bootwalla et al. (1983) unintentionally noticed the that response of broiler breeders fed a single meal at 1600 h or fed *ad libitum* was seasonally dependent. *Ad libitum* fed hens produced eggs with lower specific gravity (1.074) during the summer months, but not at moderate temperatures, compared to that of hens fed a restricted amount of feed at 0800 or 1600 h. The authors attributed this to the seasonal variation in the environmental temperatures.

We observed that the egg production of broiler breeder hens maintained under LO was in some aspects different from the response of hens maintained under HI. Hens fed twice daily produced fewer eggs at LO. These results are contrary to those reported by Balnave (1977) and Cave (1981) who reported that increasing the frequency of feeding from once daily to twice or three time daily increased hen-day egg production. Number of eggs produced by hens fed at 1800 h was not significantly different ($P>.05$) from that of hens fed at 0700 h regardless of the temperature. These results confirm those of Bootwalla et al. (1983) and Wilson and Keeling (1991). Harms (1991) on the other hand observed a decline in rate of egg production when the feeding time was changed from 0800 to 1600 h. Harms (1991) provided partial explanation for these different results; he suggested that the changing the feeding time on a gradual basis as used by Bootwalla et al. (1983) may have been a factor. Since there were similarities between the study of Wilson and Keeling (1991) and that of Harms (1991), the above explanation does not account for these different results.

Egg weight was not significantly ($P>.05$) affected by feeding time. These results are contrary to previous reports (Cave, 1981; Daniel and Balnave, 1981; Farmer et al., 1983c) that meal feeding or feeding in the afternoon increased egg weight. However, these results confirm those of Harms (1991) and Wilson and Keeling (1991). These different results could

be related to age and strain of birds, environment, and quantity of feed and time of feeding relative to light-dark cycle used these experiments. For example, Daniel and Balnnave (1981), using egg-type hens, restricted the length of time for which food was available rather than restricting the amount of feed allowance as reported in other studies. Egg specific gravity and shell thickness were not significantly ($P>.05$) affected by feeding time regardless of the temperature treatment.

Harms (1991) reported that, under thermoneutral conditions, feeding broiler breeder hens at 1600 h resulted in a significant ($P<.05$) body weight loss in one of two experiments, even though the hens that lost weight ate more and laid fewer eggs compared to the hens fed at 0800 h. By contrast, the results of the present study indicate that the hens at LO gained weight regardless of the feeding time. This disagreement could be due to the fact that the hens in the study conducted by Harms (1991) were 52 wk of age, whereas 39 wk old hens were used in the present study. When compared to hens at low temperature, hens fed at 0700 h experienced significant ($P<.05$) body weight loss (37.9 g) when housed under the high temperature. The data on the body weight loss due to high cyclic temperature agree with those reported by Campos et al. (1960) who found that at 38 C, *ad libitum* fed hens lost from 10.9 to 17 g per kg BW over a period of sixteen days. De Andrade et al. (1977) observed body weight loss for

egg-type laying hens exposed to a cyclic temperature of 26.7-35.6 C. These authors reported that this weight loss took place shortly after the hens were exposed to the high environmental temperatures.

Hens exposed to the high cyclic temperature consumed significantly ($P<.05$) more water compared to hens maintained under low cyclic temperature regardless of feeding time. Also hens fed at 0700 h consumed 100 ml/hen more water than hens fed at 1800 h, suggesting that feed consumption before peak temperature enhanced additional water intake.

The time interval between successive ovipositions was not significantly ($P>.05$) affected by feeding time and/or cyclic temperature. An exception was the shorter time interval between successive ovipositions for hens at LO and fed the split-diet. With regard to hens at HI, these results are not in agreement with those reported by Nordstrom (1973) who observed that under a constant temperature of 32 C the developing egg spends 1-2 extra hours in the shell gland making the time interval between successive eggs 27.7 h compared to 25.5 h for hens (18-mo old) at 21 C. Age of the birds have been shown to affect the hen's response to high temperatures (Arima, 197.). Therefore, it likely that differences in results are due to the use of high constant temperature and 18-mo old hens in the study conducted by Nordstrom (1973). Since feeding late in the day (Daniel and Balnave, 1981; Harms, 1991; Wilson and Keeling, 1991) and high

cyclic temperature (Miller and Sunde, 1975) have been observed to cause a delay in time of oviposition, it was anticipated that their effect will be additive. Both cyclic temperature and meal feeding were observed to cause a delay in time of oviposition but the effects were not additive.

Under thermoneutral conditions, broiler breeder hens consume their daily feed allowance in approximately 3-4 h (Farmer et al., 1983b). The results of the present experiment revealed different patterns for the changes in feed consumption due to the high cyclic temperature. At LO, resting hens fed at 0700 h or those fed the split-diet ate more and faster than hens that had laid an egg on that day (173.5 *versus* 170.7 g and 162.2 *versus* 151.7 g/hen/d, respectively). This is consistent with the work of Roland et al. (1972) who reported that more feed was consumed by layers on non-oviposition days than on oviposition days. The opposite was true for hens fed at 1800 h (164.4 *versus* 165.1 g/hen/d). These results indicate that oviposition played a significant role in regulating feed consumption since hens fed at 1800 h and had laid eggs earlier in the day, ate more and faster than those that did not lay eggs on that day.

At high cyclic temperature, resting hens fed at 0700 or 1800 h ate more but not faster than hens that had laid eggs on that day (152.6 *versus* 151.4 and 145.6 *versus* 140.9 g/hen/d). The opposite was true for the hens fed the split-diet (141.6 *versus* 152.2 g/hen/d). Therefore, it seems that oviposition

has a less pronounced role in regulating feed consumption in hens maintained at HI. Hens fed at 1800 h consumed less feed and ate faster than hens fed at 0700 h even though these hens gained weight and produced eggs with better shell quality. Because the process of oviposition and increasing temperatures have been shown to increase body temperature and reduce feed intake (Wilson et al., 1989), it seems that feeding at 0700 h, when the temperature was increasing, is more stressful compared to feeding at 1800 h when the temperature was declining. In addition, the difference in patterns of feed consumption of hens maintained at HI from that of hens maintained at LO may affect their response to the feeding time.

Experiment 2

Many researchers have reported that heat stress depresses egg shell quality (Warren and Schnepel, 1940; Harrison and Biellier, 1969; Wolfenson et al., 1979); however, it is not yet clear how heat stress interferes with egg shell formation. We examined the phenomenon by varying the time of day and thus the point in the egg formation process at which peak temperature occurred. When temperature peaked at 2400 h, egg production was reduced. Egg production partially recovered when temperature peaked at 0800 h and recovered further when temperature peaked at 1600 h. Wolfenson et al. (1979) speculated that the reduction in egg production following

night exposure of hens to high temperature may be caused by the direct effect of heat stress on LH release. It could be argued that the effect on LH release is mediated through blood calcium concentrations, since calcium has been reported to influence LH secretion (Taylor, 1972; Luck and Scanes, 1979). There are several arguments in favor of this assumption. First, peak temperature at 2400 h coincides with maximum calcium utilization for shell formation. Thus, a state of calcium stress may occur. However, peak temperature at 0800 h coincides with advanced stages of shell calcification and with feed consumption. Thus, the effect of heat stress would not be as severe and hence the less severe effect on egg production. Exposure (at 1600 h) of hens to the same temperature but at 1600 h did not cause a reduction in the rate of egg laying. In this state, peak temperature occurred when no calcium was being used for shell calcification.

Campos et al. (1960) reported that overnight exposure of hens to a temperature of approximately 37 C caused the production of eggs with greater Haugh unit values. Smith and Oliver (1972a, b) reported that hens exposed to cyclic temperature that peaked at night produced heavier eggs compared to hens exposed to peak temperature during the day. Similar results have been reported by Wolfenson et al. (1979). In the present experiment, 0800 h peak temperature resulted in a significant ($P<.05$) reduction in egg weight compared to 1600 or 2400 h peak temperature. This may be related to pattern of

feed consumption. Peak temperature at 0800 coincides with time of maximum feed consumption. Under these conditions, albumen secretion may become more dependent on body stores since feed intake is reduced. It seems likely that egg weight becomes more sensitive to heat stress when the high temperature precedes albumin secretion while it is not severely affected if the high temperature occurs after or before the food is eaten.

It has been reported that high environmental temperatures cause an immediate reduction in egg shell weight (Harrison and Biellier, 1969; Miller and Sunde, 1975). Wolfenson et al. (1979) suggested that early stages of shell formation are more sensitive to the heat stress than later stages. Emery et al. (1984) showed that high temperature peaks at 0200 h caused more damage to shell thickness than the high temperature peaks during the day. In the present experiment, the cyclic temperatures that peaked at either 0800 h or at 2400 h resulted in a significant ($P<.05$) reduction in egg specific gravity compared to cyclic temperature that peaked at 1600 h; however, Shell thickness was not different among temperature treatments. These results support the conclusion that high temperatures are most likely to adversely affect egg shell quality if imposed during shell calcification.

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PART III

EFFECTS OF TEMPERATURE AND FEEDING TIME ON EGG PRODUCTION AND BIOCHEMICAL VARIABLES OF EGG SHELL QUALITY IN BROILER BREEDER HENS

INTRODUCTION

High environmental temperatures reduce laying hen feed consumption and impair egg shell quality (Mueller, 1959; Payne, 1966a; De Andrade, 1977). Reduced feed intake does not appear to be the sole reason for the decline in egg shell quality (Sunde, 1971). Smith (1974) suggested that the heat-induced reduction in egg shell quality may be due primarily to the rise in the blood pH resulting from respiratory alkalosis. Using laying hens exposed to either low or high cyclic temperature, De Andrade et al. (1977) observed a diurnal rhythm in pCO_2, with highest values occurring at midnight coincident with lowest environmental temperature.

Activity associated with feeding, presence of feed in the digestive tract, nutrient metabolism, oviposition and high temperatures are the major factors that increase metabolic heat production in laying hens (Cain and Wilson, 1971; Ahmad et al., 1976; Wilson et al., 1989; Li et al., 1992). It is, therefore, to the bird's best physiological advantage that feed consumption does not coincide with the period of highest temperature, especially during hot weather. Therefore, hens should be fed either in the morning or in the evening when temperatures are low. Broiler breeders are usually fed a limited amount of feed once in the morning of each day. However, under thermoneutarl conditions, evening feeding has been shown to improve egg shell quality compared to morning

feeding. The justification for this is that broiler breeder hens fed in the afternoon have more dietary calcium available during the stage of egg calcification (Roland et al., 1973; Lennards and Roland, 1981). However, the effects of changing feeding time (from early morning to late afternoon) have been studied by a number of researchers, and the results are generally inconsistent. Balnave (1977) and Bootwalla et al. (1983) found that evening feeding resulted in a higher rate of egg production. Wilson and Keeling (1991) found that evening feeding did not affect egg production. In contrast, Brake and Peebles (1988) and Harms (1991) observed a decline in egg production when feeding time occurred at 1300 or 1600 h. It is not clear why egg production decreases, as reported by some studies, in evening fed hens as compared to hens fed in the morning. Age and strain of birds, housing (cage versus litter floor, feed management (gradual versus abrupt change of feeding time) have each been implicated. Nevertheless, some of the above studies did report that egg shell quality was improved due to evening feeding which could prove beneficial under high temperatures. High temperatures have also been reported to cause a reduction in concentrations of total plasma calcium (De Andrade et al., 1977; Wolfenson et al., 1979) and blood ionized calcium (Odom et al., 1986).

It is well known that estrogens and androgen play a significant role in calcium metabolism in the laying hen (Hurwitz, 1978; Eastin and Spaziani, 1978). However, little

information is available in the effects of high temperatures on the concentrations of these hormones during the egg cycle of the laying hen. Nasser et al. (1992) measured (weekly and every 6 h periodically during the temperature cycle) estrogen concentration in plasma of broiler breeder hens exposed to cyclic temperatures of 22-40 C and 22-42 C or 19-25 C from 35-44 wk of age. They reported that estradiol increased at high temperatures and peaked at 0700 h, then declined, and was lowest at 0100 h the next day.

The objective of this study was to determine the effect of environmental temperature and feeding time on various production parameters of broiler breeder hens. A second objective was to determine the effect of high environmental temperatures on pH and blood ionized calcium and plasma concentrations of calcium, androgen, and estrogen during the egg cycle of broiler breeder hens.

MATERIALS AND METHODS

Four experiments were conducted with 38-wk-old Indian River broiler breeder. The hens had received 178 g/hen-d of feed (Table 1, part II) at 0700 h. Prior to the experiment, the hens were subjected to 17 h light (0700 to 2400 h) and 7 h darkness. Two hundred hens were placed in individual laying cages in two environmentally controlled chambers. For 3 wk (adjustment and pretreatment periods) the hens continued to receive the same daily feed allowance at 0700 h and artificial light from 0700 to 2400 h. During this period, the temperature in both houses was maintained at 25 C. Hens had access to water at all times. Egg production was recorded daily, and egg weight, specific gravity and shell thickness were recorded for all eggs produced during the last 2 days of every week. Measurements of these criteria were carried out as in part II. Sequence length for each hen was also established. During the last 7 days of this period, time of oviposition was recorded hourly from 0700 to 1800 h. Eggs collected before 0700 h were treated as if they were laid after 1800 h (A1800).

Experiment 1

Sixty of the 100 hens in each house were selected and divided into four groups of 15 hens each. Hens utilized in the experiment had: 1) laid at least 12 eggs in the 20-day

pretreatment period; and 2) laid at least 3 eggs or more in a sequence. The hens of group 1, in each chamber, continued to receive their daily feed allowance at 0700 h, whereas the hens in group 2 were switched to receive their daily feed allowance at 1800 h. The remainder of the 100 hens in each chamber were also divided into two feeding groups and were set aside for future use. Two temperature treatments were imposed: treatment 1 (LO), 10-25 C; and treatment 2 (HI), 21-39 C. The daily temperature cycle is illustrated in Figure 1, part I. Relative humidity was not controlled, but ranged from 20 to 50%. Egg production was recorded daily. Egg weight, specific gravity and shell thickness were obtained from all eggs produced during the last three days every week of the 6-wk experimental period. Time of oviposition was recorded hourly from 0700 to 1800 h for 5 consecutive days during the 4th week and for four days during the last week of the experiment. Data for egg production, egg weight, specific gravity, shell thickness, and the interval between successive ovipositions were subjected to the analysis of variance using the General Linear Models Procedure of SAS Institute (1985). Differences between means were assessed using the least square difference test. The Chi-square test was used to determine the effects of feeding time and temperature cycle on time of oviposition.

Experiments 2 and 3

These experiments started at the end of the 4th week of

experiment 1 and were completed within one week. Sixteen laying hens and five non-laying hens that had been receiving and continued to receive their feed allowance at 0700 h were selected from both environmental chambers to measure the changes in blood ionized calcium, blood pH, total plasma calcium, estrogens and androgens concentrations in relation to the egg cycle and the daily periods of high and low temperatures. The laying hens used were those: 1) laying at least three eggs in a sequence; 2) had a one-day pause between sequences. The non-laying hens used were those that had stopped laying for 20 days or more.

In experiment 2, on the day prior to blood sampling, all 50 hens in each environmental chamber were observed at 15 min intervals (started at 0700 h until oviposition). Eight hens that laid the first egg in a sequence between 0900 and 1000 h and met the above conditions were fitted with brachial vein cannula as described in Part I. In order to minimize stress to the hens, no attempt was made to digitally palpate the cannulated hens at any time after the cannulation procedure was completed. The following day, these hens were observed at 10 to 15 min intervals to verify that oviposition had occurred. The first blood sample was collected within minutes after oviposition and every 4 h thereafter until the next oviposition. Blood samples (2-ml each) were kept on ice and analyzed within four hours except those samples obtained around midnight which were analyzed 6 to 8 hours after

collection. Storage of blood samples on ice for up to 13 hours did not significantly affect calcium concentrations. Less than .5 ml blood was used to obtain measurements of ionized calcium and pH. The selection of the hens, the cannulation and blood sampling were repeated utilizing a total of eight laying hens from each environmental chamber.

In experiment 3, two and three non-laying hens were selected from hens maintained under LO and HI, respectively, and fitted with a cannula in the brachial vein as described in part I. The following day, the first blood sample (2 ml) was drawn between 1130 and 1300 h and every 4 hours thereafter for 26 h. The time 1130 to 1300 h was chosen because it coincided with the time of oviposition for the laying hens in experiment 2.

Blood ionized calcium for both experiments was measured using an ion selective electrode (Beckman, LABLYTE™ 820 Electrolyte Analyzer, Beckman Instruments, Inc., Brea, California, USA). Blood pH was also measured. Plasma was prepared from whole blood (approximately 1.5 ml) by centrifugation at 1000xg for 10 min and frozen until analyzed for estrogens, androgens and total plasma calcium. The atomic absorption technique was used to determine plasma calcium. Plasma concentration of estradiol (E_2) was determined as described by Britt (1990). Estradiol antibody was provided by Dr. Norman Mason, Lilly Research Laboratories, Indianapolis, IN. The sensitivity of the assay was .15 pg.tube^{-1}. All

samples were tested in a single assay. Intra-assay coeffecient of variation was less than 5%. Androstenedione was assayed as described by Jackson et al. (1989). Androstenedione antibody was provided by Dr. P.N. Roa (Southwest Foundation for Biomedical Research, San Antonio, TX). The sensitivity of the assay was 2.5 pg.tube^{-1}. Intra-assay coeffecient of variation was less than 5%.

Experiment 4

This experiment was conducted to determine whether heat stress and/or time of feed intake had a direct effect on: 1) time of ovulation; 2) the time that the egg spent in the oviduct. A total of 113 hens that had been used in the preceding experiments were killed by cervical dislocation at 1, 4, 6, 8 and 14 to 16 h after oviposition. Prior to killing of hens, oviposition was checked every 10 to 15 min (from 0700 to 1600 h). The timing was begun when the egg was laid. In all cases, only hens which laid the first, or the second egg in a sequence were killed. This procedure was used to ensure that ovulation of the following egg was likely to occur. With regard to hens killed 1 h after oviposition, ovulation and/or the position of the ova was verified. For hens killed at 4, 6, and 8 h after oviposition, the length of the magnum and isthmus was measured, and the distance travelled by the growing egg was recorded. The oviduct containing the growing egg was separated and placed on top of a plastic meter which

was attached to a stainless steel table. No attempt was made to forcefully stretch the oviduct. In all cases, the egg shell gland was rapidly removed and placed on ice for further use.

RESULTS

Experiment 1

The number of eggs produced per hen was not affected by feeding time or temperature (Table 1); however, high temperatures caused a significant ($P<.05$) reduction in egg specific gravity and a corresponding though nonsignificant ($P<.05$) reduction in shell thickness. Hens housed under high temperatures also tended to exhibit longer intervals between successive ovipositions.

Distribution of oviposition times were influenced by feeding time (Figures 1 and 2). Differences in time of oviposition for hens (in both temperature treatments) fed at 0700 h from those fed at 1800 h was significant ($P<.05$) (Figure 3). Hens fed at 0700 h laid 59.2% of their eggs before 1400 h versus 53% for hens fed at 1800 h. The distribution of oviposition times of the first-of-sequence eggs is presented in Figures 4 and 5. Overall, neither feeding time nor temperature cycle caused a significant ($P>.05$) modification in the distribution of oviposition times of the first-of-sequence eggs.

Experiments 2 and 3

Figure 6 illustrates the effects of temperature and relative stage of egg formation on blood ionized calcium (Ca^{++}). The pattern of changes in ionized calcium were

TABLE 1. The influence of time of feeding and cyclic temperatures on egg production, egg weight, egg specific gravity and shell thickness of broiler breeder hens.

	Feeding time			
	LO		HI	
Variable	0700h	1800h	0700h	1800h
Eggs per hen[1]	13.93±.42[b]	15.19±.44[a]	14.78±.37[ab]	15.35±.38[a]
Egg weight, g	68.09±.96[a]	68.83±.98[a]	64.88±.87[b]	65.19±.87[b]
Egg specific gravity	1.0838±.0009[a]	1.0837±.0009[a]	1.0793±.0008[b]	1.0801±.0009[b]
Egg shell thickness, mm	.3347±.005[ab]	.3471±.005[a]	.3239±.004[b]	.3349±.004[ab]
Op-op, h[2]	26.94±.32[a]	25.98±.33[b]	26.34±.28[ab]	26.60±.29[ab]

[ab]Means + SEM with no common superscript within a row differ signifcantly ($P<0.05$). n = 30 hens per temperature-feeding time combination.

[1]Based on number of eggs produced/hen/20 day period. Measurements of all other variables are absed on 3-day period/wk.

[2]Op-op (oviposition-oviposition) interval was based on 9-day period. Birds were watched every 15 min. from 0700 to 1800 h every day.

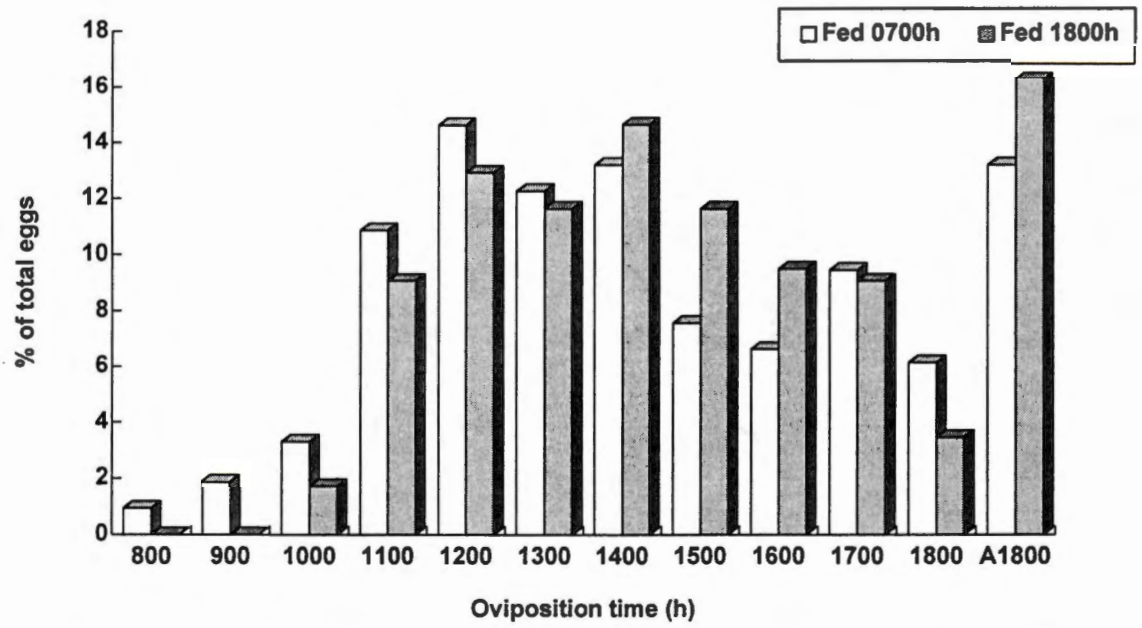

Figure 1. Frequency distribution (percentage) of oviposition times for broiler breeder hens maintained under low temperatures and fed at 0700 (n=212 eggs) and 1800 h (n=232 eggs).

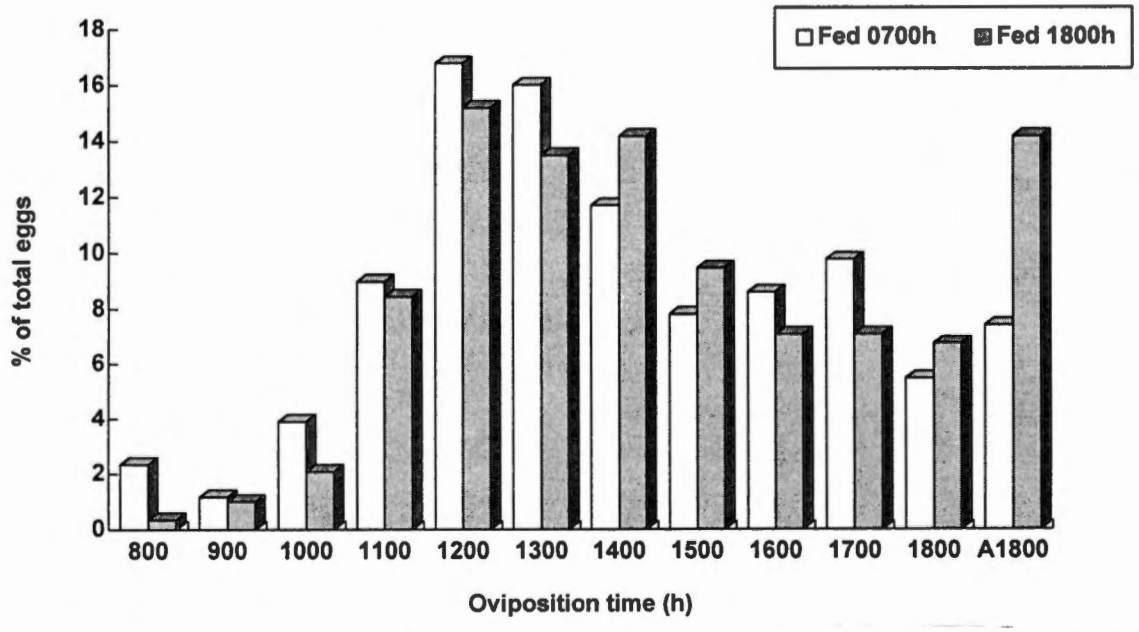

Figure 2. Frequency distribution (percentage) of oviposition times for broiler breeder hens maintained under high temperatures and fed at 0700 (n=256 eggs) and 1800 h (n=296 eggs).

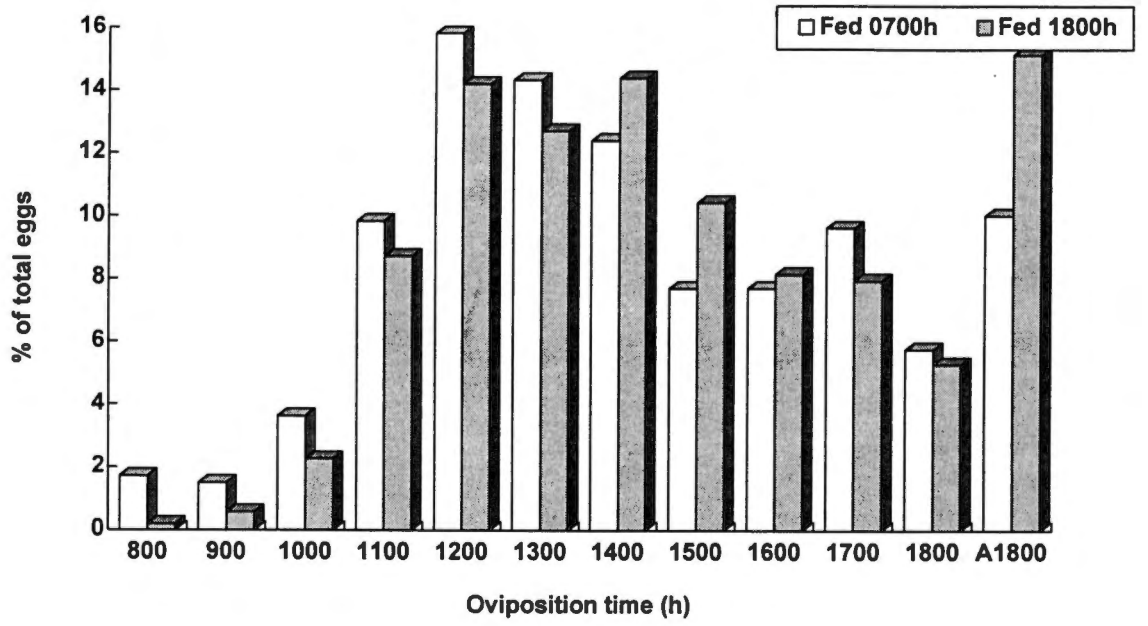

Figure 3. Frequency distribution (percentage) of oviposition times for broiler breeder hens fed at 0700 (n=468 eggs) or 1800 h (n=528 eggs).

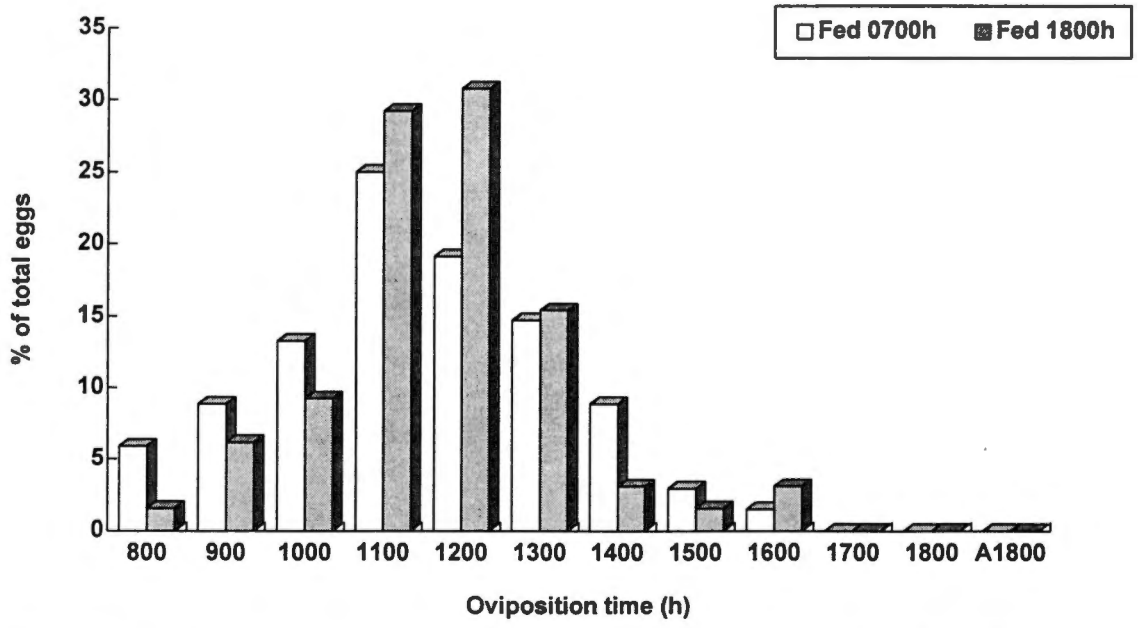

Figure 4. Frequency distribution (percentage) of oviposition times of first-of-sequence eggs for broiler breeder hens maintained under low temperatures and fed at 0700 (n=68 eggs) and 1800 h (n=65 eggs).

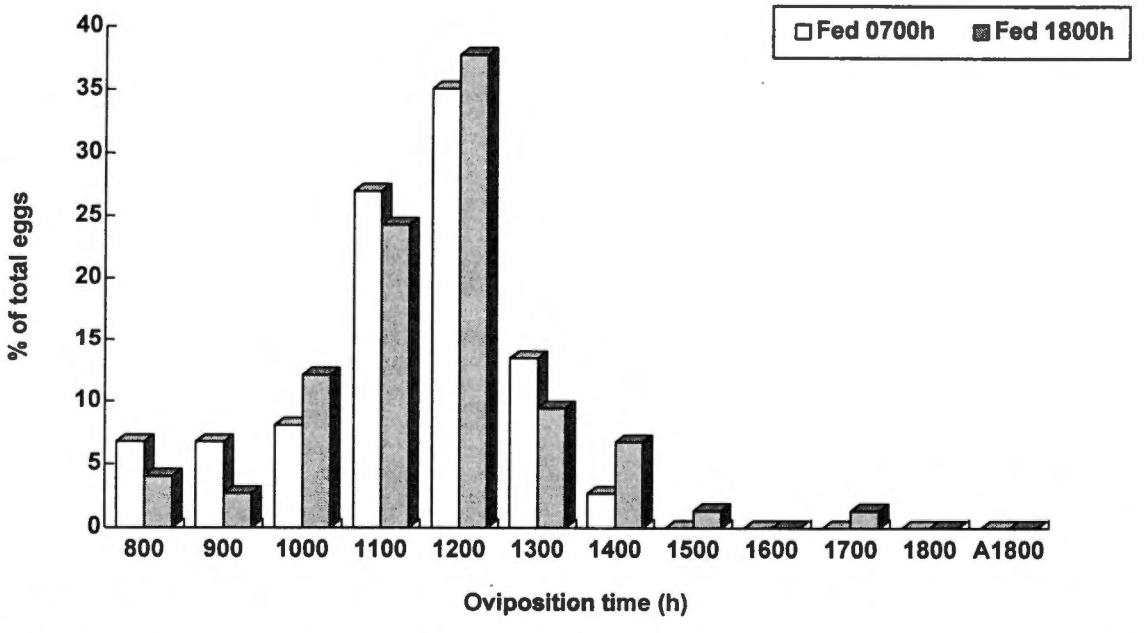

Figure 5. Frequency distribution (percentage) of oviposition times of first-of-sequence eggs for broiler breeder hens maintained under high temperatures and fed at 0700 (n=74 eggs) and 1800 h (n=74 eggs).

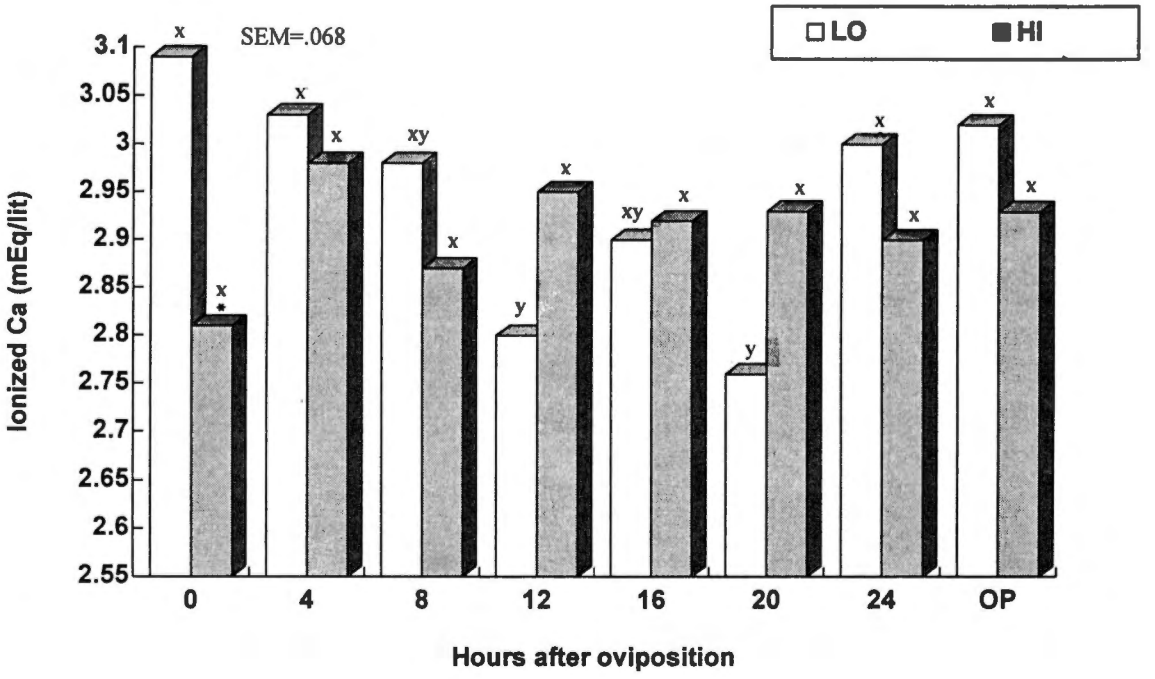

Figure 6. Blood ionized calcium during the egg cycle of broiler breeder hens (n=5 hens per treatment) maintained under low (LO) and high (HI) cyclic temperatures. ^{x,y}Means within the same temperature treatment with no common letter are significantly different (P<.05). Asterisks denotes significant difference (P<.05) between treatments at the same sampling period. Hours after oviposition (op) indicate the interval from op (1100 to 1200 h). Op-op interval is 26 to 26.5 h. Shell formation begins at 6 h post-oviposition.

affected by temperature; indeed there was a significant ($P<.05$) temperature by time interaction. Only hens maintained under low temperatures exhibited a decrease in calcium coincident with relative time of egg shell calcification. Hens at LO had higher Ca^{++} levels during the hours which preceded the entrance of the egg into the shell gland, which subsequently decreased during the period of shell calcification. Hens housed under high temperatures did not exhibit significant ($P>.05$) changes in blood Ca^{++} concentrations over time.

Total concentrations of plasma calcium during the egg cycle are shown in Figure 7. Plasma calcium of hens housed under low temperature remained constant over time. Hens housed under high temperatures exhibited a slight and significant ($P<.05$) decrease in plasma calcium after 12 h coincident with onset of shell calcification.

Blood pH was significantly ($P<.05$) affected by temperature (Figure 8) at all sampling times, blood pH was higher in hens housed under high temperatures. There was a significant ($P>.05$) temperature by time interaction.

The pattern of changes in estradiol and androstenedione concentrations during the egg cycle are given in Figures 9 and 10. In general, circulating estradiol and androstenedione levels peaked 24 h after oviposition and were not significantly ($P<.05$) affected by temperature treatment or by interactions between temperature and sampling time. If data

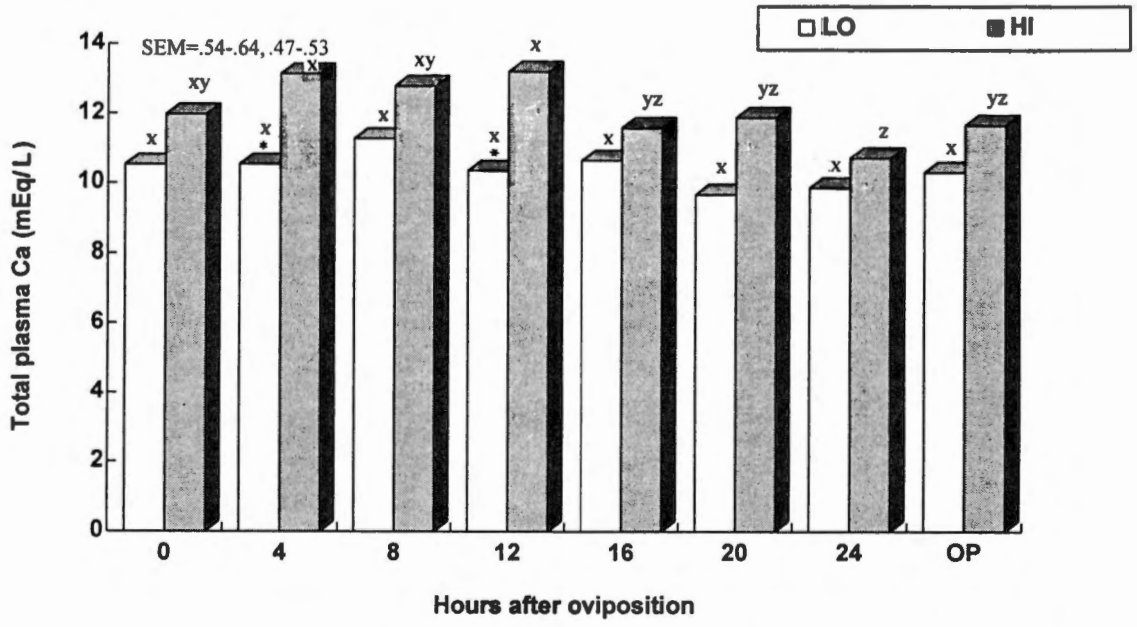

Figure 7. Plasma total calcium during the egg cycle of broiler breeder hens (n=5 hens per treatment) maintained under low (LO) and high (HI) cyclic temperatures. [x,y]Means within the same temperature treatment with no common letter are significantly different (P<.05). Asterisks denotes significant difference (P<.05) between treatments at the same sampling period. Hours after oviposition (op) indicate the interval from op (1100 to 1200 h). Op-op interval is 26 to 26.5 h. Shell formation begins at 6 h post-oviposition.

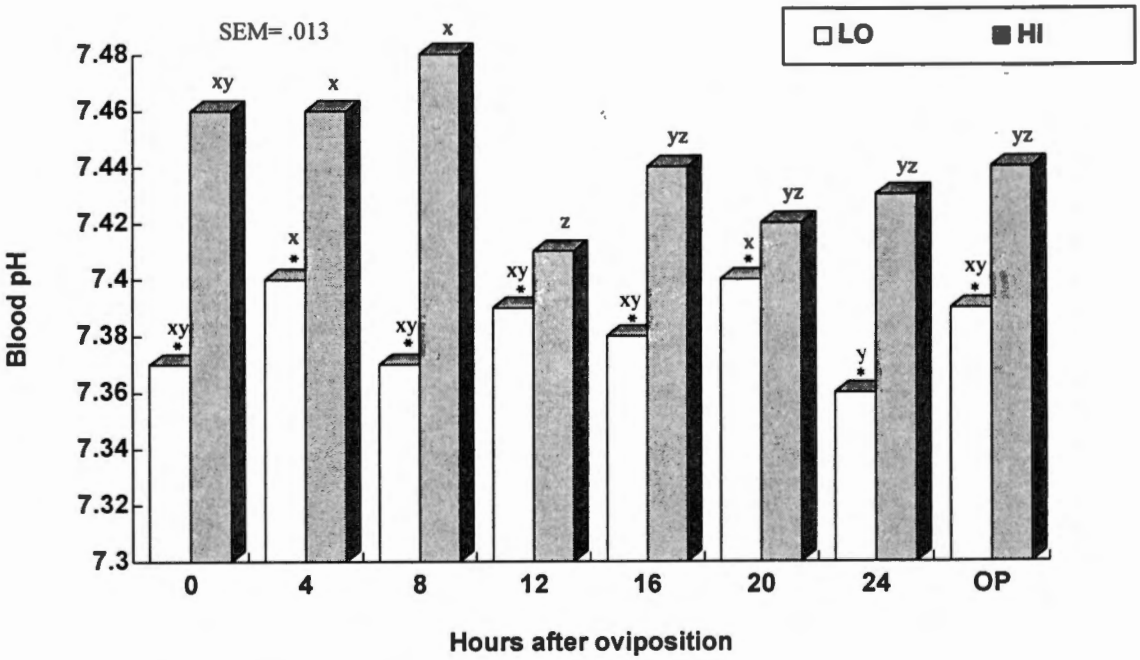

Figure 8. Blood pH during the egg cycle in broiler breeder hens (n=5 hens per treatment) maintained under low (LO) and high (HI) cyclic temperatures. [x, y]Means within the same temperature treatment with no common letter are significantly different (P<.05). Asterisks denotes significant difference (P<.05) between treatments at the same sampling period. Hours after oviposition (op) indicate the interval from op (1100 to 1200 h). Op-op interval is 26 to 26.5 h. Shell formation begins at 6 h post-oviposition.

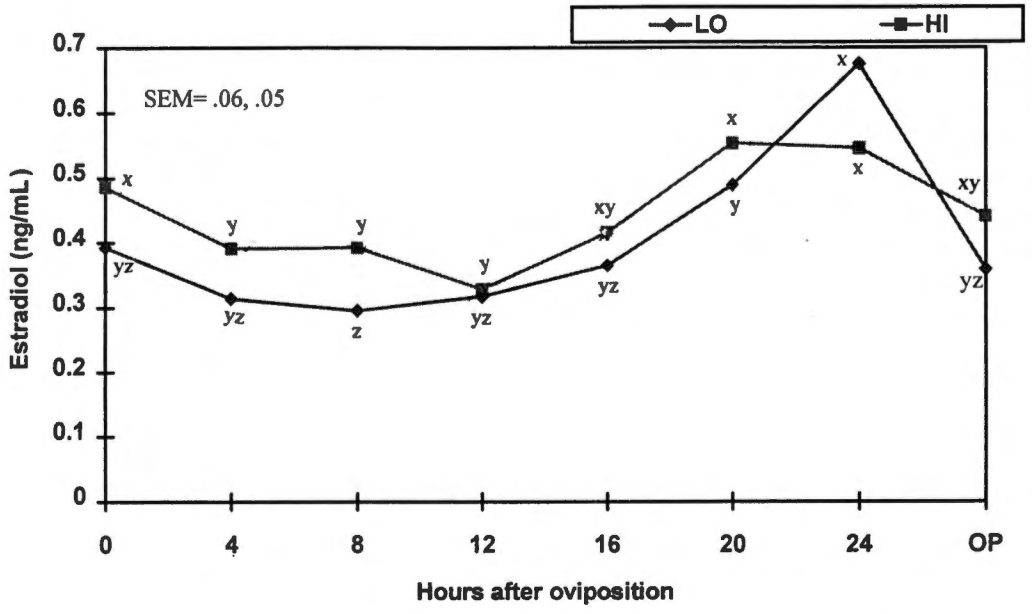

Figure 9. Plasma estradiol of broiler breeder hens (n=5 hens per treatment) maintained under low (LO) and high (HI) temperatures. [x-z]Means within the same treatment with no common letter are significantly different (P<.05). Asterisks denotes significant difference (P<.05) between treatments at the same sampling period. Hours after oviposition (op) indicate the interval from op (1100 to 1200 h).

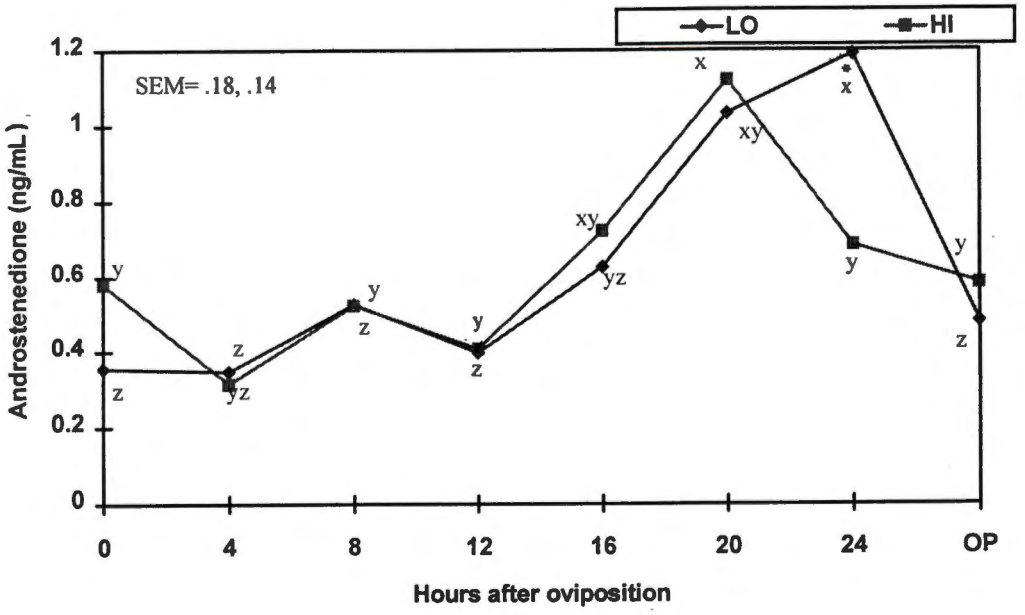

Figure 10. Plasma androstenedione of broiler breeder hens (n=5 hens per treatment) maintained under low (LO) and high (HI) temperatures. [x-z]Means within the same treatment with no common letter are significantly different (P<.05). Asterisks denotes significant differences (P<.05) between treatments at the same sampling period. Hours after oviposition (op) indicate the interval from op (1100 to 1200 h).

for estradiol were pooled across treatments, concentration of estradiol of hens at HI (0.44 *vs.* 0.40 ng/mL) was not significantly (P>.05) different from that of hens at LO. However, when base line (0-12 h) concentrations were compared, hens at HI had significantly higher (P<.05) estradiol concentration (0.40 *vs.* 0.33 ng/mL) than hens at LO. When similar comparisons were made for androstenedione, hens at HI did not differ from hens at LO. Estradiol and androstenedione concentrations varied relative to the egg cycle. Estradiol concentration dropped significantly (P<.05) and reached a minimum value at about 12 h after oviposition (14 h before the next oviposition) and rose rapidly to peak values at 6 to 4 h before the next oviposition. It is of interest to note that estradiol reached peak concentrations at an earlier time (6-4 h *versus* 2 h) in hens maintained under HI than hens kept at LO. In addition, the rate of decline to basal levels following peak levels was higher for hens under high cyclic temperature. Four out of five hens maintained under low cyclic temperature showed a peak of estradiol 3 to 2 h before oviposition. However, four out of five hens maintained under high cyclic temperature had peak estradiol levels 6 to 4 h prior to oviposition.

Plasma androstenedione showed the same pattern as estradiol: lowest concentration was found at 12 h after oviposition (14 h before the next oviposition). Inter-bird variation in the time of the peak levels was highly

significant (P<.05). In addition, peak concentration of androstenedione tended to occur earlier (8 to 6 h *versus* 8 and 4 h) in hens exposed to high cyclic temperature compared to hens exposed to low cyclic temperature. Almost all hens maintained under HI had the highest androstenedione concentration at 8 to 6 h before oviposition, while hens maintained at LO had the highest androstenedione level at 6 and 2 h before oviposition.

With regard to the non-laying hens in experiment 3, there were no significant (P<.05) differences in blood Ca^{++} due to time of collection or temperature cycle (Figure 11). An exception was: a significant (P<.05) drop was found in blood Ca^{++} of hens maintained under high cyclic temperature. This drop occurred 4 h (1900 to 2000 H) after the peak temperature. In all non-laying hens, blood calcium tended to increase at the time the lights were on (0700 h) and at 1500 to 1600 h. Total plasma calcium (Figure 12) showed little variation over the 24-26 h period and had no definite pattern. It is surprising that hens maintained under high cyclic temperature had, for almost all blood samples, numerically higher total plasma calcium levels compared to hens maintained under low cyclic temperature. Overall, the laying hens had noticeably higher total plasma calcium concentrations compared to the non-laying hens.

Blood pH of the non-laying hens maintained under LO showed little variation over the 24-26 h period (Figure 13).

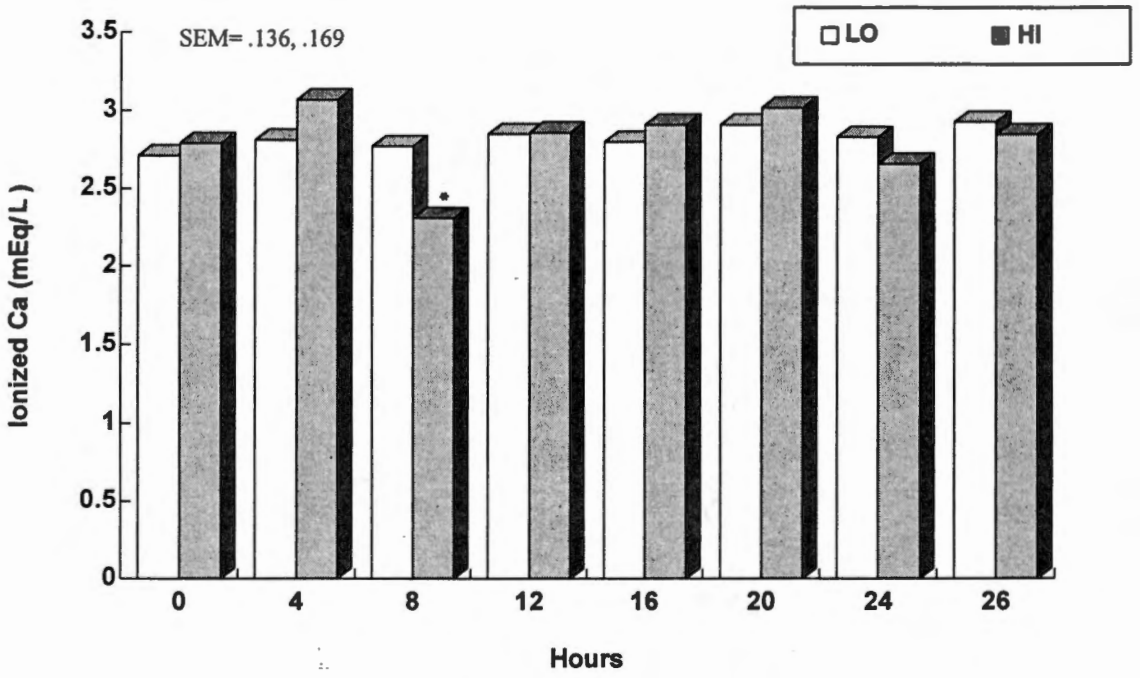

Figure 11. Blood ionized calcium of non-laying broiler breeder hens exposed to low (LO) and high (HI) temperatures. [x, y]Means within the same treatment with no common letter are significantly different (P<.05). Asterisks denotes significant difference (P<.05) between treatments at the same sampling period. LO, n=3; and HI, n=2 hens. On the X-axis 0 h (zero)=1100 to 1300 h.

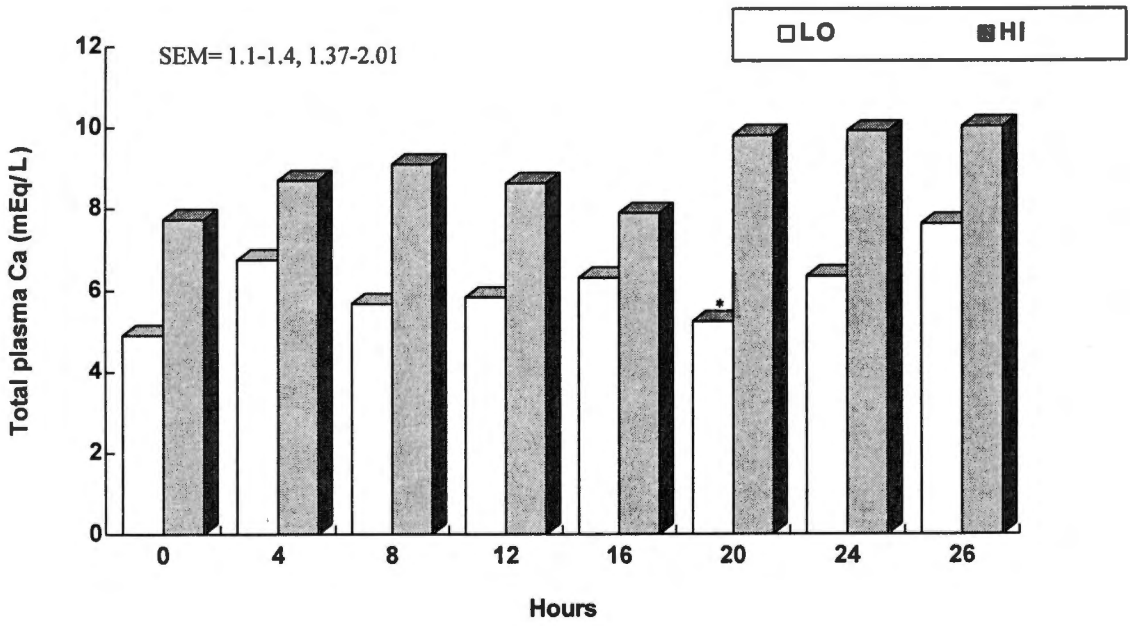

Figure 12. Total plasma calcium of non-laying broiler breeder hens exposed to low (LO) and high (HI) temperatures. ^{x,y}Means within the same treatment with no common letter are significantly different (P<.05). Asterisks denotes significant difference (P<.05) between treatments at the same sampling period. LO, n=3; and HI, n=2 hens. On the X-axis 0 h (zero)=1100 to 1300 h.

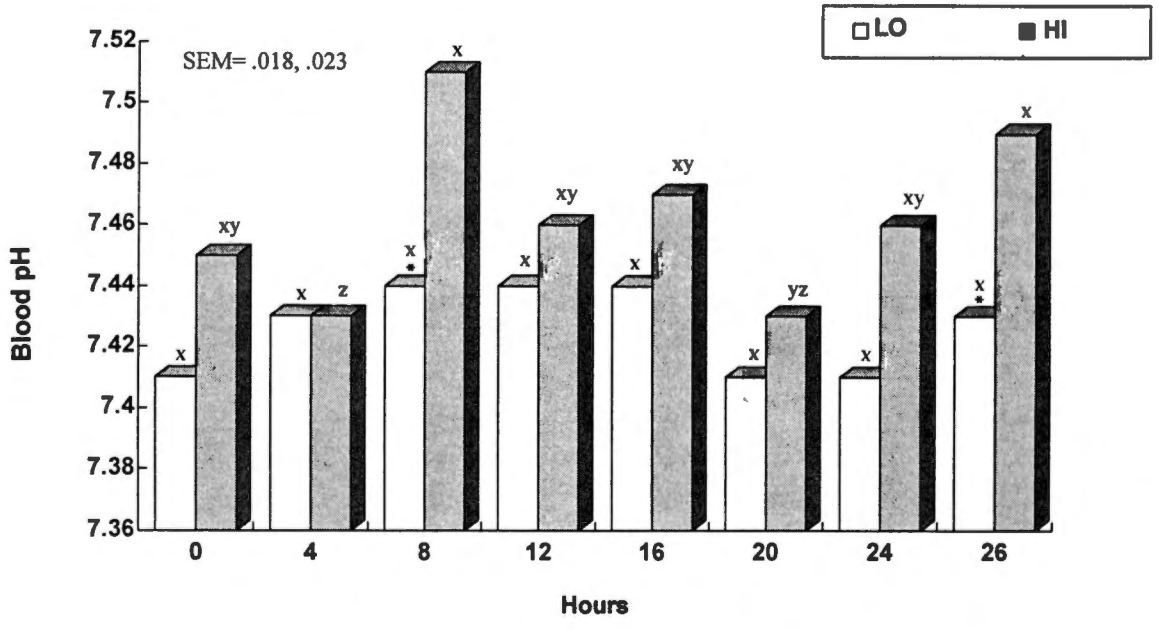

Figure 13. Blood pH in non-laying broiler breeder hens exposed to low (LO) and high (HI) temperatures. [x, y]Means within the same treatment with no common letter are significantly different (P<.05). Asterisks denotes significant difference (P<.05) between treatments at the same sampling period. LO, n=3; and HI, n=2 hens. Hours indicate the interval from 1100 to 1300 h (zero).

However, blood pH of hens maintained under HI showed significant ($P<.05$) variation over the same period of time. Values of blood pH for these hens decreased ($P<.05$) during the night, and remained low throughout the early morning hours, and increased during the hottest part of the day. Non-laying hens had higher ($P<.05$) blood pH values than laying hens, regardless of the temperatures cycle, which suggests that egg shell formation participates in reducing the blood pH of the active egg layers.

Experiment 4

A total of 113 hens were killed over a period of two weeks to determine the effect of temperature and feeding schedule on transit time of the egg through different regions of the oviduct. These data are presented in Table 2. The estimated time interval from oviposition to the entrance of the ovulated ovum into the shell gland was based on the stage of development the ovum had in the isthmus. The time the egg spent in the shell gland was estimated by subtracting the mean time elapsed before the egg entered the shell gland from the mean time between two successive ovipositions. Overall, neither the temperature nor feeding time affected the time the egg spent in the shell gland.

TABLE 2. Estimated time (h) for various stages of egg formation in broiler breeder hens fed at 0700 and 1800 h and maintained under low (LO) or high (HI) cyclic temperatures.

Stage of egg formation	Temperature	Feeding time	Estimated time (h)
Oviposition-ovulation	LO	0700 h	< 1.0
	LO	1800 h	< 1.0
	HI	0700 h	< 1.0
	HI	1800 h	< 1.0
Oviposition-entry into shell gland	LO	0700 h	> 6.5 but < 7.0
	LO	1800 h	> 6.5 but < 7.0
	HI	0700 h	> 6.5 but < 7.0
	HI	1800 h	> 6.5 but < 7.0
Time in the shell gland[1]	LO	0700 h	20.19
	LO	1800 h	19.23
	HI	0700 h	19.59
	HI	1800 h	19.85
Oviposition-next oviposition	LO	0700 h	26.94±.32
	LO	1800 h	25.98±.33
	HI	0700 h	26.34±.28
	HI	1800 h	26.60±.29

[1]Estimated as the difference between the time interval between two successive ovipositions and time of entry into the shell gland.

DISCUSSION

Experiment 1

The rate of egg production was not affected (P>.05) either by feeding time or temperature. Bootwalla et al. (1983) and Wilson and Keeling (1991) similarly reported that feeding in the afternoon did not adversely affect egg production; however, Harms (1991) reported that changing the feeding time from 0800 h to 1600 reduced rate of egg production. In the study conducted by Harms, the hens (51 and 58 wk old Arbor acre broiler breeders) were fed at either 0800 h or 1600 h, and in the study conducted by Wilson and Keeling (1991) the hens (65 wk old Arbor acre broiler breeders) were fed at either 0830 or 1730 h. Because of the lack of effect in egg production with feeding time in the present study and the study conducted by Wilson and Keeling (1991), it is apparent that evening feeding does not negatively affect egg production. Egg weight was not significantly (P>.05) affected by feeding time but was reduced due to the HI. Eggs from LO hens were significantly (P<.05) heavier than those from hens at HI. Egg specific gravity was not significantly (P>.05) affected by feeding time, which is in agreement with Wilson and Keeling (1991). Eggs from hens in the HI had significantly lower specific gravity compared to those from of hens at LO. Hens at HI and fed at 1800 had numerically, but not significantly, higher egg specific gravity (1.0793 *versus*

1.0801) than the hens fed at 0700 h. Decreased egg weight, specific gravity and shell thickness in the hens at HI (Table 1) was in concert with previous findings (Miller and Sunde, 1975; De Andrade et al., 1977; Emry et al., 1984).

Wilson and Keeling (1991) concluded that feeding in the afternoon did not change time of oviposition dwarf broiler breeders, 34 wk of age, whereas it significantly increased the proportion of eggs laid the in the afternoon in 65-wk old Arbor Acres broiler breeder hens. As concerns the Arbor Acres broiler breeders are concerned, these observations are not confirmed by our data. However, the results of the present study are in agreement with those reported by Harms (1991) who showed a slight change in the time of oviposition due to feeding 52 and 58-wk-old Arbor Acres broiler breeder hens at 1600 h. Although the age (Berg, 1945) of the hens may have been a factor, it is possible that the short (10 days) duration of the study conducted by Wilson and Keeling (1991) may have also contributed to the pattern of oviposition observed. Moreover, regardless of the number of birds used, their data were based on 4-day collection periods (the last 4 days of the study). The present study lasted longer and the data were based on a 9-day collection period. The finding, in the present study, that for the combined distribution of oviposition times at both temperatures showed that more ($P<.05$) eggs were laid in the afternoon when feeding time was changed from 0700 to 1800 h. This indicates that apart from

the delay in oviposition times that was caused by the 1800-h-feeding time, high as well as low environmental temperatures may also delay time of oviposition. Results of the effects of feeding time and cyclic temperatures on the timing of the first-of-sequence eggs were not different (P>.05); however, hens maintained under HI tended to lay a larger proportion of eggs at an earlier time compared to hens maintained under LO. Although it is not presented here, this may be due to the relatively longer sequences in some of the hens maintained under HI since these hens were able to maintain constant sequence length after the initiation of the experiment. The results of the present study are the first to show that high cyclic temperatures do not change time of oviposition of the first-of-sequence eggs.

Mc Daniel et al. (1979) demonstrated that eggs with specific gravity below 1.080 had the greatest weight loss during storage and lowest hatchabilities. In support if this, Bennet (1991) reported that a specific gravity of 1.080 or less is appropriate to designate eggs as thin shelled because hatchability of these eggs is significantly less than hatchability of eggs with higher specific gravity. At LO, changing the time of feeding broiler breeder hens from 0700 h to 1800 h increased egg specific gravity from 1.085 to 1.086 in experiment 1 (Part I) and remained constant (1.084) in experiment 1 (Part II). The corresponding values at HI were 1.081 and 1.082 in experiment 1 (Part I) and 1.079 and 1.080

in experiment 1 (Part II). Therefore, it is apparent that changing the time of feeding from 0700 h to 1800 h had important implications on egg shell quality when it is used for hens at HI compared to hens at LO.

Experiments 2 and 3

The present results for ionized calcium (Figure 6) agree with previous studies conducted under thermoneutral conditions have shown that blood ionized calcium levels in the egg-type laying hen declined as the egg entered the shell gland and remained low until 3-6 h before oviposition (Taylor and Hertelendy, 1961; Luck and Scans, 1978; Parson and Combs, 1981; Va de Velede et al., 1984). More recently, Frost et al. (1991) reported that Ca^{++} significantly ($P<.001$) decreased during shell calcification from about 6.6 mg/dL at 2 h after oviposition to about 5.5 mg/dL at 14 h postoviposition of the previous egg. However, at HI, blood Ca^{++} levels did not follow the same pattern during the egg cycle as those seen in hens maintained under low cyclic temperature, suggesting that calcium utilization for egg calcification may not be occurring in a manner similar to that under LO.

Hens maintained under high cyclic temperature had higher ($P<.05$) blood pH than hens at low cyclic temperature. Hens HI also had lower ($P<.05$) blood pH during the night than during the day, which indicates that egg shell formation and the drop in the environmental temperature contribute to recovery of

blood pH during the night. This is evident since blood pH of the non-laying hens also dropped ($P<.05$) during the night (Figure 13), but to a lesser extent. De Andrade et al. (1977) observed a diurnal rhythm in pCO_2, with the highest values occurring at midnight coincident with lowest environmental temperature which indirectly indicate a lower blood pH at this time.

With regard to the total plasma calcium, data of Luck and Scans (1978) and Parson and Combs (1981) showed no significant ($P>.05$) decline in total calcium during egg shell calcification. However, Hertelendy and Taylor (1961) observed a progressive decline in total calcium between the middle and the end of shell formation. Van de Velede et al. (1986) reported that the total plasma calcium concentration gradually decreased during egg formation from 32 to 23.3 mg/100 mL plasma. These authors pointed out that variation between hens was very high. Frost et al. (1991), on the other hand, reported that total plasma calcium increased from 15 mg/dL at 2 h after oviposition to about 21-22 mg/dL at 12 to 16 h, and gradually decreased to 17-18 mg/dL at 22-24 h after oviposition. Our data confirms that the total plasma calcium decreased during shell formation; however, this reduction was not significant ($P>.05$) for hens maintained under low cyclic temperature. These hens had the highest total plasma concentrations (11.26 mEq/L) at 6 h after oviposition which remained high at 16 h after oviposition. Significantly, there

was a gradual decline (P<.05) in plasma calcium that continued until the next oviposition. Total plasma calcium in hens at high cyclic temperature showed the same patterns; however, it significantly (P<.05) decreased during the last 10 to 8 h before oviposition, which is in agreement with Hertelendy and Taylor (1961), and Van de Velede et al. (1986) and in disagreement with the data of Frost et al. (1991). The discrepancy in the differing observation was considered by Frost et al. (1991) to be due to dilution resulting from bleeding the same bird (egg-type layers) in the studies of Parson and Combs (1981) and Van de Velede et al. (1986). Since large birds (broiler breeder hens) were used in the present research, it is unlikely that dilution was important factor in the effect of egg calcification on calcium levels. The data in the study conducted by Frost et al. (1991) had been obtained by sampling the same bird at two time periods during the egg cycle. Since there is evidence for a wide variation between individual hens in total calcium, it is more probable that the use of different birds by the above researchers may have contributed to the discrepancy in the reported data. Moreover, ionized calcium is better indicator of calcium utilization by the shell gland than total calcium since changes in total calcium could be explained by differences in feed (calcium) intake (Mueller, 1959)

The current results differ from those of De Andrade et al. (1977), Wolfenson et al. (1979) and Odom et al. (1986) in

that neither total nor ionized calcium were severely affected by cyclic temperature of 21-39 C. The short duration (3 hours) of heat exposure, and the use of a limited number of blood samples that were not correlated to the relative stages of the egg cycle in their studies may explain the discrepancy in the reported data. De Andrade et al. (1977) assumed that blood flow redistribution may be factor responsible for low egg shell quality under heat stress conditions; however, this assumption has recently been challenged by Arad et al. (1993) who reported that blood flow to the reproductive tract of the laying hen was not compromised during exposure to temperature of 35 to 45 C for 1.5 h. Therefore, it seems more probable that other physiological changes such as reduced calcium secretion (Bragg et al., 1971) or reduced calcium transport in the shell gland (Odom and Harrison, 1985) or a drop in the amount of bicarbonate ions caused by respiratory alkalosis are the major factors contributing to the reduced shell quality under heat stress conditions. More important, the production of H^+ ions in the shell gland during egg calcification facilitates the dissociation of the calcium-protein complex (Winget and Smith, 1962; Hodges, 1969) which in turn makes calcium available for shell deposition in the form of calcium carbonate. Heat stress-induced respiratory alkalosis caused a drop in HCO_3^- concentration, pCO_2 and a rise in blood pH (Mueller, 1966). Therefore, it is likely that elevated blood pH in the shell gland impairs the dissociation of protein-

bound calcium which in turn reduces the calcium available for shell formation. The finding that, in the present study, protein-bound calcium (the difference between ionized calcium concentration from total calcium concentration) was higher for the hens at HI compared to that of hens at LO indicates that dissociation of protein-bound calcium may have not occurred normally.

There are no data on the patterns of changes in blood calcium of non-laying hens and the effect of heat stress on this pattern. Blood ionized calcium (Figure 11) and total plasma calcium (Figure 12) concentrations did not significantly ($P>.05$) change during the 24-26 h period. An exception was the significant ($P<.05$) decline in the blood ionized calcium in hens maintained under HI coincident with peak temperature, suggesting that calcium mobilization did not take place in the non-laying hens to compensate for the decline caused by the high temperature. On the other hand, it is likely that laying hens responded by mobilizing more calcium to meet the demand for egg calcification.

Peterson and Common (1971) reported that estradiol peaked at 22-18 and 6-2 h before ovulation. Estradiol reached a minimum level at 14-10 h before ovulation. League et al. (1975) reported that peaks of estrogen and progesterone occurred between 7 and 4 h before ovulation. Graber and Nalbandov (1976) measured total estrogen concentration in 2-year-old White Leghorn hens. They found that the

concentration of the estrogen ranged from 25 pg/mL to over 600 pg/mL. Four peaks of estrogen were observed with the first occurring 1 h before ovulation and the others following at about 6 h intervals thereafter. Johnson and Van Tienhoven (1980) found only two peaks for estrogen (E_1 and E_2): a smaller peak at 22-18 h before ovulation and a larger one between 6 and 2 h prior to ovulation. Etches and Cheng (1981) reported that the plasma concentrations of testosterone, estradiol, and progesterone began to rise 9, 8, and 6 h, respectively, before ovulation. The concentrations of estradiol and testosterone were significantly higher ($P<.05$) than the baseline 4-5 and 6 h, respectively, before oviposition. Estradiol concentrations ranged from .33 (at 24 h before oviposition) to .25 (at 9 h before oviposition) to .4 ng/mL at 5 h before oviposition. Etches (1993) showed that androstenedione peaked 6-4 h before oviposition, and its concentrations were .4, .5, .9, .74, .8, and 1.4 ng/mL at 24, 20, 16, 12, 8, and 4 h, respectively, before oviposition. In general, we observed similar changes in concentrations of estradiol and androstenedione relative to oviposition. Only one peak was observed for estradiol or androstenedione, possibly due to the fact that in this study blood samples were obtained every 4 hours preventing detection of other peaks. Nasser et al. (1992) measured (every 6 h during the temperature cycle) plasma estradiol of broiler breeder hens (34-44 wk old) that were exposed to cyclic temperatures(22-40 C and 22-44 C or 19-25 C) and observed that

estradiol increased at high temperatures. HI increased measures of baseline levels of estradiol, but did not affect levels of androstenedione in the present study. There in no data on the effects of high temperatures plasma androstenedione of the laying hen. Because plasma concentration of estradiol and androstenedione (as an indicator of plasma androgen) were not reduced, it unlikely that high temperatures diminished the effects of these hormones on calcium metabolism.

Experiment 4

Nordstrom (1973) measured the duration of egg formation in 18 mo old SCWL laying hens before (at a constant temperature of 21 C) and after hens were exposed to a constant temperature of 32 C for 3 weeks. The time interval between successive eggs was longer (27.7 *versus* 25.6 h) when the hens were moved to the high temperature. The results of the present experiment disagree with those of Nordstrom (1973) in that the high cyclic temperature resulted in no effect on the time that the egg spent in the shell gland. The egg was observed to move somewhat faster through the magnum in hens maintained under HI as compared to the hens under LO. This may explain why less albumen is secreted in the egg from hens exposed to high temperatures (Bennion and Warren, 1933). In disagreement with the data reported by Nordstrom (1973), the present experiment indicates that the high cyclic temperature

did not result in a significant (P>.05) change in the mean time interval between successive eggs.

It is of interest that the time interval between successive eggs seems somewhat longer in broiler breeder hens as compared to that observed in egg-type laying hens. This interval was 26.6 h for hens fed at 0700 h compared to 25.8 and 25.4 h reported by Warren and Scott (1935) and Melek et al. (1973), respectively. These differences are probably due to the shorter sequence length for the broiler breeder hens. Berg (1945) reported that the time interval between eggs decreased as the length of the sequence increases.

The estimated mean time that an egg spends in the shell gland was 20.1 h, which is comparable to 20.4 h reported by Warren and Scott (1953) but slightly higher than 19.76 h that reported by Melek et al. (1973). The length of the magnum and the isthmus has been reported to be 49.4 cm (Warren and Scott (1935), 42 cm (Taylor and Hertelendy (1960). In the present experiment, however, the length of these two regions measured 50.7 cm, with a range from 45 to 55 cm. Although comparable data in the broiler breeder hens do not exist, the longer magnum and isthmus may be explained by the heavier body weight for these hens. Further information is necessary since the different lengths in the segments of the oviduct of the broiler hen would most likely affect the length of the time that the egg spends in each of these regions. Previous estimates of the total time that the egg spent in the

infundibulum, magnum, and isthmus were: 4.42 h (Warren and Scott, 1935); 5.15 h (Melek et al., 1973). Assuming that the interval from oviposition to ovulation is .5 to 1 h in the broiler breeder hen, it is evident from the data for the broiler breeder hens killed at 8 h after oviposition, that the egg spent at least 5.5-6 h in the infundibulum, magnum and isthmus. Therefore, it is possible that the longer time interval between successive egg in the broiler breeder hen may also be due to the extra time that the egg spends in these segments. However, to accurately determine whether the sequence length or the length of the oviduct is responsible for the longer time interval between successive eggs, it is important that a comparison is made between broiler breeders and egg-type laying hens producing sequences with a similar length.

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PART IV

SUMMARY AND CONCLUSIONS

SUMMARY AND CONCLUSIONS

Two studies were conducted to determine the effect of feeding time and temperature on various production criteria of broiler breeder hens.

Two ranges in temperature were used: low (10-25 C) and high (21-39 C). In study 1, hens were randomly assigned to feeding times of 0700 h, 0700 and 1800 h, and 1800 h. In the second study, hens were assigned to feeding times of 0700 h and 1800 h.

The criteria studied were egg production, egg shell quality, patterns of feed consumption, blood ionized calcium, body weight change, time of ovulation and oviposition, and the transit time of the egg through different regions of the oviduct. In separate experiments, blood pH, blood concentrations of calcium, estradiol, and androstenedione were also measured in 0700-h-fed hens at the two temperatures.

The results obtained indicated that feeding time and temperature did not significantly ($P>.05$) affect hen-day egg production, except for a decrease ($P<.0\%$) in number of eggs produced by hens at high temperature and fed their daily allowance in two meals (0700 and 1800 h). Egg weight was reduced ($P<.05$) due to the high temperature. Egg specific gravity and shell thickness were also reduced ($P<.05$) due to the high temperature and slightly improved due to feeding at 1800 h. Significant body weight loss ($P<.05$) occurred in hens

fed at 0700 h and maintained under the high temperature. Oviposition time was not significantly ($P<.05$) changed due to temperature; however, it was significantly ($P<.05$) changed due to feeding at 1800 h. Neither feeding time nor temperature affected time of ovulation, or the transit time of the egg through the different regions of the oviduct. Different consumption patterns were observed for hens in relation to feeding time, oviposition, temperature and meal feeding. Both high temperature and meal feeding were observed to reduce ($P<.05$) feed intake but the effects were not additive. High temperature resulted in lower ($P<.05$) rate of feed consumption. High temperatures increased ($P<.05$) baseline levels of plasma estradiol, but did not affect ($P>.05$) plasma levels of androstenedione. High temperatures did not significantly reduce ($P>.05$) the concentrations of blood ionized calcium, plasma total calcium, but increased ($P<.05$) blood pH especially when the temperature was increasing during the day. The hens maintained under low temperatures exhibited a cyclic pattern in ionized calcium, and plasma total calcium over the egg cycle, whereas the hens maintained at high temperatures exhibited a cyclic pattern in pH and plasma total calcium but not blood ionized calcium during the same time.

In conclusion, high temperatures caused a drop ($P<.05$) in egg specific gravity below the recommended level (1.080) required to achieve a reasonable hatchability. At LO, egg specific gravity remained well above 1.080 regardless of

feeding time. Feeding broiler breeder hens at 1800 h improved egg specific gravity. Although this improvement was small, it can be effective means for improving hatchability especially for eggs from hens at high environmental temperatures. The economics of this effect, however, requires further evaluation. The data from the present experiments also indicate that high temperatures caused a significant ($P<.05$) drop in feed consumption; thus nutrient intake for these hens is below requirement levels. The literature suggests that egg shell quality of heat stressed hens can be improved by dietary supplements such as vitamins and minerals. Because of the possibility that the response to dietary supplements depends on the degree of the reduction in feed intake caused by high temperatures, it is logical that any practice directed at increasing feed intake will be as effective as dietary supplements and will result in significant economic savings.

These results indicate that the supply of calcium available for shell calcification was not diminished in hens acclimated to high cyclic temperatures. Moreover, concentrations of estradiol and androstenedione which are involved in calcium metabolism were not adversely affected by high temperatures. High temperatures cause an increase in breathing rate (panting). The birds exhale carbon dioxide during panting. This induces respiratory alkalosis which results in an increase in bicarbonate excretion. Bicarbonate is required for shell formation. Moreover, during normal

shell calcification there is production of H^+ ions as a result of CO_2 hydrolysis and formation of the shell carbonate. The calcium required for shell formation is transported to the shell gland as protein-bound complex (i.e. albumin and vitellogenin). The production of H^+ ions and the resulted decline in blood pH in the shell gland is required for the dissociation of calcium-protein complex, and the subsequent formation of $CaCO_3$. High temperatures cause a reduction in pCO_2. This results in an increase in blood pH, which slows down the dissociation of the protein-bound complex and limits the amount of ionized (free) calcium, in the shell gland, available for shell formation.

Further studies are required to fully understand: 1) the effect of feeding time in combination with other management practices (i.e. carbonated drinking water) on egg production, shell quality, and hatching eggs; 2) the effect of heat stress on calcium uptake (by the shell gland) and excretion (by the kidney) during the egg cycle of the laying hen.

VITA

Maen Helmi Samara was born September 2nd, 1958 in Brouqin, the West Bank. In 1977, he graduated from High School of Salfeet. He received a Bachelor's degree in Animal Production and Protection from the University of Jordan, Amman, Jordan, 1981. In the same year he was employed as a manager in a farm of broiler breeders by PROVIMI, JORDAN. In 1984, he was employed as a technical manager by Poultry Farmer's Cooperative, Ramalla/ East Jerusalem. In 1986, he was awarded a British Council Scholarship where he got his M.S. in Animal Production, from the University of Reading, England. In 1988, he worked as chief supervisor of animal production sector by a local Agriculture Relief Committee, East Jerusalem. In 1990, he joined the faculty of Agriculture at Hebron University, West Bank. In the same year he was awarded a AMIDEAST scholarship. In Spring 1991, he entered the Graduate School of the University of Tennessee, Knoxville. In January 1992, he was married. His first son was born in October 1992 and his second child will be born in December 1994. He was granted the Ph.D. degree in Animal Science with concentration in Poultry Nutrition in December 1994.

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