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Ahemeral Light-Dark Cycles on Reproduction in the Ring-Necked Pheasant (Phasianus colchicus)

presented by

Austin Glasspole Blake

has been accepted towards fulfillment
of the requirements for

Ph.D. degree in Poultry Science

Robert K Ringer
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# AHEMERAL LIGHT-DARK CYCLES ON REPRODUCTION IN THE RING-NECKED PHEASANT (*PHASIANUS COLCHICUS*)

By

Austin Glasspole Blake

A DISSERTATION

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Department of Animal Science
1984

ABSTRACT

AHEMERAL LIGHT-DARK CYCLES ON
REPRODUCTION IN THE RING-NECKED
PHEASANT (PHASIANUS COLCHICUS)

By

Austin Glasspole Blake

The effects of two ahemeral light-dark (L:D) cycles on ring-necked pheasant reproduction were investigated in this experiment. At 10 months of age, 36 females and 12 males each, were either exposed to a conventional 24 hour (14L:10D), an ahemeral 22 hour (14L:8D), or an ahemeral 26 hour (14L:12D) L:D cycle. Most ovipositions occurred later under the 22 hour L:D cycle and earlier under the 26 hour cycle than under the conventional cycle. There was oviposition entrainment such that at the end of the peak oviposition time approximately 60% of the total ovipositions had occurred regardless of the light treatment. The values obtained at LH surge under 22, 24, and 26 hour L:D cycles were 4.1, 7.5, and 4.7 ng/ml, respectively, while progesterone values were 6.9, 8.2, and 9.5 ng/ml, respectively. Initiation of the progesterone surge always preceded the LH surge. The LH and progesterone surge occurred approximately 6-9 hours prior to ovulation. The shifts in oviposition times under the ahemeral L:D cycles were due to changes in the phase of LH but the surge remained fixed relative to ovulation time.

Total lag time for each egg sequence was greater in the ahemeral than the conventional cycle, resulting in egg formation

time (EFT) of 25.8, 25.5, and 26.5 hours under the 22, 24, and 26 hour L:D cycles, respectively. The EFT under the 26 hour L:D cycle was closely synchronized (within 0.5 hours) with the length of the L:D cycle resulting in longer egg sequences and a trend for greater egg production than observed under the other cycles.

The ahemeral 26 hour L:D cycle significantly ($P \leq 0.05$) improved percent fertility of pheasant eggs. However, percent hatchability of fertile eggs, egg weight, percent hen-day egg production, feed intake, and body weight were not significantly ($P > 0.05$) affected by ahemeral L:D cycle treatments. Conversely, the ahemeral 22 hour L:D cycle significantly ($P \leq 0.05$) reduced egg shell quality.

This dissertation is dedicated to my father, EGBERT AGUSTUS BLAKE (1918-1981) who contributed immensely to my education.

ACKNOWLEDGEMENTS

I would like to express my candid gratitude to Dr. Robert Ringer, my Major Professor and Chairperson of my graduate committee for his encouragement, understanding, advice, and help given to me during my Ph.D. program at Michigan State University. Also, my most sincere appreciation to the other members of my graduate committee, namely: Drs. Steven Bursian, Richard Balander, and James Ireland who have all contributed intellectually to my education by sharing their knowledge and experience with me and by making constructive criticism of my work. My most sincere appreciation to Prof. Brian Follett for his benevolence in consenting to have my LH analysis done in his laboratory at the University of Bristol, England.

Thanks to Dr. John Gill and Dr. Clyde Anderson for their help and advice in computing the data and interpreting the results of my doctoral research.

The author also acknowledges the other faculty and staff members, and fellow graduate students of the Animal Science Department who have contributed to the successful completion of this study. Special thanks is extended to Dr. Theo Coleman for the invaluable support he has given the author; and to Dr. Cal Flegal for providing some of the research materials used in this project.

Finally, I would like to thank my wife, Sonea, my son, Austin, Jr., and the rest of my beloved family for their support, encouragement, and patience given to me during my university training.

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## ABBREVIATIONS

| | |
|---|---|
| AI | artificial insemination |
| AL | average lag |
| ANOVA | analysis of variance |
| Avg | average |
| b | bird |
| B | broken |
| Bv | basal value |
| cc | cubic centimeter |
| cm | centimeter |
| cpm | counts per minute |
| CV | coefficient of variation |
| d | day |
| DNR | Department of Natural Resources |
| $E_2$ | estradiol |
| EFT | egg formation time |
| ESG | egg specific gravity |
| EST | egg shell thickness |
| Genstat | General statistics |
| gm(g) | gram(s) |
| h | hour(s) |
| L:D | light:dark |
| LH | luteinizing hormone |
| LHRH | luteinizing hormone releasing hormone |
| ml | milliliter |
| mm | millimeter |
| MPO | mean peak oviposition |

| | |
|---|---|
| MSU | Michigan State University |
| n | number of observations |
| ng | nanogram(s) |
| no. | number |
| OT | oviposition time |
| $P_4$ | progesterone |
| PCV | pack cell volume |
| PHDEP | percent hen-day egg production |
| PSE | percent shell-less eggs |
| Pt | peak time |
| Pv | peak value |
| RIA | radioimmunoassay |
| rpm | revolution per minute |
| SD | standard deviation |
| SED | standard error of the difference between two means |
| SEM | standard error of the mean |
| SL | shell-less |
| TL | total lag |
| TOP | total oviposition |
| vs. | versus |

KEY TO SYMBOLS

| | |
|---|---|
| °C | degrees centigrade |
| °F | degrees fahreinheit |
| µl | microliter |
| % | percentage |
| / | per |

# CHAPTER 1

## INTRODUCTION

Ahemeral light-dark cycles are those cycles in which the light and dark periods do not add up to 24 hours (h). Since the early 1970's, there have been a number of reports on the effects of ahemeral lighting on the chicken (Gallus domesticus). However, there are no reports on ahemeral lighting in other avian species, with the exception of an unreported preliminary study conducted at the Michigan State University Poultry Research Station (MSUPRS), in which turkeys (Meleagris gallopava) were used. Consequently, the effect of ahemeral lighting in the ring-necked pheasant (Phasianus colchicus) is not known.

With the growing popularity of the ring-necked pheasant as a game bird, both in Michigan and other parts of the United States of America (Reynnells, 1979), and the continued increase in pheasant research for the main purpose of improving body weight and egg production, it has become necessary to learn more about pheasant reproduction.

Thus, the main objective of this study was to subject ring-necked pheasants to ahemeral lighting, and consequently measure the effect of ahemeral lighting on their feed intake, body weight changes, and reproductive responses. It was hoped that the information obtained from the research would be of use in increasing the efficiency of pheasant egg production and the continued propogation of this species.

# CHAPTER 2

# REVIEW OF THE LITERATURE

The subject attempted to cover in this review is a very broad one; thus, only articles relevant to the research under investigation has been selected. This review is therefore not all encompassing.

## A. Pheasant Propogation at Michigan State University (MSU)

Increasing urban population and high hunting pressures have resulted in a decline in the pheasant population in Michigan from 1950 to 1970 (Varghese and Flegal, 1978). A joint project between the Michigan Department of Natural Resources (DNR) and the former MSU Poultry Science Department was initiated in 1970 with the objective of increasing pheasant production via several management procedures.

The foundation stock of pheasants for this project was obtained from the DNR flock in Mason, Michigan, and was designated as the DNR strain. To this strain, selection pressure was applied over the years to improve egg production. The high egg-producing birds that were obtained from these efforts were designated MSU strain (Carpenter, 1980).

At the beginning of the project, egg production averaged 33 percent (%) on a per hen housed basis during a 120 day egg production period. Due to genetic selection, egg production increased to approximately 66% by 1979 (Carpenter, 1980; Carpenter and Flegal, 1981). Compared to pheasants in the wild, which lay 10-12 eggs during the breeding season (Streib,

et al., 1973), this represents a great improvement in pheasant egg production. Artificial insemination was successful in improving fertility from 30 to 53% over a three year period (Wing, 1976). Fertility and hatchability of fertile eggs as high as 69.5 and 75.3%, respectively, were reported by Carpenter (1980). Differences in egg weight were reported between both strains of pheasants with the DNR strain producing an average higher egg weight (34 g) than the MSU strain (33 g) (Carpenter, 1980).

B. Photoperiodicity in Birds

The effects of light intensity and color, continuous lighting, intermittent lighting, ahemeral lighting, and various photoperiod lengths on reproduction have been examined primarily in domestic chickens, turkeys, and Japanese quails (Coturnix coturnix japonica). There are few reports on the effect of light on reproduction in the ring-necked pheasants (Bissonnette and Csech, 1936; Clark et al., 1937; Adams et al., 1968).

1. The circadian rhythm

According to van Tienhoven (1968) and Saunders (1977), a circadian rhythm is that which persists when all environmental periodicities are excluded, and in the free-running condition shows a natural period which is close to 24 hours. It functions in providing synchrony between the organism and environmental periodic changes and also in the integration of the organism's internal environment.

The period of a circadian rhythm can be entrained by external stimuli, such that the rhythm adopts the period of the

stimulus (Bünning, 1973; Saunders, 1977; Rusak and Zucker, 1979). The major entraining cue (agent, zeitgeber, signal) for the reproductive cycle of many species is light (Saunders, 1977; Rusak and Zucker, 1979; Ringer, 1982), with the change from light to dark and vice-versa as the most important signal (Saunders, 1977). However, Morris (1973) stated that entrainment depends on the contrast between the bright and dim phase and not upon the absolute level of light intensity. A bright: dim ratio of 27 to 30:1 hours was quite adequate for entrainment of oviposition in chickens (Morris, 1973). Temperature is also an important entraining cue but is known to be less powerful than light (Bünning, 1973; Bhatti and Morris, 1977; Saunders, 1977; Rusak and Zucker, 1979; Ringer, 1982).

2. Mechanism of light

Generally, the photo response in birds involves the eye, hypothalamus, anterior pituitary (adenohypophysis) and the gonads (Ringer and Sheppard, 1960; Benoit, 1964; van Tienhoven, 1981; Ringer, 1982). According to Ringer and Sheppard (1960) and Ringer, (1982) the energy of light penetrates the eye and its surrounding tissues to initiate an impulse which travels via the optic nerve to the hypothalamus. The neurons in the hypothalamus are then stimulated to produce releasing hormones. These neurohormones are carried from the area of the median eminence via the portal blood vessels to the anterior pituitary where they stimulate the release of gonadotropins. Gonadotropins travel to the gonad (target organ) via the general circulation (Figure 1).

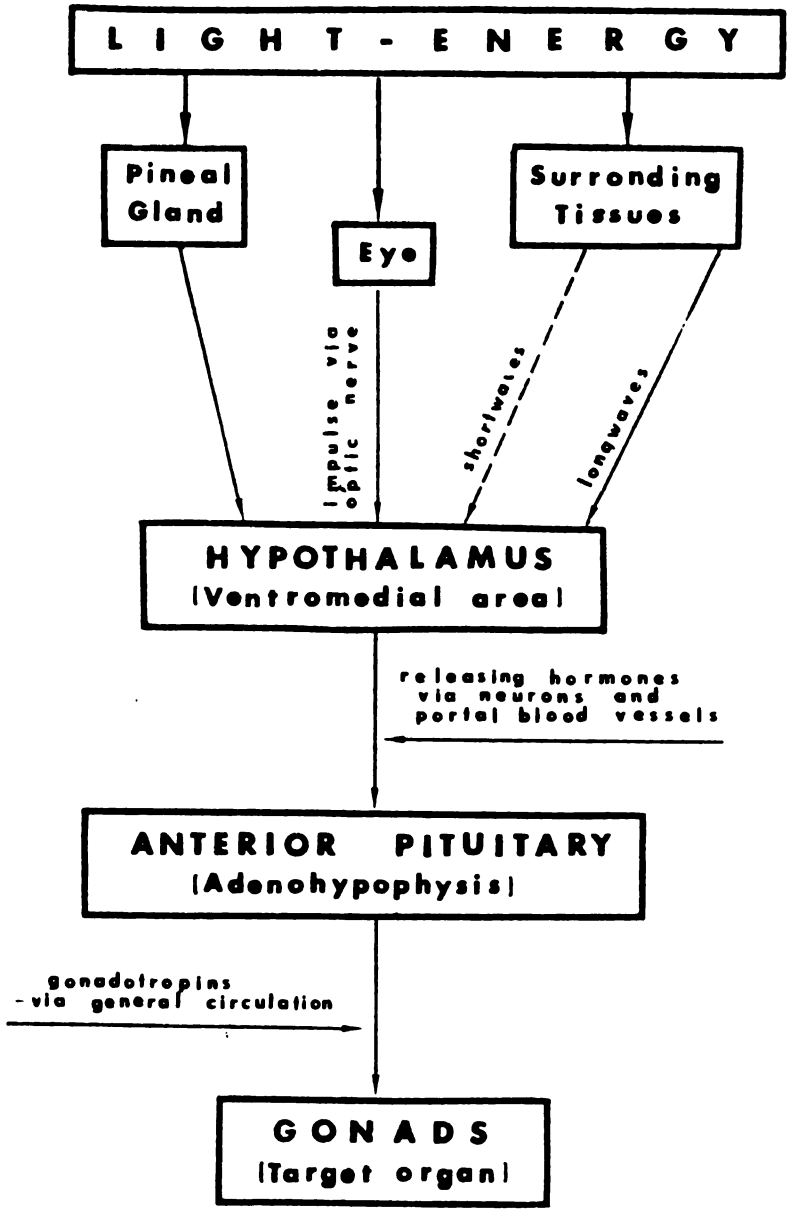


Figure 1. Mechanism of light in avian species.

Although the eye appears to be the organ of photoreception, Benoit (1964) reported that "deeper" photoreceptors may play a role in light-induced gonadal stimulation. The removal of the eyes or sectioning of the optic nerves in immature male ducks does not prevent photoperiodic stimulation of the gonads. The hypothalamus is one of these "deeper" receptors since its stimulation with long wave length radiation also resulted in testicular growth (Benoit, 1964). Rusak and Zucker (1979) reported that the hypothalamus was an extraocular photoreceptor in white-crowned sparrows (Zonotrichia leucophrys gambelie), with the ventromedial portion being the area of greatest sensitivity. The work of Oishi and Lauber (1973) suggested that the eyes and the pineal body probably act as guides to light for the brain's photoreceptor, the hypothalamus (Figure 1).

During the period of sustained photoperiod in the breeding season, most birds will exhibit photorefraction; the bird no longer responds to the stimulatory effect of light, and its reproductive activity is terminated (Bissonnette, 1938; Hammond, 1957; van Tienhoven, 1968; Ringer, 1982). The commercialization and genetic selection of chickens reared under controlled lighting has led to an absence of photorefraction for this species. Pheasants under similar environmental conditions as chickens will show photorefraction (Flegal, 1981; Ringer, 1981).

## 3. The Influence of light-dark cycles on egg production

Three types of light-dark (L:D) cycles are described in the scientific literature. One is the conventional cycle, in which the light and dark periods total 24 hours. This has been the cycle used in poultry operations. Another is the continuous cycle in which the light is given continuously. The third is the ahemeral cycle. In this cycle the light and dark periods do not total 24 hours (Foster, 1972; Morris, 1973).

For the purpose of this review the emphasis will be placed on ahemeral L:D cycles. However, the 24 hour conventional cycle will be briefly discussed because it has been used as the standard against which ahemeral light is evaluated.

### a) The effect of 24 hour light-dark cycles

The majority of earlier experiments and practices using lighting in poultry operations have been conducted under natural daylength or under controlled lighting using 24 hour L:D cycles. There was an assumption that the period of the biological rhythm for ovulation and/or egg formation was 24 hours. However, more recent work showed that the rhythm for ovulation is greater than 24 hours (Warren and Scott, 1936; Wolford et al., 1964a; Woodard and Mather, 1964). A 24 hour L:D cycle appears to be out of synchrony with the above biological rhythm. Alternatives to a 24 hour L:D cycle may therefore be in order.

Under conventional L:D cycles a period of 10 or more hours of light per day is required to induce maximum sexual maturity

in chickens and turkeys. Beyond 17 hours of light per day no further increase in egg production will be obtained. A decrease in photoperiod after sexual maturity attainment in chickens and turkeys will depress egg production rate, but an increase will do the opposite (Ringer, 1982; Tucker and Ringer, 1982). A delay in sexual maturity can be achieved with decreased photoperiods (North, 1978; Tucker and Ringer, 1982). The absence of light inhibits the onset of sexual maturity in pheasants (Clark et al., 1937). Pheasants will respond to night lighting by coming into early egg production (Bissonnette and Csech, 1936), and will also increase egg production when they are exposed to artificial lighting (Clark et al., 1937; Adams et al., 1968).

b) The effect of ahemeral light-dark cycles

The use of ahemeral L:D cycles in poultry operations is one method of attempting to synchronize the length of the L:D cycle with the hen's interval between ovipositions (Foster, 1972). Morris (1978a) hypothesized that under ahemeral cycles (25 to 30 hours) the bird's biological clock is reset by "sunrise" or "sunset" in each cycle. Physiological rhythms then occur in a manner as if the next sunrise or sunset will occur approximately 24 hours later, although the light may actually go "on" or "off" later than this.

An increase in chicken egg weight is usually obtained using ahemeral L:D cycles that are longer than 26 hours or shorter than 24 hours (Table 1). Melek et al. (1973), Morris (1973),

Table 1. The effect of various ahemeral light-dark (L:D) cycles on egg production characteristics in chickens relative to the 24 hour light-dark cycle.

| L:D cycle length (h) | % Ep[1] | Ewt[2] | Em[3] | Esg[4] | Est[5] | Eswt[6] | Reference |
|---|---|---|---|---|---|---|---|
| 21 | ↓ | – | ↓ | – | – | – | Shanawany (1982) |
| | 0 | ↑ | – | – | – | ↑ | Ousterhout and Zimmermann (1983) |
| 22 | ↑ | – | – | – | – | – | Yassin and Biellier (1978) |
| | ↑ | – | – | – | – | – | Koelkebeck and Biellier (1979) |
| | – | 0 | 0 | – | – | – | Rezvani and Biellier (1981) |
| 23 | ↓ | – | – | – | – | – | Foster (1968) |
| | – | ↑ | – | – | 0 | ↑ | Morris (1973) |
| | ↑ | – | – | – | – | – | Koelkebeck and Biellier (1979) |
| | ↓ | ↑ | ↑ | – | – | – | Shanawany (1982) |
| 25 | ↑ | – | – | – | – | – | Foster (1968) |
| | ↓ | ↑ | – | – | – | – | Fox et al. (1971) |
| | ↑ | ↑ | ↑ | – | – | – | Morris (1973) |
| | – | – | – | ↑ | – | – | Koelkebeck and Biellier (1980) |
| | 0 | – | ↑ | – | – | – | Shanawany (1982) |
| 26 | ↑ | – | – | – | – | – | Foster (1969) |
| | ↓ | ↑ | – | – | – | – | Fox et al. (1971) |
| | ↓ | ↓ | – | – | – | – | Fox and Shaffner (1972) |
| | ↑ | ↑ | ↑ | – | – | – | Morris (1973) |
| | ↓ | ↑ | – | – | ↑ | – | Cooper and Barnett (1976) |
| | ↓ | ↑ | ↓ | ↑ | ↑ | – | Koelkebeck and Biellier (1979) |
| | – | – | – | – | – | ↑ | Nordstrom (1981) |
| | – | ↑ | – | – | – | – | Rezvani and Biellier (1981) |
| | ↓ | ↑ | ↑ | – | – | – | Shanawany (1982) |
| | – | 0 | – | – | – | ↑ | Nordstrom and Oustershout (1983) |
| 27 | ↓ | ↑ | – | – | – | – | Fox et al. (1971) |
| | ↓ | ↑ | – | – | ↑ | – | Morris (1973) |
| | ↓ | ↑ | – | – | ↑ | ↑ | Yassin and Biellier (1978) |
| | – | ↑ | – | – | – | ↑ | Nordstrom (1981) |
| | ↑ | 0 | – | 0 | – | – | Proudfoot (1980) |
| | ↓ | ↑ | ↑ | – | ↑ | – | Shanawany (1982) |

Table 1 (con't)

| L:D chcle length (h) | % Ep[1] | Ewt[2] | Em[3] | Esg[4] | Est[5] | Eswt[6] | References |
|---|---|---|---|---|---|---|---|
| 28 | ↓ | ↑ | – | – | ↑ | – | Cooper and Barnett (1976) |
| | ↓ | – | – | – | – | – | Foster (1969) |
| | 0 | ↑ | – | – | – | – | Leeson et al. (1979) |
| | 0 | ↑ | – | – | – | ↑ | Nordstrom and Andrews (1981) |
| | ↓ | ↑ | ↑ | – | ↑ | – | Shanawany (1982) |
| | – | ↑ | – | – | – | ↑ | Nordstrom and Oustershout (1983) |
| | 0 | ↑ | – | – | – | ↑ | Oustershout and Zimmermann (1983) |
| 30 | ↓ | ↑ | – | – | ↑ | – | Morris (1973) |
| | ↓ | ↑ | – | – | ↑ | – | Cooper and Barnett (1976) |
| | ↓ | ↑ | – | – | – | – | Shanawany (1982) |
| 33 | – | ↑ | – | – | ↑ | ↑ | Morris (1973) |
| | ↓ | ↑ | ↓ | – | – | – | Shanawany (1982) |

[1] Percent egg production

[2] Egg weight

[3] Egg mass

[4] Egg specific gravity

[5] Eggshell thickness

[6] Eggshell weight

↑ = increase

↓ = decrease

0 = no change

and Shanawany (1982) reported that the longer the length of the L:D cycle, the greater is the increase in weight of eggs produced under the cycle. According to these authors, the increase in egg weight was attributed to an increase in yolk size and an increase in albumen and shell deposition. This was due to the extra oviducal term of eggs when ahemeral L:D cycle treatments were applied. Increases in egg weight under ahemeral cycles less than 24 hours was also due to extra oviducal term of eggs since Biellier et al. (1978) reported a mean egg formation time of 26.6 hours for hens kept under a 23 hour L:D cycle.

Compared to conventional 24 hour L:D cycles, hens kept under ahemeral L:D cycles, in most cases, will decrease egg production (Morris, 1973; Cooper and Barnett, 1976; Proudfoot, 1980; Shanawany, 1982) (Table 1). The decrease in egg production for ahemeral L:D cycles have been reported to be almost linear between 24 and 21, and between 25 and 33 hours L:D cycles (Shanawany, 1982). The decline reported was offset by total egg mass (egg weight x number of eggs laid) due to the greater increase in egg weight under L:D cycles greater than 24 but less than 28 hours (Shanawany, 1982). However, Foster (1969; 1972) reported that an ahemeral 26 hour L:D cycle allowed hens to lay in longer sequences or clutches (a number of eggs laid on successive days, then interrupted for one day or more before laying is resumed) which eventually led to increased egg production.

Improvement in egg shell quality under ahemeral lighting is more pronounced for L:D cycles of 27 or more hours compared to L:D cycles of less than 27 hours (Table 1), and very effective for older hens (Morris, 1973; Yassin and Biellier, 1978; Shanawany, 1982; Nordstrom and Ousterhout, 1983). A threshold seems to exist for improvement in egg shell strength when long ahemeral cycles are utilized. Morris (1973) reported that the maximum value for shell thickness was obtained under a 27 hour L:D cycle. This author stated that the improvement in egg shell thickness/quality, via the use of ahemeral lighting greater in length than 24 hours, was due to the eggs spending a longer time in the shell gland of chickens, compared to those chickens kept under a 24 hour L:D cycle, resulting in a longer time for calcium accumulation after the last oviposition.

Lacassagne et al. (1973, cited by Morris, 1978b) reported that hatchability was better for chickens when a 27 hour L:D cycle was used rather than a 24 hour cycle. No explanation was offered for this improvement in hatchability. In a study by Proudfoot (1980), an ahemeral 27 hour L:D cycle failed to cause an increase in hatchability of chicken eggs compared to the response obtained under a 24 hour L:D cycle. The fertility responses obtained under both L:D cycles were very similar. A need to investigate the effect of ahemeral L:D cycles on hatchability, fertility, and embryo mortality in future ahemeral L:D cycle experiments does exist since data on these parameters are not presently available for ring-necked pheasants and are limited for chickens.

Limited information is available on the effect of ahemeral lighting on feed intake, body weight, and mortality in birds. Nordstrom (1981), however, reported a significant improvement in feed efficiency for chickens kept under an ahemeral 27 hour L:D cycle compared to chickens kept under a 24 hour L:D cycle. To the contrary, Proudfoot (1980) reported that a 27 hour L:D cycle had a depressing effect on feed efficiency. In bobwhite quail, Kirkpatrick (1957) reported similar feed intake values under 24, 36, and 40 hour L:D cycles. Proudfoot (1980) and Nordstrom (1981) reported that there was no significant effect of ahemeral L:D cycles on mortality or body weights of chickens.

## C. The Role of Luteinizing Hormone, Progesterone, and Estradiol in the Hen's Ovulatory Cycle

### 1. Hormone functions

#### a) Luteinizing hormone

Luteinizing hormone (LH) is produced by the anterior pituitary gland and functions in stimulating the maturation and rupture of the follicles after follicular growth has been stimulated by follicle stimulating hormone. The secretion of progesterone from the ovary is also stimulated by LH (Sharp, 1980).

#### b) Progesterone

In birds, progesterone ($P_4$) is produced by the follicle (Sturkie and Mueller, 1976). Its secretion increases rapidly as the ruptured follicle becomes luteinized. There

seems to be a significant correlation between follicular development and progesterone secretion (Sturkie and Mueller, 1976). Furr et al. (1973) and Sharp (1980) reported that progesterone stimulates the pituitary to release LH. These authors suggested that progesterone could be a positive feedback hormone for the release of gonadotropins from the pituitary which controls the ovulatory cycle.

c) Estradiol

Estradiol ($E_2$) is produced by the bird's ova, and has been reported to be involved in the growth and development of ovarian follicles (Senior, 1974; Sturkie and Mueller, 1976; van Tienhoven, 1981). It has been suggested by Senior (1974) that estradiol may be essential for the synthesis of yolk protein precursors involved in the conservation of calcium for medullary bone, prior to the onset of laying, in preparation for egg-shell formation. Estrogen is also involved in the growth and differentiation of the oviduct (van Tienhoven, 1981). Similar to progesterone, estradiol has a positive feedback effect on LH release during the hen's ovulatory cycle (Sharp, 1980).

2. Hormone rhythms

The rhythmicity of the above hormones tends to follow a consistent pattern between hens, within the ovulatory cycle (assumed to be approximately 24 hours), although peak time and the circulating concentrations of hormones tend to vary between species and also between studies (Table 2).

Table 2. Hormonal concentration and peak time during the ovulatory cycle of some avian species under various light-dark (L:D)cycles.

| Species | | LH | | | Progesterone | | | Estradiol | | | Reference |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Pt[1] | Pv[2] | Bv[3] | Pt[1] | Pv[2] | Bv[3] | Pt[1] | Pv[2] | Bv[3] | |
| Chicken | (8) | 5 | 3.5 | 1.0 | 3 | 4.5 | 0.5 | -- | -- | -- | Furr et al. (1973) |
| | (8) | 5-4 | 5.5 | 1.5 | 3-2 | 5.5 | 0.5 | -- | -- | -- | Etches and Cunningham (1976) |
| | (8) | 6 | 2.0 | 0.6 | -- | -- | -- | -- | -- | -- | Etches and Cunningham (1977) |
| | (8) | 6 | 2.0 | 0.7 | 4-3 | 5.0 | 0.5 | 6 | 0.15 | 0.075 | Follett and Davies (1978) |
| | (8) | 6 | 2.7 | 1.7 | 6 | 4.5 | 0.5 | -- | -- | -- | Williams and Sharp (1978) |
| | (4) | - | -- | -- | 5-1 | 4.5 | 0.5 | -- | -- | -- | Liou and Biellier (1979) |
| | (8) | 6 | 7.0 | 2.7 | 6 | 4.5 | 0.3 | 6-4 | 0.3 | 0.14 | Johnson and van Tienhoven (1980) |
| | (5) | - | -- | -- | 5-3 | 3.6 | 0.1 | 5-3 | 0.79 | 0.5 | Liou et al. (1980) |
| | (8) | - | -- | -- | 6-4 | 3.7 | 0.5 | -- | -- | -- | Tanaka and Kamiyoshi (1980) |
| | (8) | 5-4 | 4.5 | 1.0 | 5-4 | 6.0 | 1.0 | 5,24 | 0.25 | 0.05 | Tanabe and Nakamura (1980) |
| | (6) | 8-4 | 4.5 | 0.5 | -- | -- | -- | -- | -- | -- | Wilson et al. (1983) |
| | (8) | 6 | 6.0 | 0.5 | -- | -- | -- | -- | -- | -- | White and Etches (1984a) |
| | (7) | 6 | 5.5 | 2.0 | -- | -- | -- | -- | -- | -- | White and Etches (1984a) |
| Duck | (8) | 3 | 8.0 | 2.0 | 3 | 11.0 | 1.5 | 3 | 0.35 | 0.1 | Tanabe and Nakamura (1980) |
| Japanese Quail | (8) | 6 | 5.0 | 1.5 | -- | -- | -- | -- | -- | -- | Follett and Davies (1978) |
| | (8) | 6 | 2.25 | 0.7 | 4 | 3.1 | 0.75 | 6,22 | 0.175 | 0.1 | Tanabe and Nakamura (1980) |
| | (8) | 6-3 | 6.62 | 3.59 | 3 | 5.33 | 0.24 | 6,21 | 0.1 | 0.075 | Gulati et al. (1981) |
| Turkey | (8) | 8-2 | 5.0 | 2.5 | 8-2 | 4.0 | 0.7 | -- | -- | -- | Mashaly et al. (1976) |
| | (8) | 7-5 | 5.0 | 2.0 | -- | -- | -- | -- | -- | -- | Follett and Davies (1978) |
| | (8) | - | -- | -- | 6-4 | 6.9 | 0.69 | 5-7 | 0.21 | 0.077 | Opel and Arcos (1978) |
| | (8) | 8 | 2.7 | 0.3 | 12 | 8.0 | 1.0 | -- | -- | -- | Sharp et al. (1981) |

[1] Peak time (hours) of the hormone, prior to ovulation

[2] Peak value (ng/ml)

[3] Basal value (ng/ml)

[4] Used a 18L:5D cycle

[5] Used a 16L:6D cycle

[6] Used a 16L:8D cycle

[7] Used a 14L:14D cycle

[8] Used a 14L:10D cycle

The LH peak or surge during the hen's ovulatory cycle is known to either coincide with or precedes that of progesterone (Furr et al., 1973; Mashaly et al., 1976; Williams and Sharp, 1978; Johnson and van Tienhoven, 1980; Tanabe and Nakamura, 1980; Gulati et al., 1981). However, in most cases the LH and progesterone peaks have been reported to occur at approximately 6 and 3 hours prior to ovulation, respectively (Etches and Cunningham, 1976, 1977; Follett and Davies, 1978; Tanabe and Nakamura, 1980; Gulati et al., 1981; White and Etches, 1984a). Sharp et al. (1981) also noticed that the progesterone peak may occur as early as 12 hours prior to ovulation in turkeys. The peak of LH and progesterone in birds is absent on days when no ovulation takes place (Cunningham and Furr, 1972; Mashaly et al., 1976; Follett and Davies, 1978). Generally, the basal values of LH and progesterone during the hen's ovulatory cycle tend to be as low as 0.5 ng/ml while peak value can be as high as 8.0 ng/ml. At the time of ovulation, the progesterone and LH levels return to base line concentrations (Furr et al., 1973; Wilson and Sharp, 1973; Mashaly et al., 1976; Etches, 1979; Johnson and van Tienhoven, 1980).

Peaks in blood concentrations of estradiol occur within 3-6 hours prior to ovulation (Opel and Arcos, 1978; Johnson and van Tienhoven, 1980; Liou et al., 1980; Tanabe and Nakamura, 1980; Gulati et al., 1981). In addition, other peaks of estradiol have been reported to occur during the ovulatory cycle of chickens (Tanabe and Nakamura, 1980), and Japanese quails

(coturnix) (Tanabe and Nakamura, 1980; Gulati et al., 1981). These peaks occur at approximately 24 and 21 hours prior to ovulation in chickens and coturnix, respectively. Estradiol has been reported to have values as high as 0.8 ng/ml (Liou et al., 1980) and as low as 0.05 ng/ml during the ovulatory cycle of the bird (Tanabe and Nakamura, 1980).

3. Mechanism of ovulation

In the mature chicken, ovarian follicles develop in response to an increase secretion of LH and FSH (Tucker and Ringer, 1982). As a follicle grows it produces an increased amount of progesterone and estradiol. The increase in estradiol is due to the steroidogenic effect of LH on the rapidly growing follicle (Sharp, 1980). Larger amounts of progesterone are also produced as the follicle nears maturity (Sharp, 1980; Tanabe and Nakamura, 1980; Tucker and Ringer, 1982).

Cunningham and Furr (1972) observed that the administration of progesterone in the fowl was followed 9 to 10 hours later by premature ovulation, provided that the pituitary gland remained in situ for at least 1 hour following the administration of this hormone. In experiments in which lesions were made in the hypothalamus of hens, more than 8 hours before the expected time of premature ovulation, progesterone failed to induce ovulation (Cunningham and Furr, 1972). Ovulation is also known to be blocked by anti-progesterone, but not anti-estradiol serum (Sharp, 1980).

Ovulation occurs when the final state of follicular maturation coincides with the circadian rhythm, controlling the

"open period" in which LH is released over a period of 8 hours each night. In normal L:D cycle treatments, this period is entrained to 24 hours (Cunningham and Furr, 1972; Morris, 1973; Follett and Davies, 1978; Etches, 1979). The onset of darkness normally sets the phase of an internal circadian clock which governs the regular recurrence of LH "open period" which is controlled by the secretion of luteinizing hormone releasing hormone (LHRH) from the hypothalamus (Morris, 1973; 1978a). The first LH release occurs early in the "open period" and is released progressively later each day, resulting in average ovulation and subsequent oviposition time occurring later on successive days. However, there is no shift in the "open period" (Tucker and Ringer, 1982). If follicular maturation occurs after the "open period" there will be no ovulation, resulting in a pause in egg laying (Etches, 1979; Tucker and Ringer, 1982; Wilson et al., 1983). Usually, ovulation occurs within 15 to 30 minutes after ovulation for chickens, coturnix, and turkeys (Warren and Scott, 1936; Wolford et al., 1964a; Woodard and Mather, 1964).

The attainment of the preovulatory peak of LH which leads to the eventual rupture of the follicle seems to follow a cascade of events. The largest follicle, as it grows, becomes increasingly sensitive to gonadotropin stimulation and will ovulate in response to an LH surge. A small increase in the levels of LH will stimulate the ovarian follicle to increase progesterone and estrogen secretion. This in turn causes the preovulatory LH surge, leading to ovulation 4-6 hours later

(Williams and Sharp, 1978; Sharp, 1980). According to Wilson and Sharp (1976), Follett and Davies (1978), Sharp (1980), and van Tienhoven (1981), progesterone exerts a positive feedback effect on the release of LH. There is a first phase called the "priming phase" in which the circulating levels of estrogen and progesterone act to prime the hypothalamus in order to obtain a positive feedback to the incremental change in plasma concentration of progesterone (inductive phase). This leads to release of LHRH by the hypothalamus which then causes the preovulatory release of LH (Figure 2).

Enzyme activity has also been implicated in the ovulation of the chicken ovarian follicle (Nakajo et al., 1973). Nakajo et al. (1973) injected collagenase and non-specific proteases into the wall of follicles and ovulation occurred in almost all of the follicles within 2 to 3 hours. Doi et al. (1980) and Tanabe and Nakamura (1980) suggested that LH acts to synthesize progesterone which in turn produces these enzymes to break the follicular membrane. This is based upon the fact that the content of progesterone is highest in the largest follicle and starts to increase 10 hours prior to ovulation, and reaches a peak in the largest follicle 2 hours prior to ovulation. This was not observed in smaller follicles. To make any definite conclusions regarding enzyme activity in ovulation, enzyme concentrations during the ovulatory cycle need to be measured.

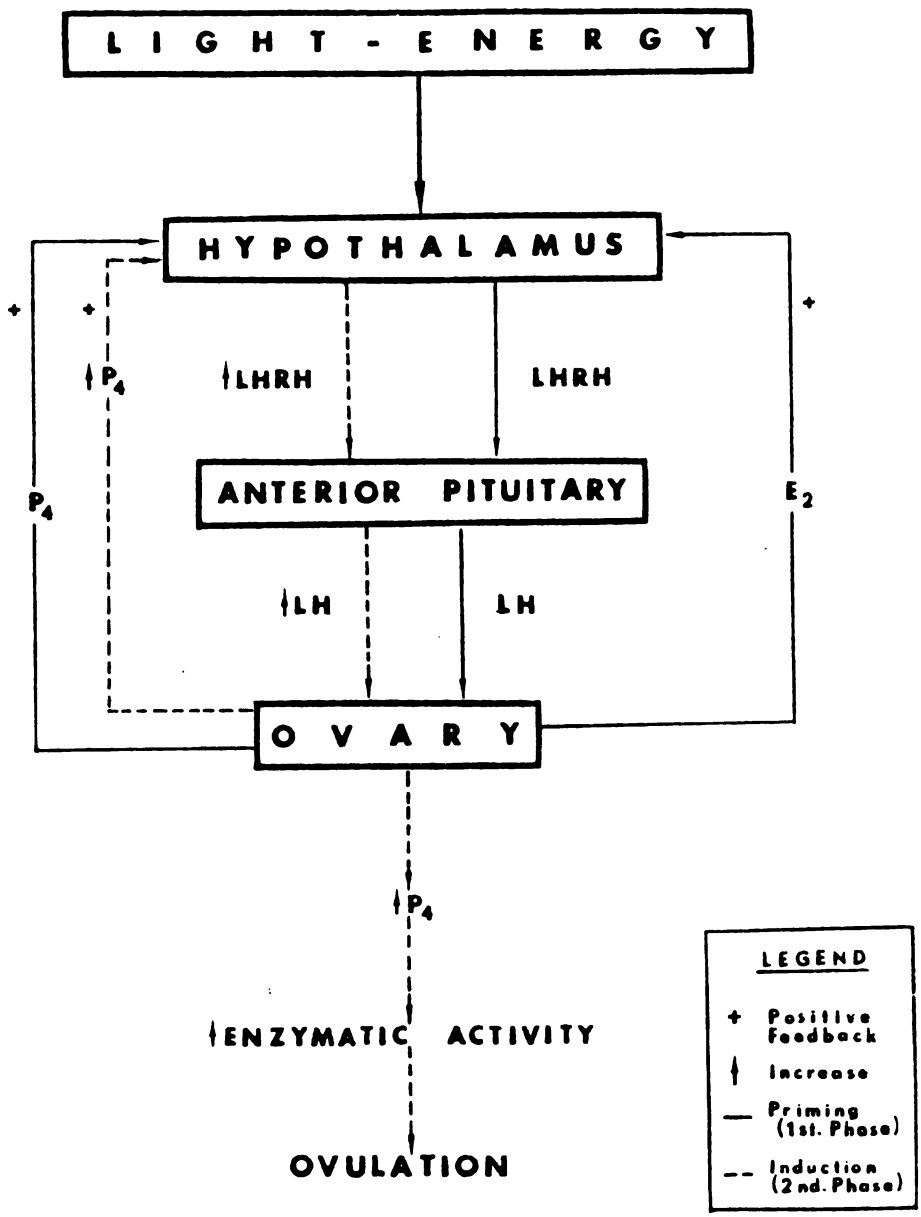


Figure 2. Mechanism of ovulation in avian species.

## D. Egg Production Patterns of Avian Species

When one studies egg production patterns of avian species factors such as lag time, oviposition time, and egg formation time should be considered because they affect the pattern of egg production. At least one of these factors has been reported in chickens, turkeys, coturnix, and bobwhites. However, similar reports cannot be found for the ring-necked pheasant.

### 1. Oviposition time

Oviposition time is the time of day or the clock hour at which a hen lays an egg. In most instances, oviposition occurs as a circadian rhythm, exhibiting some species variation. Oviposition can be entrained by light (Payne and Ortman, 1956; Foster, 1972; Morris, 1973; Bhatti and Morris, 1978). The predominant phase-setting signal for the entrainment is "sunset" with "sunrise" having a minor influence (Morris, 1973). The length of the L:D cycle appears to affect the time of oviposition in chickens and coturnix. According to Ostmann and Biellier (1958), Bhatti and Morris (1978), and Follett and Davies (1978), increasing the length of the L:D cycle resulted in progressive advancement of oviposition time. Under constant lighting, a uniform distribution of oviposition time was achieved in chickens and coturnix (Warren and Scott, 1936; McNally, 1947; Arrington et al., 1962). Feeding cycles were also shown to alter oviposition pattern, from a uniform to a cyclic distribution in coturnix, when feed was given during the

day (light) period in a continuous light cycle (Arrington et al., 1962).

Generally, in chickens, turkeys, and coturnix, most ovipositions occur during the light period (Warren and Scott, 1936; Arrington et al., 1962; Woodard et al., 1963; Tanabe and Nakamura, 1980). Most ovipositions (60%) in chickens will take place within the first 8 hours of the light period, peaking at approximately 5 hours into the light phase (Arrington et al., 1962; Wilson and Huang, 1962; Tanabe and Nakamura, 1980). In turkeys, ducks, and coturnix most ovipositions (80% or more) occur later in the L:D cycle compared to chicken oviposition time (Stockton and Asmundson, 1950; Wilson and Huang, 1962; Woodard et al., 1963; Wolford et al., 1964b; Tanabe and Nakamura, 1980). The peak oviposition time occurs in ducks at 7 hours into the dark phase (Tanabe and Nakamura, 1980), in turkeys at 8 hours into the light phase (Woodard et al., 1963), and in coturnix at 12 hours into the light phase (Wilson and Huang, 1962; Tanabe and Nakamura, 1980). For turkeys, it was postulated by Wolford et al. (1964b) that afternoon oviposition time may result from the fact that egg formation in the turkey required 2-4 hours longer (26-28 hours) than in the chicken (24-26 hours). A longer interval of time between lutenizing hormone release and ovulation could also be involved. This postulation does not apply to coturnix, although a greater percentage of their ovipositions occur in the late afternoon but the time required for egg formation was similar to that for chickens (Sturkie and Mueller, 1976).

2. Lag time

The lag time reported for chickens and turkeys may be defined as the interval of time occurring between successive ovipositions in the same egg sequence minus 24 hours (Woodard et al., 1963; Morris, 1973; Follett and Davies, 1978). For example, if the first egg in a clutch is laid at 8:30 a.m. and the second egg is laid at 9:30 a.m. the following day, then the lag time would be 1 hour. Lag time may be due to successive follicles maturing progressively later in a sequence. The cummulative or total lag (Foster, 1972) represents the extent by which the last egg in a sequence is laid later in the day, in the L:D cycle, than the first egg in the sequence. For chickens and turkeys the total lag tended to increase to a maximum of 8 hours as the number of eggs in a sequence is increased (Wolford et al., 1964b; Foster, 1972; Sturkie and Mueller, 1976). This value corresponds to the period between early morning and mid-afternoon during which eggs are normally laid. These reports were for hens kept under a 24 hour L:D cycle and it is not known how ahemeral cycles would influence lag time in birds.

Generally, as sequence length increases average lag time decreases although total lag increases (Atwood, 1929, Wolford et al., 1964b; Morris, 1973; Follett and Davies, 1978). The greatest lag time between successive eggs in a sequence occurred between the first two eggs and also between the last two eggs (Wolford et al., 1964b; Sturkie and Mueller, 1976).

3. Egg formation time

Egg formation time in birds is defined as the length of time required for all the necessary components of an egg to be added to a post-ovulation ovum as it travels through the reproductive tract to the time of oviposition. For chickens, coturnix, and turkeys egg formation time or intra-clutch interval (the time interval between two successive eggs in a clutch) is approximately 24 to 28 hours (Atwood, 1929; Warren and Scott, 1936; Wolford et al., 1964a; Woodard and Mather, 1964; van Tienhoven, 1981; Tucker and Ringer, 1982). For chickens kept under a 24 hour L:D cycle, Warren and Scott (1935) and Sturkie and Mueller (1976) reported the average time for passage of an ovum through various parts of the reproductive tract to be as follows: infundibulum, 18 minutes; magnum, 2.9 hours; isthmus, 1.4 hours; shell gland, 20.8 hours. Similar data have been reported for the turkey (Wolford et al., 1964a) and coturnix (Woodard and Mather, 1964). However, Wolford et al. (1964a) and Sharp et al. (1981) reported that the ovum spends approximately 23 hours in the shell gland of the turkey.

The length of the L:D cycle affects the egg formation time. For example, Morris (1973) reported that using a 14 hour photoperiod in L:D cycles of 24, 27, and 30 hours, an average egg formation time of 24.9, 27.1, and 29.0 hours were recorded, respectively. Also, changing from a 24 hour alternating L:D sequence to continuous light resulted in a change in egg formation time from 24.8 to 26.8 hours (Arrington et al., 1962).

# CHAPTER 3

## OBJECTIVES

The objectives of this investigation on ring-necked pheasants are listed below.

1. To measure the effect of ahemeral lighting on egg production and egg characteristics.
2. To determine the effect of ahemeral lighting on egg sequence size, lag time, egg formation time, and the rhythm of oviposition.
3. To measure the effect of ahemeral lighting on percentage fertility and hatchability of fertile eggs.
4. To measure the effect of ahemeral L:D cycles on feed intake and body weight changes.
5. To measure and also relate luteinizing hormone and progesterone cyclicity to the time of ovulation.

# CHAPTER 4

## MATERIALS AND METHODS

The purpose of this study was to measure the effect of two ahemeral L:D cycles and a conventional L:D cycle on feed intake, body weight, and reproduction in two strains of ring-necked pheasants during the breeding season.

### A. Experimental Design

This experiment was initiated on January 2, 1982 and terminated on May 21, 1982 for a period of 20 weeks. A total of 108 female pheasants were used in a 2 x 3 factorial design. Light and strain were the treatment factors. The light treatments were, a control 24h L:D cycle (14L:10D), controlled by an Intermatic timer[1], and two ahemeral L:D cycles which included a 22h (14L:8D) and a 26h (14L:12D) L:D cycle controlled by Cramer timers[2]. Each light treatment consisted of 36 female pheasants. This included two strains (MSU and DNR) of 18 birds each. In addition, 12 MSU males were kept in each light treatment for the purpose of producing semen to be used in the articifial insemination (AI) of females.

The rearing procedures used from hatching to the time of the beginning of the experiment were similar to those outlined by Carpenter (1980) and Hussein (1983). The rations fed are

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[1] Intermatic Time Controls, Intermatic Incorporation, Spring Grove, IL 60081.

[2] Conrac, Cramer Division, Old Saybrook, CT.

shown in the Appendix A (Tables A1, A2, and A3). Water and feed were provided ad libitum. Each light treatment was assigned to a separate room. Each room was equipped with a set of stacked battery cages, 20.3 x 40.6 cm, having three rows of 8 cages. In addition, one row of 12 cages of the same dimensions were hooked to one side of each room. Similarly, another row of cages was added to each room into which 12 MSU male pheasants were caged. In each row of cages, 4 females from each strain were placed such that each strain was grouped together. There was one feed and water trough per 4 females per strain. The position of the strains in the rows were alternately arranged from one row to another. Since there were only 18 females per strain it was necessary to have within each strain in a L:D cycle one group which had only 2 females. All the male pheasants were supplied with water from a single trough but were fed in groups of two, such that there was one feed trough per two cages (Appendix B, Figure B1).

Starting at 7 months of age, all birds were randomly placed in cages within a particular L:D cycle and were preconditioned for 12 weeks to that cycle length using a non-stimulatory photoperiod of 4 hours per cycle (Figure 3) supplied from two incandescent 60 watt light bulbs. At age 10 months, the photoperiod was increased to 14 hours which was thought to be stimulatory. An 18 percent pheasant breeder ration (Table 3) and water were supplied ad libitum. The increase in photoperiod for each cycle was for the purpose of bringing females and male pheasants into egg and semen

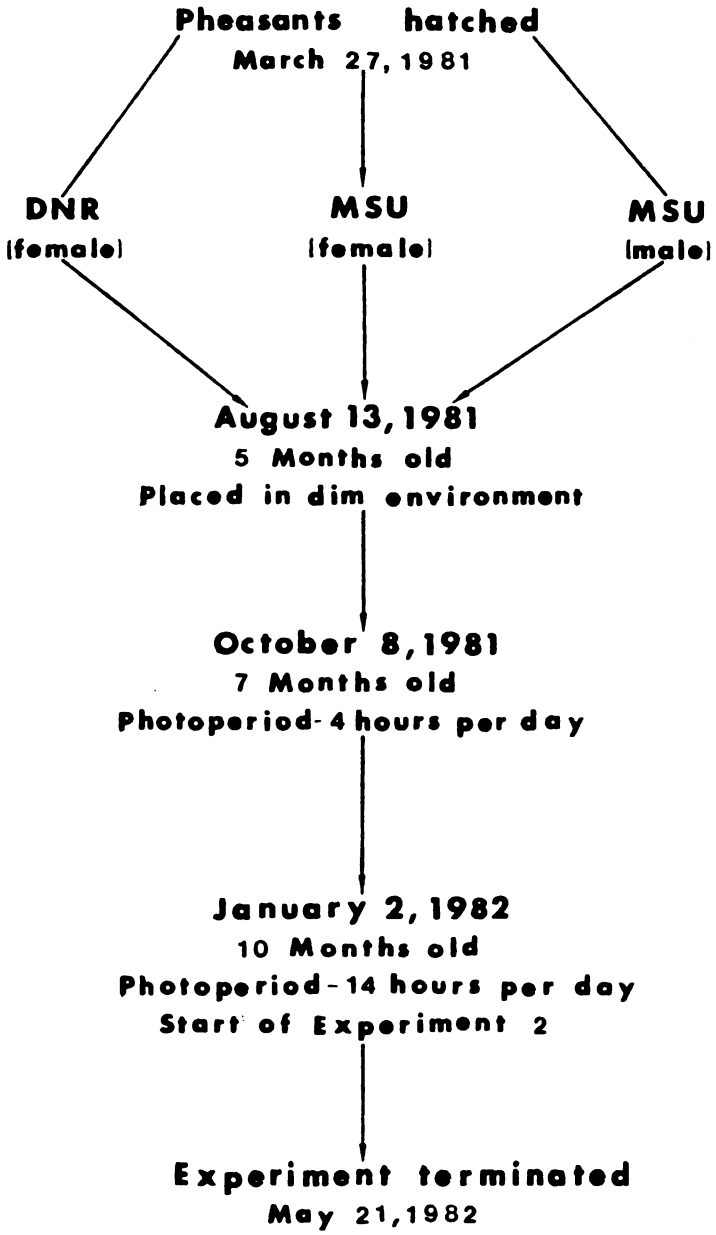


Figure 3. Chronology of study for ring-necked pheasants treated with conventional and ahemeral light-dark cycles.

Table 3. Pheasant breeder ration fed to birds from time of stimulatory lighting to end of egg production.

| Ingredient | Percent |
|---|---|
| Corn | 53.25 |
| Soybean meal, 44% | 15.00 |
| Oats | 7.50 |
| Wheat middling | 7.50 |
| Alfalfa, 17% | 3.00 |
| Fish meal, 60% | 2.50 |
| Meat and bone meal, 50% | 3.00 |
| Whey, dried | 2.00 |
| Salt | 0.25 |
| Dicalcium phosphate | 1.25 |
| Limestone | 3.75 |
| Premix[1] | 0.75 |

[1] Provides the following micronutrients per pound of premix: vitamin A, 600,000 U.S.P. units; vitamin $D_3$, 166,667 I.C. units; riboflavin, 400 mg; pantothenic acid, 800 mg; niacin, 3,334 ng; choline chloride, 41,344 mg; folic acid, 116.7 mg; vitamin $B_{12}$, 1 mg; vitamin E, 500 I.U; menadione sodium bisulfite, 134 mg; thiamine mononitnate, 66.7 mg; manganese, 1.533%; iodine, 0.02%; copper, 0.161%; cobalt, 0.0051; zinc, 1.0%; and iron, 0.5%. Premix obtained from Dawe's laboratories, Inc., 4800 South Richmond Street, Chicago IL 60632.

CALCULATED ANALYSIS

| | |
|---|---|
| Crude protein ..... | 18.00 |
| Fat ..... | 3.44 |
| Fiber ..... | 4.65 |
| Calcium ..... | 2.40 |
| Phosphorus, available ..... | 0.68 |
| M.E. cal/lb. ..... | 1225.00 |

production, respectively. The light intensity at the feed trough level was approximately 86 international lux (8 foot candle). This study lasted for 140 days.

B. Data Collected

1. Body weight

Body weights of all birds were obtained with the use of a Toledo balance[3]. Weights were recorded to the nearest gram. Measurements were made at the beginning of the experiment and thereafter at the 10th and 20th week.

2. Feed intake

Feed consumption for male and female pheasants was determined at 28 day periods. A Homs platform scale[4] was used. No feed intake data were collected for the first 28 days of the study.

3. Egg production

Daily egg production records were kept for each hen. At the end of each 28 day period, a summary was made. The data were analyzed as percent hen-day egg production (average number of eggs laid per hen ÷ 28 x 100). A daily record of the number of cracked (C) or shell-less (SL) eggs was maintained for analysis. The number of cracked and shell-less eggs were expressed in percentage (number C or SL ÷ number of eggs laid per 28 day x 100).

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[3] Toledo Scale Company, Toledo, OH.

[4] Douglas Homs Corporation, Belmont, CA.

4. Egg weight and egg mass

Average egg weight was obtained for each hen by collecting the eggs laid during the last three days of each of five consecutive 28 day periods. Egg weights were recorded to the nearest gram by the use of a Toledo balance[5] designed for individual egg weighing. Egg mass was determined by multiplying the respective average egg weight by total egg production.

5. Egg specific gravity and egg shell thickness

The eggs that were collected for weight measurements were subsequently used to determine the effect of the three lighting regimes on shell quality. This involved the determination of egg specific gravity and shell thickness.

Egg specific gravity was obtained by the floatation of an egg in a sodium chloride solution (kept at 15.6°C (60°F)) using a multiple bucket system. This method of determination was similar to that used by Novikoff and Gutteridge (1949), Njoku (1978), and Rahn (1982). All eggs used in this test were refrigerated at 15.6°C for 24 hours prior to sampling.

The eggs that were used for the determination of egg specific gravity were used for the analysis of egg shell thickness. Both analysis were done within a few hours of each other. The method used for the measurement of egg shell thickness was similar to that described by Reynnells (1979) and Flaga (1981).

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[5] Toledo Scale Company, Toledo, OH.

6. Fertility and hatchability

During the third and fourth 28 day periods of this experiment, all eggs, except those used to test for egg shell quality, were incubated following AI of the hens. Eggs were incubated once per week for 8 consecutive weeks.

Individual hens were artificially inseminated by use of the procedures described by Burrows and Quinn (1935) and Carpenter (1980). Females within each light treatment were inseminated with 0.05 ml of pooled semen from males maintained under the same L:D cycle. The volume of semen used was calculated to contain approximately $378 \times 10^6$, $383 \times 10^6$, and $389 \times 10^6$ spermatozoa per insemination for hens at 22, 24, and 26 hour L:D cycles, respectively. This was based upon an average percentage spermatocrit which was determined to be 16.3, 16.4, and 16.9 for males and 22, 24, and 26 hour L:D cycles, respectively. Determinations were made by the use of a standard curve (Figure 4) correlating hemacytometer values (sperm cell count) (Appendix C) with that of percentage spermatocrit.

The procedures used for the incubation of the pheasant eggs were similar to those of Reynnells (1979), Carpenter (1980), Fuentes (1981), and Hussein (1983). The number of fertile eggs were expressed as a percentage of the total number of eggs set, while the number of hatched eggs, pipped eggs, and unpipped eggs with dead embryos were expressed as a percentage of the fertile eggs.

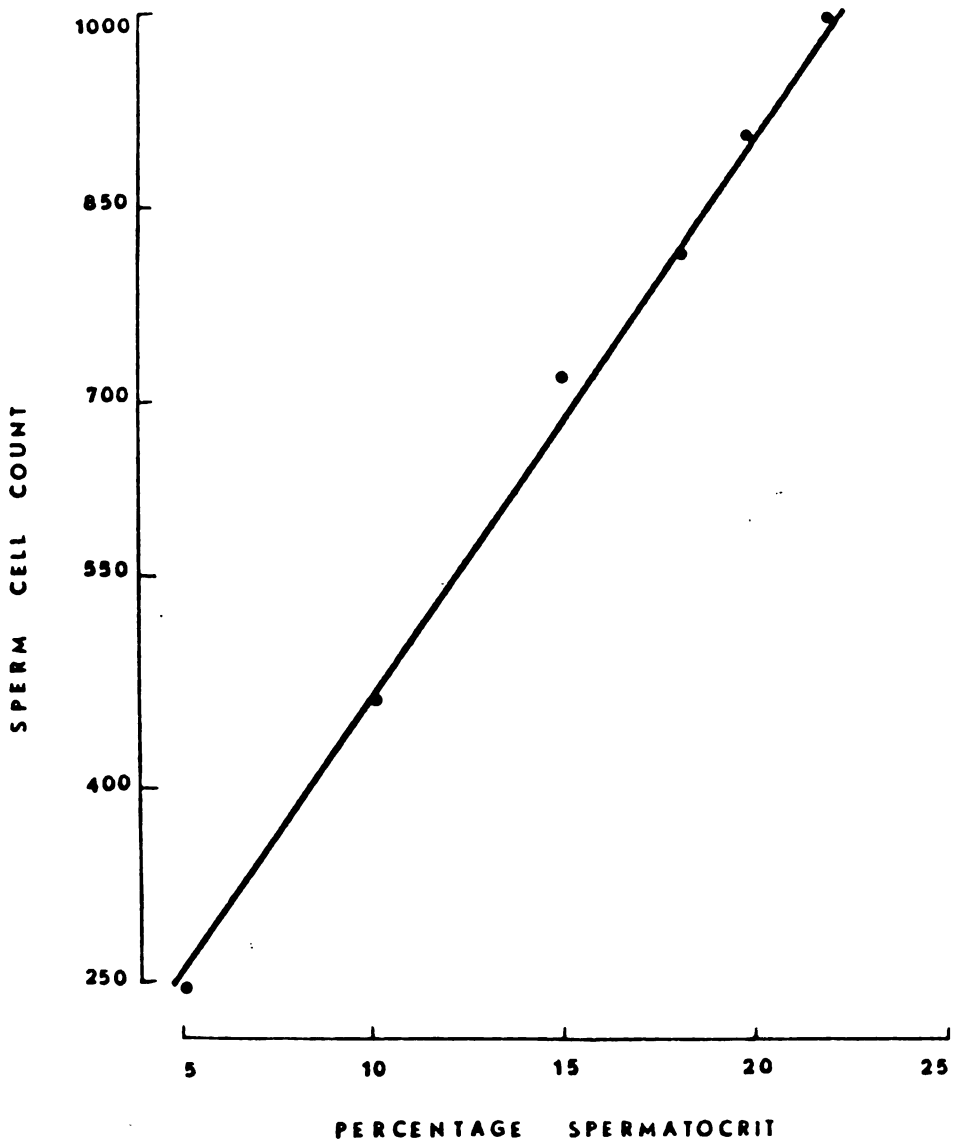


Figure 4. Pheasant sperm cell concentration relative to percent spermatocrit.

7. Oviposition time

Starting midway through the third period of egg production and continuing for 42 days, the time of oviposition to the nearest 15 minutes was recorded for all hens. The collection of oviposition time allows for the determination of lag time, egg formation time (average lag time + 24 hours), the rhythm of oviposition, and egg sequence size.

8. Hormones

Blood samples to be used in the radioimmunoassays (RIA) for the determination of LH and progesterone were obtained during the same period when daily oviposition times were recorded. The procedures for collection of blood are outlined in Appendix D.

a) LH

Pheasant plasma LH concentrations were measured via a micromodification (a final assay volume of 100 µl) of a RIA developed by Follett et al. (1972)[6]. All plasma samples collected from hens exposed to the three L:D cycle treatments were analyzed in one assay. Each sample was analyzed in triplicates of 16 µl. The intra-assay coefficient of variation was 8.6%. The standard curve that was used is represented in Figure 5.

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[6] The LH assay was done in Professor Brian K. Follett's Laboratory, Department of Zoology, University of Bristol, Woodland Road, Bristol, BS8 1UG.

Assay validation: To validate the LH RIA, parallelism of the chicken LH standard and pheasant plasma samples were determined. The pheasant samples were obtained from five individual pheasant hens. Concentrations of LH were measured in different dosages of plasma, which ranged from 2.5 to 20 µl, in increments of 100%. A dose response curve was set up using each hen's LH value to be utilized in comparison with the standard curve. The dose response curves were parallel to the standard curve (Figure 6).

b) Progesterone

The RIA procedures used for the analysis of pheasant plasma progesterone concentrations were previously reported for turkeys (Mashaly and Wentworth, 1974) and pheasants (Mashaly et al., 1982). The antibody, designated Lot #112179-38, used in the assay was raised against 3-carboxymethyloxine:bovine serum albumin in female New Zealand White rabbits[7].

Progesterone was extracted from plasma with 2 ml of toulene: hexane (1:2). The extraction efficiency was 82.7 ± 1.85 (SEM) %. Extracts were separated from the plasma by placing the extraction tubes containing samples (100 µl) and the solvents in a dry-ice and methanol bath for freezing. The aqueous layer formed contained the solvent and extracted progesterone. This aqueous layer was used for the determination of

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[7] The progesterone antibody was obtained from Dr. M.M. Mashaly, Poultry Science Department, Pennsylvania State University, University Park, PA 16802.

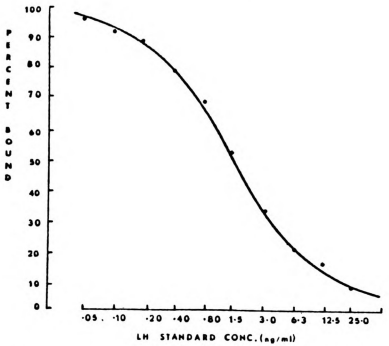



Figure 5. Representative dose-response curve of standard LH.

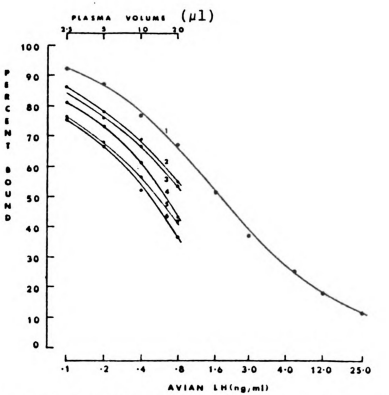



Figure 6. A representative dose-response curve of standard LH (1) and the dose-response curves of LH concentrations in pheasant plasma. EAch curve (2-6) represents the response for a separate bird.

progesterone concentration. The extraction efficiency was determined by preparing four separate extraction tubes, each containing 10 µl of $^{3}H$-progesterone (used as progesterone tracer at 5,000 cpm/10 µl) and 100 µl of plasma sample from the same pool for extraction. The extracts were evaporated in scintillation vials and then quantified for radioactivity, which was expressed as a percentage of the total cpm of the progesterone tracer. This was used to determine the extraction efficiency in the assay.

Triplicate standard curves (.0, .0, .02, .04, .06, .10, .20, .40, .60, 1.0, and 2.0 ng of progesterone) were prepared for each assay (a total fo three assays were done). A representative standard curve is shown in Figure 7. Prior to the removal of unbound $^{3}H$-progesterone by dextran-coated charcoal, the tubes were placed in a refrigerated centrifuge at 4°C for 30 minutes. This resulted in a 32% binding of the antibody. Centrifugation was done at 2230 x g for 15 minutes after the 30 minutes equilabration time. The intra-assay and inter-assay coefficient of variation was 9.0 and 0.0%, respecitvely.

Assay validation: The validation of the progesterone assay was similar to that described by Mashaly et al. (1982). These researchers compared parallelism of the progesterone standard with pooled, stripped and unstripped pheasant plasma. Pooled pheasant plasma was stripped of steroids, using 25% dextran-coated charcoal. The dosages used for the determination of progesterone concentrations in the stripped plasma

were 20, 40, 80, and 100 µl, while the dosages of unstripped plasma used were 20, 40, 100 and 200 µl. The results are shown in Figure 8. The dose-response curve for the pooled unstripped, but not the pooled stripped plasma was parallel to the standard curve.

C. Statistical Analysis

A statistical computing package, Genstat (Alvery et al., 1982), was used to compute the analysis of variance (ANOVA) for split-plot models. The statistical model is represented in Appendix E. The procedures outlined by Gill (1978b) for analysis of factorial split-plot experiments were used (Appendix E) except that a one-way ANOVA was used in the computation of the data obtained from the hormone analysis and the distribution of oviposition time.

The Bonferroni t-statistics (Gill, 1978a,c) was used to test for significant differences between treatment means when multiple comparisons were desired. The Dunnett's t-statistics (Gill, 1978a,c) was used to test for significant differences between means in the one-way ANOVA, where it was desired to test the peak time versus other times.

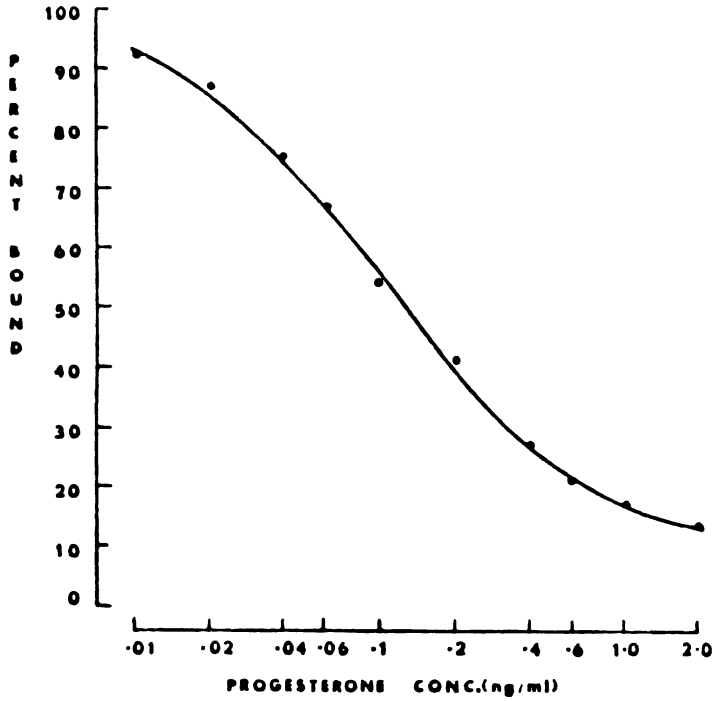



Figure 7. Representative dose-response curve of standard progesterone.

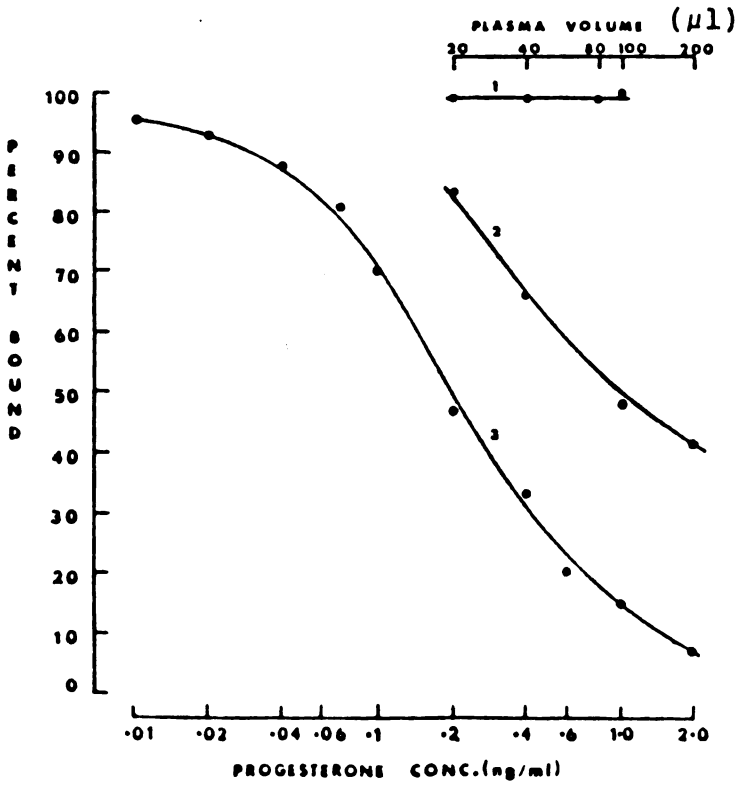



Figure 8. A representative dose-response curve of standard progesterone (3) and dose-response curve of pooled stripped (1) and unstripped (2) pheasant plasma.

# CHAPTER 5

## RESULTS AND DISCUSSIONS

All results expressed on a per day basis in this study were calculated based upon a 24 hour day.

### A. Male Feed Intake and Body Weight

The effects of the control 24h (14L:10D), 22h (14L:8D), and ahemeral 26h (14L:12D) L:D cycle on male ring-necked pheasant feed intake and body weight are summarized in Tables 4 and 5, respectively. There were no significant ($P > 0.05$) differences in feed intake or body weight between light treatments.

Males reared under the 24 hour and 22 hour L:D cycles had similar feed intakes. The highest average feed intake was recorded for males under the ahemeral 26 hour L:D cycle (Table 4). It was speculated that increases in feed intake during Period 1 could be due to the males' "anticipation" of increasing their activity since photoperiod length was increased to 14 hours at the beginning of the experiment. The fluctuations recorded in feed intake for males kept under the respective L:D cycles cannot be fully explained. It is not known if the weekly handling of these birds for semen collection during Periods 2 and 3 could stress them, resulting in reduced feed intake and subsequent body weight reductions (Table 5).

Table 4. Average feed intake (gm/bird/day) of male pheasants under 22, 24, or 26 hour light-dark cycles at 4 week intervals[1,2].

| Period | 14L:8D | 14L:10D | 14L:12D |
|---|---|---|---|
| 1 | 100.1 | 114.3 | 105.9 |
| 2 | 84.5 | 69.1 | 84.6 |
| 3 | 42.8 | 36.8 | 47.4 |
| 4 | 97.8 | 105.6 | 120.9 |
| Avg. | 81.3[a] | 81.5[a] | 89.7[a] |

[a] Means with the same symbol do not differ significantly ($P > 0.05$).

[1] The standard error of difference (SED) (homogenous variances, split plot design, see Gill, 1978b) between any two treatment means within a period is $\pm$ 13.7 gm. The SED between any two period means within a treatment is $\pm$ 11.1 gm.

[2] Mean of 12 birds per treatment.

Table 5. Average body weight (gm/bird) of male pheasants under 22, 24, or 26 hour light-dark cycles at 10 week intervals[1,2].

| Period | 14L:8D | 14L:10D | 14L:12D |
|---|---|---|---|
| 0[3] | 1489.0 | 1527.4 | 1525.6 |
| 1 | 1411.4 | 1387.5 | 1487.4 |
| 2 | 1466.2 | 1418.1 | 1547.7 |
| Avg. | 1455.5[a] | 1444.3[a] | 1520.2[a] |

[a] Means with the same symbol do not differ significantly ($P > 0.05$).

[1] The standard error of difference (SED) (repeated measurements; split plot design, see Gill, 1978b) between any two treatment means within a period is $\pm$ 116.3 gm. The SED between any two period means within a treatment is $\pm$ 42.8 gm.

[2] Means of 12 birds per treatment.

[3] Day 1 of experiment.

B. Female Feed Intake and Body Weight

Ahemeral light treatments had no significant ($P > 0.05$) effect on feed intake (Table 6) or body weight (Table 7) of female pheasants. Although the average female body weight was lower than the males body weight (Table 5) in this study, females consumed an average of 14 gm/bird/day (g/b/d) more feed than the males. The higher average feed intake by the females, compared to the males, was due to the greater reproductive activity associated with egg laying. Similar to the males, the period of highest feed intake for females under any of the L:D cycles was Period 1. It was also thought that this was due to the females' "anticipation" of increasing their reproductive activity because of the stimulatory photoperiod that was provided. The feed intake averages in this experiment greatly exceed the average (65.5 g/b/d) reported by Fuentes (1981) for laying ring-necked pheasants kept under a 24 hour L:D cycle. This difference in feed intake was not due to the difference in percent hen-day egg production (PHDEP). The average PHDEP (63.6%) for hens in Fuentes study was only 5.1% more than for pheasants in this experiment.

As shown in Table 7, the DNR strain body weight was significantly ($P \leq 0.05$) higher than the body weight of the MSU strain. However, there was no significant ($P > 0.05$) difference in feed intake between these strains. There were also no significant ($P > 0.05$) light x strain interactions for feed intake or body weight.

Table 6. Average feed intake (gm/bird/day) for two strains of female pheasants under 22, 24, or 26 hour light-dark cycles at 4 week intervals[1,2,3].

| Strain | Period | Light treatment 22 (14L:8D) | 24 (14L:10D) | 26 (14L:12D) | Avg. |
|---|---|---|---|---|---|
| DNR | 1 | 130.4 | 145.2 | 135.8 | 137.1 |
| | 2 | 108.8 | 111.3 | 88.4 | 102.8 |
| | 3 | 77.0 | 83.9 | 61.5 | 74.1 |
| | 4 | 107.3 | 108.3 | 106.6 | 107.4 |
| | Avg. | 105.9 | 112.2 | 98.1 | 105.4[a] |
| MSU | 1 | 114.0 | 117.2 | 106.6 | 112.6 |
| | 2 | 87.0 | 91.8 | 99.9 | 92.9 |
| | 3 | 61.6 | 75.7 | 75.1 | 70.8 |
| | 4 | 93.5 | 86.4 | 89.7 | 90.5 |
| | Avg. | 89.5 | 92.8 | 92.8 | 91.7[a] |
| Overall Average | | 97.7[b] | 102.5[b] | 95.5[b] | |

[a] Means with the same symbol within a column do not differ significantly ($P > 0.05$).

[b] Means with the same symbol within a row do not differ significantly ($P > 0.05$).

[1] The standard error of difference (SED) (repeated measurements; split plot design, see Gill, 1978b) between any two light treatment means within a period is ± 19.8 gm. The SED between any two period means within a treatment is ± 9.0 gm.

[2] The SED between two strain means within a period is ± 16.2 gm. The SED between any two period means within a strain is ± 7.3 gm.

[3] Means of 18 birds per strain or 36 birds per light treatment.

Table 7. Average body weight (gm/bird) for two strains of female pheasants under 22, 24, or 26 hour light-dark cycles at 10 week intervals[1,2,3].

| Strain | Period | Light treatments 22 (14L:8D) | 24 (14L:10D) | 26 (14L:12D) | Avg. |
|---|---|---|---|---|---|
| DNR | 0[4] | 1341.7 | 1356.3 | 1329.7 | 1342.6 |
| | 1 | 1331.8 | 1292.7 | 1283.1 | 1302.5 |
| | 2 | 1297.6 | 1239.9 | 1246.3 | 1202.1 |
| | Avg. | 1323.7 | 1296.3 | 1286.4 | 1302.1[a] |
| MSU | 0[4] | 1176.6 | 1215.9 | 1207.8 | 1200.1 |
| | 1 | 1163.5 | 1180.2 | 1199.1 | 1181.0 |
| | 2 | 1141.6 | 1212.0 | 1141.0 | 1164.9 |
| | Avg. | 1160.6 | 1202.7 | 1182.6 | 1182.0[b] |
| Overall Average | | 1242.1[c] | 1249.5[c] | 1234.5[c] | |

[a,b] Means with different symbols within a column differ significantly ($P \leq 0.05$).

[c] Means with the same symbol within a row do not differ significantly ($P > 0.05$).

[1] The standard error of difference (SED) (repeated measurements; split plot design, see Gill, 1978b) between any two light treatment means within a period is ± 53.5 gm. The SED between any two period means within a treatment is ± 13.5 gm.

[2] The SED between two strain means within a period is ± 43.5 gm. The SED between any two period means within a treatment is ± 10.9 gm.

[3] Means of 18 birds per strain or 36 birds per light treatment.

[4] Day 1 of experiment.

Comparisons cannot be made between the feed intake results obtained in this study and other studies. The author is not aware of any other report on the effect of ahemeral L:D cycles on female pheasants feed intake.

C. Percent Hen-Day Egg Production

The first egg collected under all three light treatments occurred 7 days after the length of the light period within each treatment was increased to 14 hours of light per cycle. All hens under the 24 hour L:D cycle started laying by January 31, 1982, for the ahemeral 22 hour L:D cycle, it was on January 29, 1982, and for the ahemeral 26 hour L:D cycle, it was January 27, 1982, except for one hen which did not lay until February 7, 1982. The data on percent hen-day egg production (PHDEP) are shown in Table 8 and are also presented graphically in Figures 9 and 10 for the effect of light and strain treatments, respectively. There was a trend for pheasants exposed to ahemeral L:D cycles to improve PHDEP. This trend was not significant ($P > 0.05$) compared to the PHDEP for hens exposed to the 24 hour L:D cycle (Table 8). Also the trend for ahemeral L:D cycles to improve pheasant egg production does not agree with the results obtained in chickens where ahemeral L:D cycles has shown to decrease egg production (Morris, 1973; Cooper and Barnett, 1976; Koelkebeck and Biellier, 1979; Proudfoot, 1980). However, Foster (1968; 1972) suggested that if chickens were selected such that their intra-clutch intervals were synchronized with the length

Table 8. Average percent hen-day egg production for two strains of pheasants under 22, 24, or 26 hour light-dark cycles at 28 day intervals[1,2,3].

| Strain | Period | Light treatment 22 (14L:8D) | 24 (14L:10D) | 26 (14L:12D) | Avg. |
|---|---|---|---|---|---|
| DNR | 1 | 12.3 | 8.7 | 10.5 | 10.5 |
| | 2 | 81.7 | 72.6 | 83.1 | 79.2 |
| | 3 | 75.6 | 77.2 | 82.1 | 78.3 |
| | 4 | 72.6 | 68.7 | 75.2 | 72.2 |
| | 5 | 51.4 | 53.5 | 54.2 | 53.0 |
| | Avg. | 58.7 | 56.1 | 61.0 | 58.6[a] |
| MSU | 1 | 13.8 | 9.2 | 10.3 | 11.4 |
| | 2 | 76.5 | 75.0 | 80.5 | 77.5 |
| | 3 | 73.1 | 67.8 | 86.1 | 75.6 |
| | 4 | 72.5 | 70.4 | 82.5 | 75.2 |
| | 5 | 54.6 | 48.4 | 53.5 | 52.2 |
| | Avg. | 58.1 | 54.2 | 62.6 | 58.3[a] |
| Overall Average | | 58.4[b] | 55.2[b] | 61.8[b] | |

[a] Means with the same symbol within a column do not differ significantly ($P > 0.05$).

[b] Means with the same symbol within a row do not differ significantly ($P > 0.05$).

[1] The standard error of difference (SED) (repeated measurements; split plot design, see Gill, 1978b) between any two light treatment means within a period is $\pm$ 7.31. The SED between any two period means within a treatment is $\pm$ 3.7.

[2] The SED between two strain means within a period is $\pm$ 5.96. The SED between any two period means within a strain is $\pm$ 3.01.

[3] Mean of 18 birds per strain or 36 birds per light treatment.

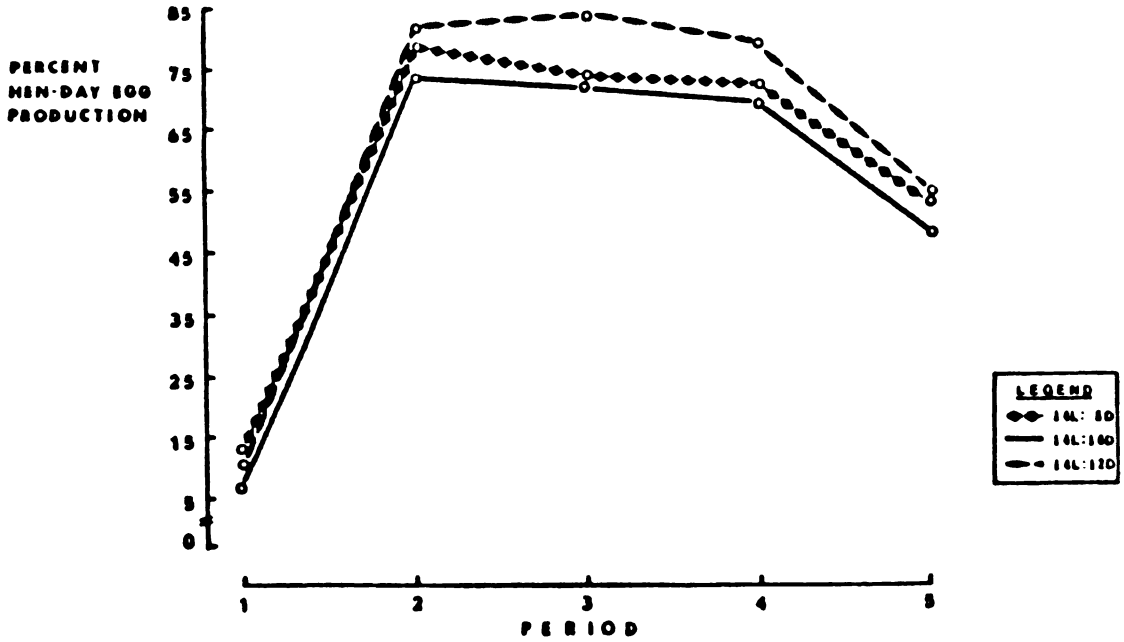



Figure 9. Effect of 22, 24, or 26 hour light-dark cycles on average percent hen-day egg production of pheasants at 28-day intervals. The SED between any two treatment means within a period is ± 7.31. The SED between any two period means within a treatment is ± 3.7. Arc sin $\sqrt{\%}$ transformation was used for statistical analysis.

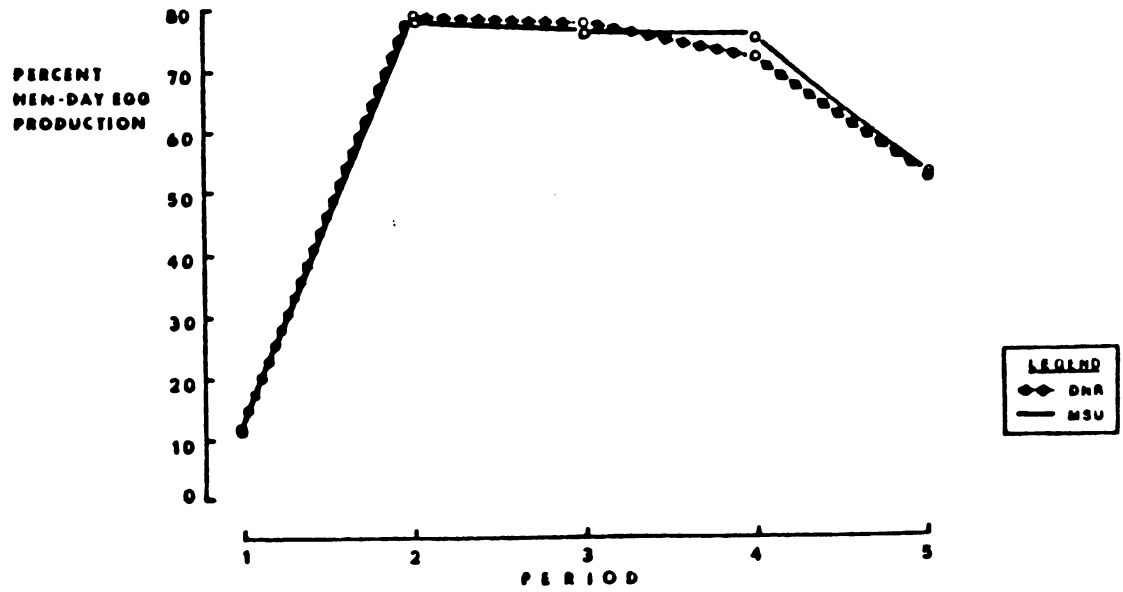



Figure 10. Strain differences in average percent hen-day egg production (PHDEP) of pheasants at 28-day intervals. The SED between any two treatment means within a period is ± 5.96 PHDEP. The SED between any two period means within a treatment is ± 3.01 PHDEP. Arc sin $\sqrt{\%}$ transformation was used for statistical analysis.

of the L:D cycle under which they were to be kept, an improvement in egg production could result. Interestingly, the hens used in this experiment were not selected for intra-clutch interval. Thus, pheasants probably respond differently to ahemeral L:D cycles than do chickens. Neither strain nor light x strain interactions had any significant ($P > 0.05$) effect on PHDEP.

The PHDEP obtained under the light L:D cycle treatments and for the strain effects peaked at approximately one month after laying started (Figures 9 and 10). This has also been reported to occur in chickens kept under a 24 hour L:D cycle (Bowman, 1960; Bowman and Jones, 1961). An interesting phenomenon of two eggs per L:D cycle was observed occasionally for some hens in this experiment. The occurrence of 2 eggs a day, preceded and followed by another egg, occurred under the 24, 22, and 26 hour L:D cycles on 9, 24, and 17 occasions, respectively. The occurrence of 2 eggs a day preceded or followed by no egg, occurred under the 24, 22, and 26 hour L:D cycles on 13, 36, and 9 occasions, respectively. There were also other incidences of 2 eggs a day by hens for which one of the eggs was shell-less, thus these were not included. This phenomenon could be inherent since it occurs under all three L:D cycle treatments. However, it appears that the occurrence of 2 eggs per L:D cycle can be altered by light. Every precaution was taken to assure that eggs from neighboring cages could not roll over to these hen's cages. In

most cases when a hen laid 2 eggs a day, the other neighboring hens also laid. At the end of the study, several hens were sacrificed and necropsied, in order to determine if more than one egg was present in their reproductive tract. Two hens were found to have a hard shell egg in their shell gland along with a shell-less egg with a shell membrane intact at the end of the isthmus. It was therefore evident that these hens were occasionally laying two eggs per L:D cycle. Neither ahemeral L:D cycles nor strain treatments had any significant ($P > 0.05$) effect on percent cracked eggs (Table 9) and percent shell-less eggs (Table 10). As the experiment progressed, the percentage of cracked eggs obtained under the ahemeral L:D cycles continued to increase. This was due to the continued decrease in egg shell thickness which will be discussed in a latter section. The percentage of shell-less eggs (PSE) laid by hens under the three L:D cycles also continued to increase as the experiment progressed. The highest values obtained were during Period 5 (Table 10), which was most evident for the MSU strain. According to Romanoff and Romanoff (1949), shell-less eggs are laid because of either a failure of the shell secreting glands of the oviduct or violent peristalsis which hurried the eggs through the shell gland before a shell can be formed. It is not known if the stress of handling the hens during the process of artificial insemination and the withdrawal of blood could have resulted in increase peristalsis activity of the hens oviduct, resulting in the trend for these hens to increase PSE towards the latter part of the experiment.

Table 9. Average percent cracked eggs for two strains of pheasants under 22, 24, or 26 hour light-dark cycles at 28 day intervals[1,2,3].

| Strain | Period | Light treatment 22 (14L:8D) | 24 (14L:10D) | 26 (14L:12D) | Avg. |
|---|---|---|---|---|---|
| DNR | 1 | 2.79 | 0.02 | 0.05 | 0.91 |
| | 2 | 1.00 | 0.59 | 0.00 | 0.53 |
| | 3 | 3.76 | 0.68 | 0.22 | 1.55 |
| | 4 | 4.42 | 0.43 | 5.10 | 3.31 |
| | 5 | 1.57 | 0.36 | 8.96 | 3.63 |
| | Avg. | 2.71 | 0.42 | 2.87 | 2.00[a] |
| MSU | 1 | 2.03 | 0.09 | 0.26 | 0.62 |
| | 2 | 0.44 | 0.74 | 1.00 | 0.72 |
| | 3 | 0.00 | 0.20 | 0.74 | 0.30 |
| | 4 | 1.57 | 0.71 | 1.51 | 1.26 |
| | 5 | 4.78 | 0.09 | 1.93 | 2.21 |
| | Avg. | 1.76 | 0.37 | 1.09 | 1.07[a] |
| Overall Average | | 2.24[b] | 0.40[b] | 1.98[b] | |

[a] Means with the same symbol within a column do not differ significantly ($P > 0.05$).

[b] Means with the same symbol within a row do not differ significantly ($P > 0.05$).

[1] The standard error of difference (SED) (repeated measurements; split plot design, see Gill, 1978b) between any two light treatment means within a period is $\pm$ 2.25. The SED between any two period means within a treatment is $\pm$ 1.64.

[2] The SED between two strain means within a period is $\pm$ 1.84. The SED between any two period means within a strain is $\pm$ 1.34.

[3] Mean of 18 birds per strain or 36 birds per light treatment.

Table 10. Average percent shell-less eggs for two strains of pheasants under 22, 24, or 26 hour light-dark cycles at 28 day intervals[1,2,3].

| Strain | Period | Light treatment 22 (14L:8D) | 24 (14L:10D) | 26 (14L:10D) | Avg. |
|---|---|---|---|---|---|
| DNR | 1 | 1.63 | 0.90 | 0.93 | 0.55 |
| | 2 | 1.44 | 0.00 | 0.47 | 0.63 |
| | 3 | 1.83 | 4.54 | 7.53 | 4.63 |
| | 4 | 3.81 | 9.71 | 4.62 | 5.71 |
| | 5 | 0.76 | 7.93 | 8.70 | 5.80 |
| | Avg. | 1.89 | 4.42 | 4.45 | 3.46[a] |
| MSU | 1 | 0.13 | 0.41 | 6.02 | 2.10 |
| | 2 | 0.77 | 1.85 | 0.26 | 0.96 |
| | 3 | 3.17 | 6.34 | 2.29 | 3.93 |
| | 4 | 3.09 | 2.72 | 3.89 | 3.23 |
| | 5 | 10.83 | 13.20 | 8.22 | 10.75 |
| | Avg. | 3.6 | 4.90 | 4.13 | 4.19[a] |
| Overall Average | | 2.74[b] | 4.66[b] | 4.29[b] | |

[a] Means with the same symbol within a column do not differ significantly ($P > 0.05$).

[b] Means with the same symbol within a row do not differ significantly ($P > 0.05$).

[1] The standard error of difference (SED) (repeated measurements; split plot design, see Gill, 1978b) between any two light treatment means within a period is $\pm$ 3.6. The SED between any two light treatment means within a treatment is $\pm$ 2.44.

[2] The SED between two strain means within a period is $\pm$ 2.9. The SED between any two period means within a strain is $\pm$ 1.9.

[3] Mean of 18 birds per strain or 36 birds per light treatment.

D. Egg Mass and Egg Weight

The average egg mass and egg weight are shown in Tables 11 and 12, respectively. Egg mass was not significantly ($P > 0.05$) affected by light or strain treatments. However, the DNR strain had a significantly ($P \leq 0.05$) higher egg weight compared to the MSU strain. Carpenter (1980) also reported higher egg weights for the DNR strain compared to the egg weight of the MSU strain. Ahemeral L:D cycles did not significantly ($P > 0.05$) affect ring-necked pheasant egg weight compared to the egg weight of ring-necked pheasants exposed to the control 24 hour L:D cycle. The average egg weight response under the ahemeral 26 hour L:D cycle was significantly ($P \leq 0.05$) less than the average egg weight response under the ahemeral 22 hour L:D cycle.

The tendency for the average egg mass to decrease for hens exposed to the ahemeral 22 hour L:D cycle compared to those hens exposed to the control 24 hour L:D cycle is in agreement with the reports of Rezvani and Biellier (1981) and Shanawany (1982). These researchers reported a decrease or no change in egg mass for chickens reared under ahemeral L:D cycles of less than 24 hours. However, the decrease in egg mass for the 26 hour L:D cycle does not agree with previous studies which showed that ahemeral L:D cycles greater than 24 hours increased egg mass for chickens compared to the egg mass obtained under a 24 hour L:D cycle (Morris, 1973; Shanawany, 1982).

The significantly lower egg weight response under the ahemeral 26 hour L:D cycle was the reason for the lower

Table 11. Average total egg mass (gm/bird) for two strains of pheasants under 22, 24, or 26 hour light-dark cycles at 28 day intervals[1,2,3].

| Strain | Period | Light treatment 22 (14L:8D) | 24 (14L:10D) | 26 (14L:12D) | Avg. |
|---|---|---|---|---|---|
| DNR | 1 | 119.6 | 78.4 | 97.2 | 98.4 |
| | 2 | 792.3 | 731.2 | 789.0 | 770.8 |
| | 3 | 732.2 | 799.2 | 731.8 | 754.4 |
| | 4 | 703.5 | 747.1 | 682.9 | 711.2 |
| | 5 | 701.1 | 657.2 | 554.3 | 637.5 |
| | Avg. | 609.7 | 602.6 | 571.0 | 594.5[a] |
| MSU | 1 | 124.2 | 64.3 | 95.0 | 94.5 |
| | 2 | 727.5 | 748.2 | 711.3 | 729.0 |
| | 3 | 673.5 | 774.7 | 775.3 | 741.2 |
| | 4 | 651.9 | 730.8 | 732.0 | 704.9 |
| | 5 | 577.8 | 617.3 | 561.6 | 585.6 |
| | Avg. | 551.0 | 587.1 | 575.0 | 571.0[a] |
| Overall Average | | 580.3[b] | 594.8[b] | 573.0[b] | |

[a] Means with the same symbol within a column do not differ significantly ($P > 0.05$).

[b] Means with the same symbol within a row do not differ significantly ($P > 0.05$).

[1] The standard error of difference (SED) (repeated measurements; split plot design, see Gill, 1978b) between any two light treatment means within a period is ± 143.7 gm. The SED between any two period means within a treatment is ± 74.4 gm.

[2] The SED between two strain means within a period is ± 117.4 gm. The SED between any two period means within a strain is ± 60.8 gm.

[3] Means of 18 birds per strain or 36 birds per light treatment.

Table 12. Average egg weight (gm) for two strains of pheasants under 22, 24, or 26 hour light-dark cycles at 28 day intervals[1,2,3].

| Strain | Period | Light treatment 22 (14L:8D) | 24 (14L:10D) | 26 (14L:12D) | Avg. |
|---|---|---|---|---|---|
| DNR | 1 | 34.7 | 33.9 | 33.0 | 33.6 |
| | 2 | 34.4 | 34.2 | 33.6 | 34.1 |
| | 3 | 34.1 | 34.3 | 31.7 | 33.4 |
| | 4 | 34.8 | 33.5 | 31.7 | 33.3 |
| | 5 | 34.5 | 34.1 | 32.1 | 33.6 |
| | Avg. | 34.5 | 34.0 | 32.4 | 33.6[a] |
| MSU | 1 | 32.6 | 32.9 | 31.9 | 32.5 |
| | 2 | 32.6 | 31.6 | 31.6 | 32.3 |
| | 3 | 31.9 | 32.7 | 31.7 | 32.1 |
| | 4 | 32.4 | 30.9 | 31.5 | 31.6 |
| | 5 | 30.6 | 32.0 | 30.5 | 31.0 |
| | Avg. | 32.2 | 32.0 | 31.4 | 31.9[b] |
| Overall Average | | 33.4[c] | 33.0[c,d] | 31.9[d] | |

[a,b] Means with different symbols within a column differ significantly ($P \leq 0.05$).

[c,d] Means with different symbols within a row differ significantly ($P \leq 0.05$).

[1] The standard error of difference (SED) (repeated measurements; split plot design, see Gill, 1978b) between any two light treatment means within a period is ± 1.23 gm. The SED between any two period means within a treatment is ± 0.16 gm.

[2] The SED between two strain means within a period is ± 1.0 gm. The SED between any two period means within a strain is ± 0.32 gm.

[3] Means of 18 birds per strain or 36 birds per light treatment.

average egg mass obtained under the 26 hour L:D cycle compared to the average egg mass obtained under the 22 hour L:D cycle. Thus, the slightly higher PHDEP response under the 26 hour L:D cycle (Table 8) was not enough to off set the lower average egg weights of pheasants exposed to the 26 hour L:D cycle. Failure of hens reared under the 26 hour ahemeral L:D cycle to increase egg weight compared to the control hens was not consistent with reports on egg weights for chickens exposed to other ahemeral L:D cycles (Foster, 1969; Fox et al., 1971; Morris, 1973; Cooper and Barnett, 1976; Koelkebeck and Biellier, 1979; Shanawany, 1982).

In chickens kept under a 24 hour L:D cycle, Bennion and Warren (1933) reported that during the annual production, egg weight starts to increase and reach a peak where it plateaus for a while and then starts to decline in a fluctuating manner. This pattern of egg weight response for chickens was not observed for the pheasant hens under any of the three L:D cycles utilized in this experiment. Instead, egg production pattern fluctuates throughout the duration of the study. It is possible that pheasants egg weight pattern of response during the egg laying cycle is different from the response of chickens.

## E. Egg Specific Gravity and Egg Shell Thickness

Measurements of egg specific gravity (ESG) and egg shell thickness (EST) were used to evaluate the effect of ahemeral L:D cycles and strain treatments on ring-necked pheasants

egg shell quality. The ahemeral 26 hour L:D cycle did not have any significant ($P > 0.05$) effect on ESG (Appendix F, Table F1) or EST (Appendix F, Table F2). The average ESG and EST for hens exposed to the ahemeral 22 hour L:D cycle was significantly ($P \leq 0.05$) lower compared to the ESG and EST obtained for hens under the control 24 hour L:D cycle. Compared to the DNR strain, the MSU strain significantly ($P \leq 0.05$) reduce ESG and EST. There was no significant ($P > 0.05$) light x strain interactions.

The effects of light and strain treatments on ESG are presented graphically in Figures 11 and 12, respectively.

The information obtained for both strains' ESG is new since there were no previous references pertaining to their ESG. According to Koelkebeck and Biellier (1979; 1980), ahemeral L:D cycles of more than 24 hours increase chicken ESG compared to those for chickens under 24 hour L:D cycle. However, in this study the ESG of pheasants kept under 26 hour L:D cycle was slightly less than for those pheasants kept under the 24 hour L:D cycle. Thus, the response by these pheasants was not the same as that reported by the above researchers for chickens.

Egg shell thickness responses in this study, either due to the effects of ahemeral L:D cycles (Figure 13) or strain treatments (Figure 14) was of a similar pattern as the response obtained for ESG. There was a high correlation coefficient of 0.73 between these two parameters, thus, either can be used as a reliable estimate of egg-shell strangth. This was also

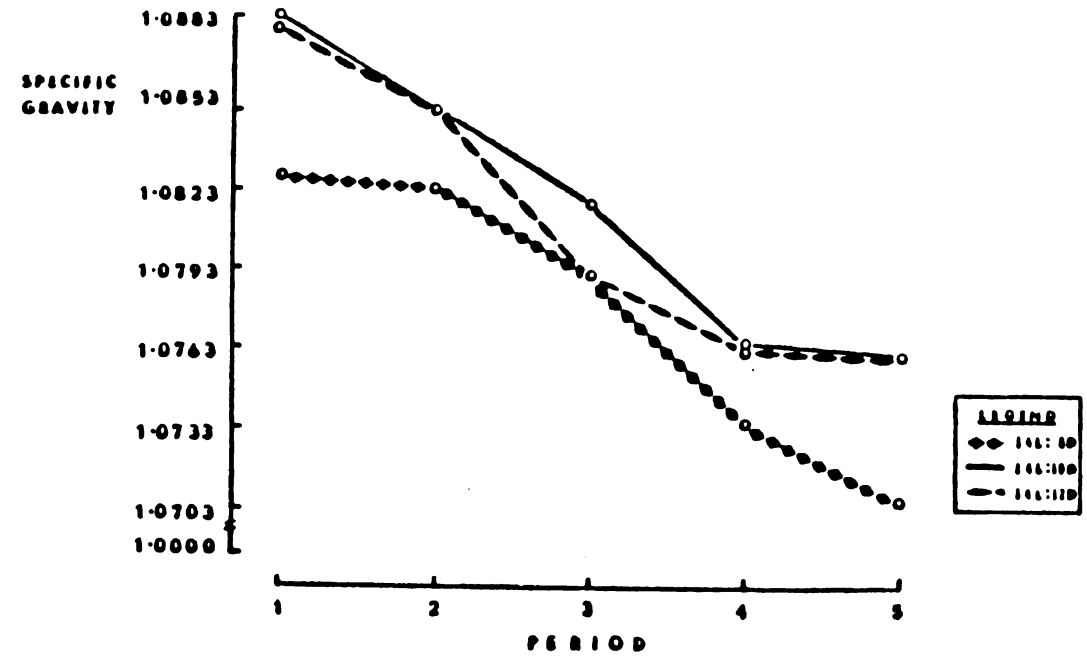



Figure 11. Effect of 22, 24, or 26 hour light-dark cycles on average egg specific gravity of pheasants at 28-day intervals. The SED between any two treatment means within a period is ± 0.0022. The SED between any two period means within a treatment is ± 0.0011.

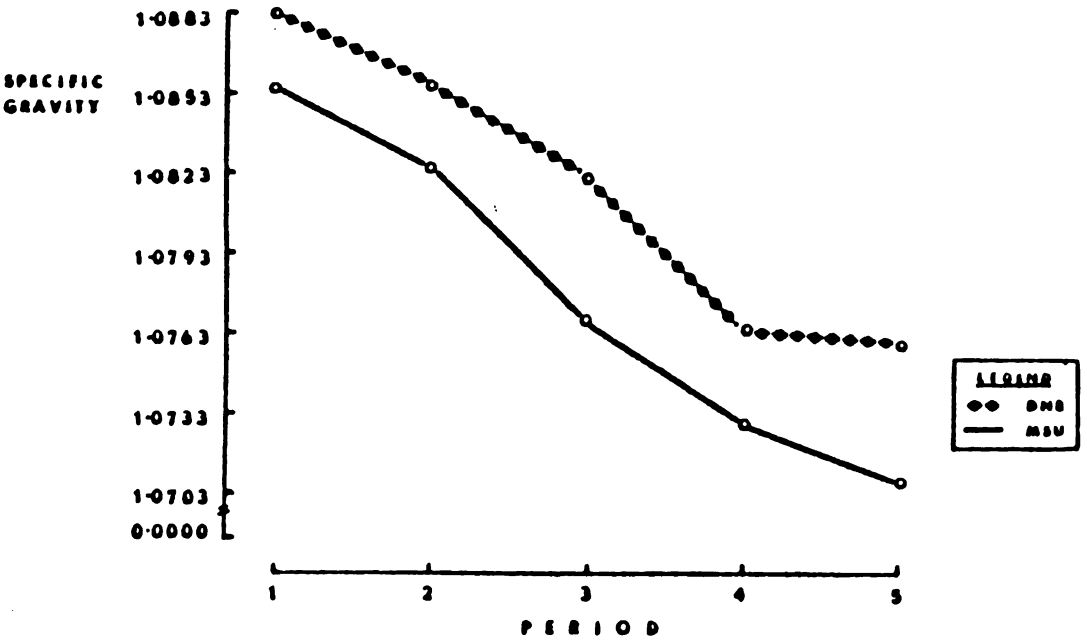



Figure 12. Strain differences in average egg specific gravity of pheasants at 28-day intervals. The SED between any two treatment means within a period is ± 0.0018. The SED between any two period means within a treatment is ± 0.0009.

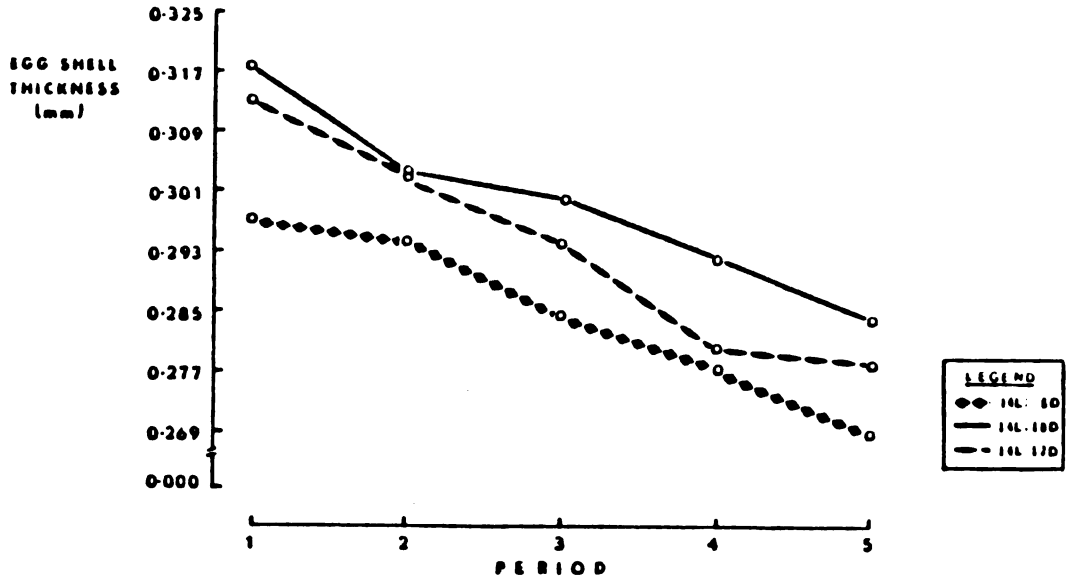



Figure 13. Effect of 22, 24, or 26 hour light-dark cycles on average egg-shell thickness of pheasants at 28-day intervals. The SED between any two treatment means within a period is ± 0.0101 mm. The SED between any two period means within a treatment is ± 0.0048 mm.

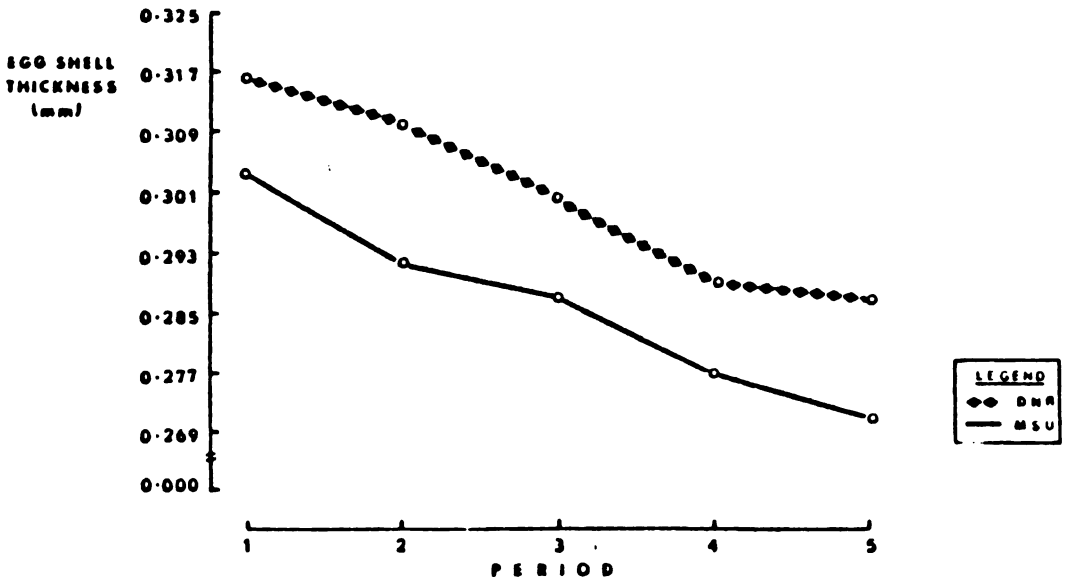



Figure 14. Strain effect on average egg shell thickness (mm) of pheasants at 28-day intervals. The SED between any two treatment means within a period is ± 0.0063 mm. The SED between any two period means within a treatment is ± 0.0039 mm.

reported by Rodda (1972) and Ahmad et al. (1976) for chickens. The overall averages for EST (Appendix F, Table F2) were within the range (0.260-0.302 mm) reported by Romanoff and Romanoff (1949) and Reynnells (1979) for ring-necked pheasants. There was a progressive decrease in ESG and EST as the experiment progressed with time. This was also reported for chickens (Petersen, 1965; Wolford and Tanaka, 1970; North, 1978; Hamilton et al., 1979; Roland, 1982). The failure of the 26 hour L:D cycle to improve pheasant EST compared to the 24 hour L:D cycle was inconsistent with the reports of Cooper and Barnett (1976), Koelkebeck and Biellier (1979), and Shanawany (1982) in chickens. The inability of ring-necked pheasants to improve egg-shell quality, when reared under a long ahemeral L:D cycle, is not understood since there was an increase in the oviducal term of eggs under the 26 hour ahemeral L:D cycle. This will be discussed in more detail in the section pertaining to egg formation time.

F. Percent Fertility and Percent Hatchability

The average percent fertility (89.3%) (Appendix F, Table F3) for ring-necked pheasants kept under the ahemeral 26 hour L:D cycle represented a significant ($P \leq 0.05$) improvement compared to the average percent fertility for hens exposed to the 24 hour L:D cycle. Percent fertility was not significantly ($P > 0.05$) affected by the ahemeral 22 hour L:D cycle. There were no significant ($P > 0.05$) effects of strain or light x strain interactions on percent fertility. The average

reported for the controls in this study was within the range (61-85%) reported by Reynnells (1979), Carpenter (1980), and Hussein (1983). Throughout the study, percent fertility under the light (Figure 15) and strain (Figure 16) treatments tended to fluctuate periodically, except under the 22 hour L:D cycle where percent fertility continued to decline steadily. This pattern of fluctuation shown for pheasant egg fertility was also observed for bobwhites (Kulenkamp et al., 1967). The fluctuation in fertility observed in this study was not due to different volumes of spermatozoa since special efforts were made to inseminate each hen once per week with a precise amount of semen (0.05 ml). Also, the same person was used at each insemination time. Each insemination took place after all hens under the same L:D cycle had completed oviposition on the day that artificial insemination was scheduled to take place. Thus, spermatozoa could be stored in the utero-vaginal junction to be released later to secondary storage sites in the upper part of the reproductive tract where fertilization takes place. The observed variation in fertility was probably due to differences in semen quality from week to week (Kulenkamp et al., 1967).

The effect of ahemeral L:D cycles on percent fertility of pheasant eggs in this expeirment could be due to a male effect. The males used to produce semen for the artificial insemination of hens kept under a particular L:D cycle, were also kept under the same L:D cycle. The males exposed to

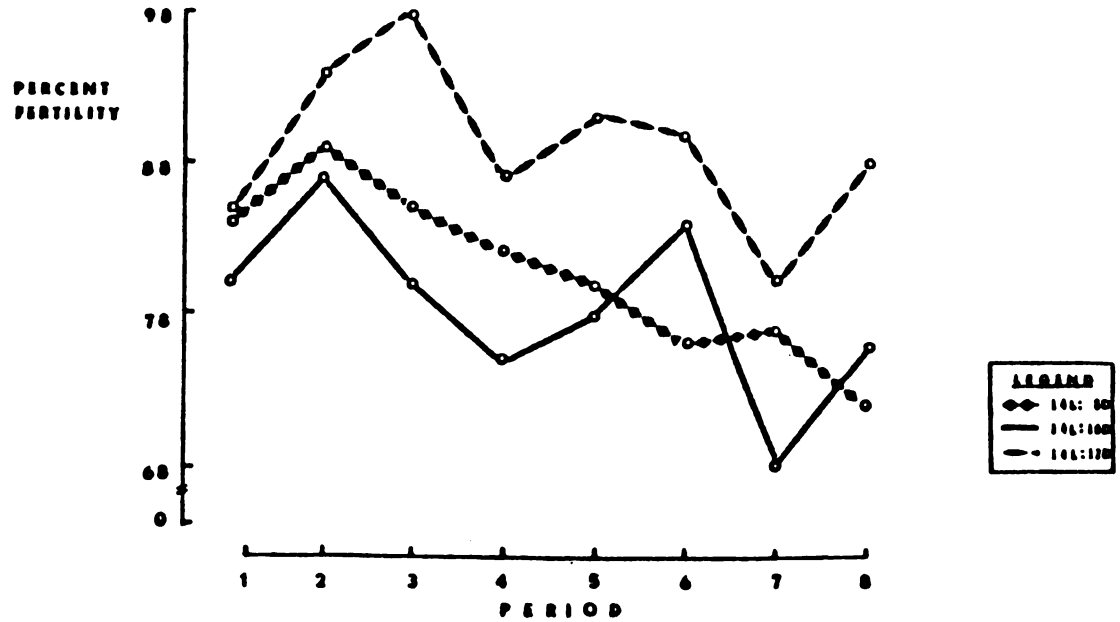



Figure 15. Effect of 22, 24, or 26 hour light-dark cycles on average percent (%) fertility of pheasant eggs at 7-day intervals. The SED between any two treatment means within a period is ± 9.2%. The SED between any two period means within a treatment is ± 5.2%. Arc sin $\sqrt{\%}$ transformation was used for statistical analysis.

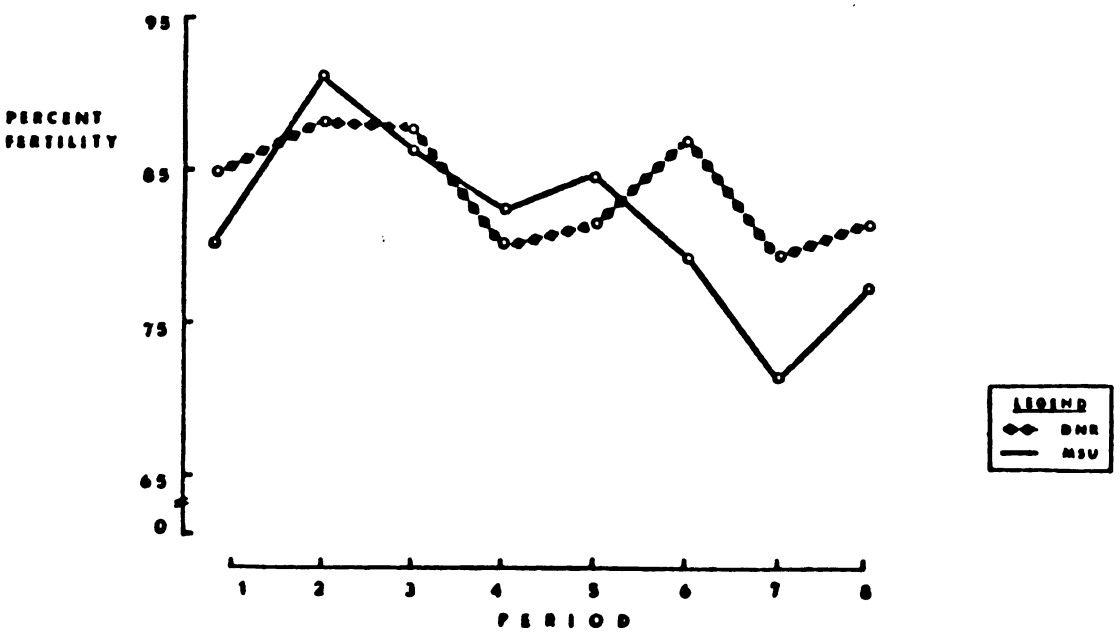



Figure 16. Strain differences in average percent (%) fertility of pheasant eggs at 7-day intervals. The SED between any two treatment means within a period is ± 7.5%. The SED between any two period means within a treatment is ± 4.2%. Arc sin $\sqrt{\%}$ tranformation was used for statistical analysis.

the 26 hour L:D cycle were the first to produce semen of acceptable quality (thick creamy appearance). These were followed by the males under the 22 hour L:D cycle, then by those under the control 24 hour cycle. In addition, on a per volume basis, the concentration of semen from males under the 26 hour L:D cycle was greater than that obtained for males under the other L:D cycles. The percentage spermatocrit measured for male pheasants under the 24, 22, and 26 hour L:D cycles were 16.3, 16.4, and 16.9, respectively. The volume of semen used per insemination was calculated to contain approximately 383 x $10^6$, 378 x $10^6$, and 389 x $10^6$ spermatozoa for hens at 24, 22, and 26 hour L:D cycles, respectively. These numbers of spermatozoa were approximately three times more than the minimum spermatozoa numbers (100 x $10^6$) recommended for optimum fertilization in pheasants (Reynnells, 1979) and chickens (Parker, 1949; Sturkie and Opel, 1976). It appears that the response obtained for percent fertility was not dose related but was due to the effect of ahemeral L:D cycles. Thus, more research needs to be conducted on the effect of ahemeral L:D cycles on male pheasant reproduction.

Throughout the experiment, percent hatchability (Figures 17 and 18) fluctuated similar to the pattern established for percent fertility. Although not statistically ($P > 0.05$) significant (Appendix F, Table F4) the percent hatchability (73.6%) for hens exposed to the ahemeral 26 hour L:D cycle was 10% greater than for the hens exposed to the other light treatments. The 63% hatchability of fertile eggs obtained

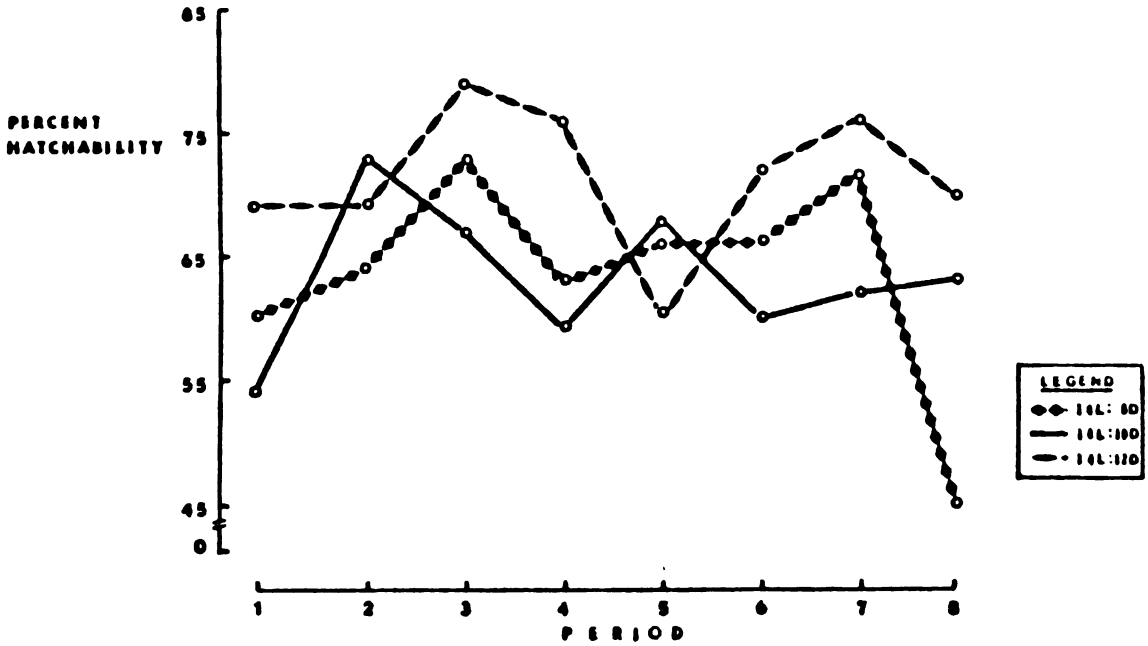



Figure 17. Effect of 22, 24, or 26 hour light-dark cycles on average percent (%) hatchability of fertile pheasant eggs at 7-day intervals. The SED between any two treatment means within a period is ± 12.9%. The SED between any two period means within a treatment is ± 7.6%. Arc sin $\sqrt{\%}$ transformation was used for statistical analysis.

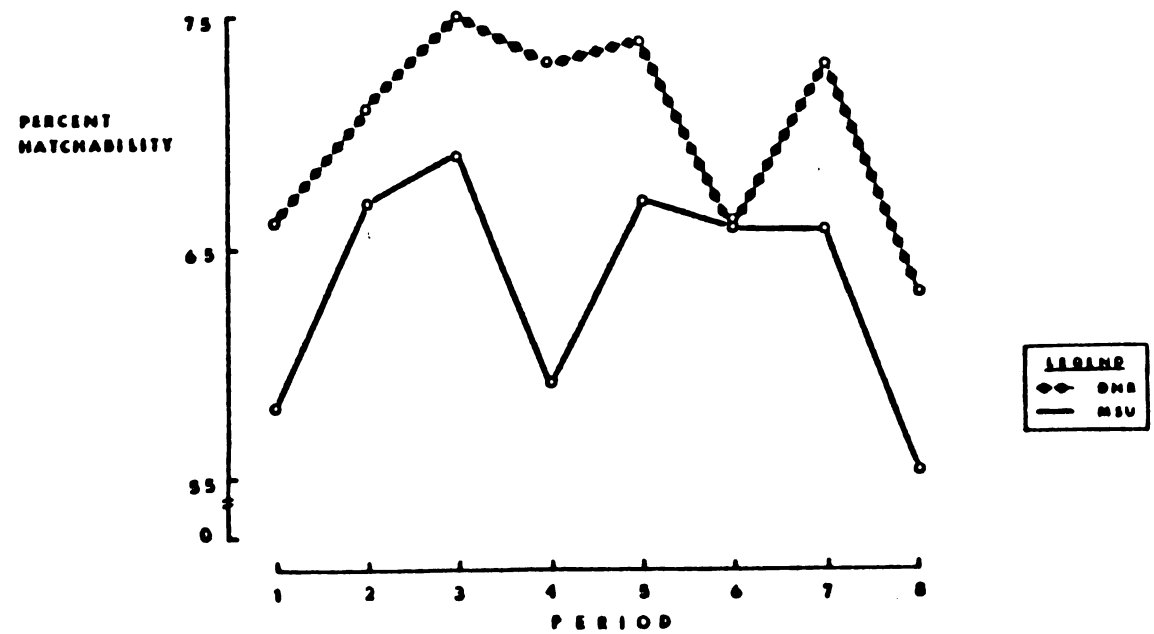



Figure 18. Strain differences in average percent (%) hatchability of fertile pheasant eggs at 7-day intervals. The SED between any two treatment means within a period is ± 10.5%. The SED between any two period means within a treatment is ± 6.2%. Arc sin $\sqrt{\%}$ transformation was used for statistical analysis.

under the control 24 hour L:D cycle was within the range (58-83%) reported by Carpenter (1980) and Hussein (1983). The increase in hatchability of chicken eggs obtained for hens kept under an ahemeral 27 hour L:D cycle (Lacassagne et al., 1973) agrees with the result obtained for pheasants in this study. Improvement in hatchability under the 26 hour ahemeral L:D cycle could be due to a carry over effect (probably biochemical) from the improved fertility response. Further investigation is needed in this area in order to determine what effect long ahemeral L:D cycles may have on fertilization and hatchability of pheasant eggs.

Neither ahemeral L:D cycle treatments nor strain had any significant ($P > 0.05$) effect on percent pipped eggs (Table 13) or percent dead embryos (Table 14) of fertile eggs. The tendency was for the DNR strain and the ahemeral 26 hour L:D cycle to produce smaller responses compared to the other strain and L:D cycles, respectively.

## G. Oviposition Time

### 1. Intra light-dark cycle test

#### a) Control 24 hour L:D cycle

The average percent oviposition distribution at 2-hour intervals for the ring-necked pheasants reared under the 24h L:D cycle (14L:10D) is shown in Table 15. Figure 19 is a graphic presentation of the data. Approximately 95% of the total oviposition occurred during the light period, 76.3% of which were afternoon ovipositions (Figure 19). This was consistent with other reports on oviposition time for turkeys

Table 13. Average percent pipped[1] eggs in two strains of pheasants under 22, 24, or 26 hour light-dark cycles at 7 day intervals[2,3,4].

| Strain | Period | Light treatment 22 (14L:8D) | 24 (14L:10D) | 26 (14L:12D) | Avg. |
|---|---|---|---|---|---|
| DNR | 1 | 12.4 | 11.1 | 10.1 | 11.2 |
| | 2 | 21.8 | 13.7 | 17.1 | 17.5 |
| | 3 | 10.9 | 10.0 | 9.7 | 10.2 |
| | 4 | 15.7 | 7.6 | 11.4 | 11.6 |
| | 5 | 10.3 | 9.8 | 8.7 | 9.6 |
| | 6 | 26.6 | 10.7 | 5.9 | 14.4 |
| | 7 | 7.3 | 13.4 | 11.1 | 10.6 |
| | 8 | 31.3 | 14.1 | 4.5 | 16.6 |
| | Avg. | 17.0 | 11.3 | 9.8 | 12.7[a] |
| MSU | 1 | 14.8 | 11.9 | 12.9 | 13.2 |
| | 2 | 26.9 | 6.7 | 25.9 | 19.9 |
| | 3 | 12.9 | 5.5 | 10.2 | 9.5 |
| | 4 | 14.2 | 15.6 | 14.3 | 14.7 |
| | 5 | 18.8 | 8.3 | 5.2 | 10.8 |
| | 6 | 12.3 | 6.8 | 15.7 | 11.6 |
| | 7 | 9.2 | 22.1 | 11.9 | 14.4 |
| | 8 | 17.9 | 7.5 | 9.5 | 11.6 |
| | Avg. | 15.9 | 10.6 | 13.2 | 13.2[a] |
| Overall Average | | 16.5[b] | 10.9[b] | 11.5[b] | |

[a] Means with the same symbol within a column do not differ significantly P(> 0.05).

[b] Means with the same symbol within a column do not differ significantly P(> 0.05).

[1] Of fertile eggs.

[2] The standard error of difference (SED) (repeated measurements; split plot design, see Gill, 1978b) between any two light treatment means within a period is ± 6.1%. The SED between any two light treatment means within a treatment is ± 4.6%.

[3] The SED between the two strain means within a period is ± 4.9%. The SED between any two period means within a strain is ± 3.8%.

[4] Mean of 18 birds per strain or 36 birds per light treatment.

Table 14. Average percent dead embryos[1] in two strains of pheasants under 22, 24, or 26 hour light-dark cycles at 7 day intervals[2,3,4].

| Strain | Period | Light treatment 22 (14L:8D) | 24 (14L:10D) | 26 (14L:12D) | Avg. |
|---|---|---|---|---|---|
| DNR | 1 | 23.1 | 32.0 | 18.6 | 24.6 |
| | 2 | 13.9 | 11.5 | 9.7 | 11.7 |
| | 3 | 11.6 | 13.9 | 8.4 | 11.3 |
| | 4 | 16.4 | 27.0 | 9.2 | 17.6 |
| | 5 | 12.3 | 20.3 | 11.8 | 14.8 |
| | 6 | 6.9 | 32.3 | 19.8 | 19.7 |
| | 7 | 12.9 | 15.8 | 8.8 | 12.5 |
| | 8 | 23.8 | 14.2 | 22.3 | 20.1 |
| | Avg. | 15.1 | 20.9 | 13.6 | 16.5[a] |
| MSU | 1 | 29.9 | 27.7 | 20.3 | 25.9 |
| | 2 | 9.8 | 13.5 | 8.3 | 10.6 |
| | 3 | 18.2 | 37.8 | 7.6 | 21.3 |
| | 4 | 29.0 | 26.1 | 17.8 | 24.3 |
| | 5 | 25.9 | 18.7 | 18.5 | 21.0 |
| | 6 | 20.6 | 21.9 | 13.2 | 18.6 |
| | 7 | 21.1 | 17.4 | 16.1 | 18.2 |
| | 8 | 36.7 | 37.3 | 22.9 | 32.3 |
| | Avg. | 23.9 | 25.1 | 15.6 | 21.5[a] |
| Overall Average | | 19.5[b] | 22.9[b] | 14.6[b] | |

[a] Means with the same symbol within a column do not differ significantly (P > 0.05).

[b] Means with the same symbol within a row do not differ significantly (P > 0.05).

[1] Of fertile eggs.

[2] The standard error of difference (SED) (repeated measurements; split plot design, see Gill, 1978b) between any two light treatment means within a period is ± 9.9%. The SED between any two light treatment means within a period is ± 5.2%.

[3] The SED between two strain means within a period is ± 8.2%. The SED between any two period means within a strain is ± 3.8%.

[4] Mean of 18 birds per strain or 36 birds per light treatment.

Table 15. Average percent[1] of total ovipositions (% ToP) at 2-hour intervals "after lights on" for pheasants under a 22 (14L:8D), 24 (14L:10D), or 26 (14L:12D) hour light-dark cycle.

| Time (hour) | Light treatment | | | | | |
|---|---|---|---|---|---|---|
| | 14L:10D | | 14L:8D | | 14L:12D | |
| | n | % ToP | n | % ToP | n | % ToP |
| 0- 2 | 33 | 1.55 ± 0.82* | 34 | 2.83 ± 0.64 | 34 | 9.55 ± 2.34$^{B}$ |
| 2- 4 | 33 | 0.91 ± 0.34 | 34 | 2.68 ± 0.61 | 34 | 23.24 ± 3.15$^{a,B}$ |
| 4- 6 | 33 | 2.45 ± 0.77 | 34 | 1.16 ± 0.43 | 34 | 29.71 ± 3.27$^{a,B}$ |
| 6- 8 | 33 | 21.29 ± 2.5$^{B}$ | 34 | 2.25 ± 0.61 | 34 | 17.10 ± 2.92$^{B}$ |
| 8-10 | 33 | 37.92 ± 2.96$^{a,B}$ | 34 | 7.88 ± 1.18 | 34 | 7.41 ± 1.23 |
| 10-12 | 33 | 17.13 ± 2.04$^{B}$ | 34 | 19.14 ± 2.07$^{a,B}$ | 34 | 2.99 ± 0.63 |
| 12-14 | 33 | 13.9 ± 1.95 | 34 | 23.99 ± 1.48$^{a,B}$ | 34 | 2.12 ± 0.67 |
| 14-16 | 33 | 4.06 ± 0.98 | 34 | 16.68 ± 1.54$^{a,B}$ | 34 | 1.29 ± 0.35 |
| 16-18 | 33 | 0.79 ± 0.34 | 34 | 9.04 ± 1.19 | 34 | 2.47 ± 0.59 |
| 18-20 | 33 | 0.0 ± 0.0 | 34 | 7.44 ± 1.26$^{B}$ | 34 | 0.0 ± 0.0 |
| 20-22 | 33 | 0.0 ± 0.0 | 34 | 6.91 ± 0.89$^{B}$ | 34 | 0.0 ± 0.0 |
| 22-24 | 33 | 0.0 ± 0.0 | -- | -- -- | 34 | 2.77 ± 1.23$^{B}$ |
| 24-26 | -- | -- -- | -- | -- -- | 34 | 1.35 ± 0.46 |

[1] Arc sin $\sqrt{\%}$ transformation used for statistical analysis.

[a] Mean peak oviposition times with a superscript (lower case), within a column, differ significantly ($P \leq 0.01$) from the other oviposition times without a superscript.

[B] Mean peak oviposition times with a superscript (upper case), within a row, differ significantly ($P \leq 0.01$) from the other oviposition times without a superscript.

n = Number of observations.

* Values are means ± standard error.

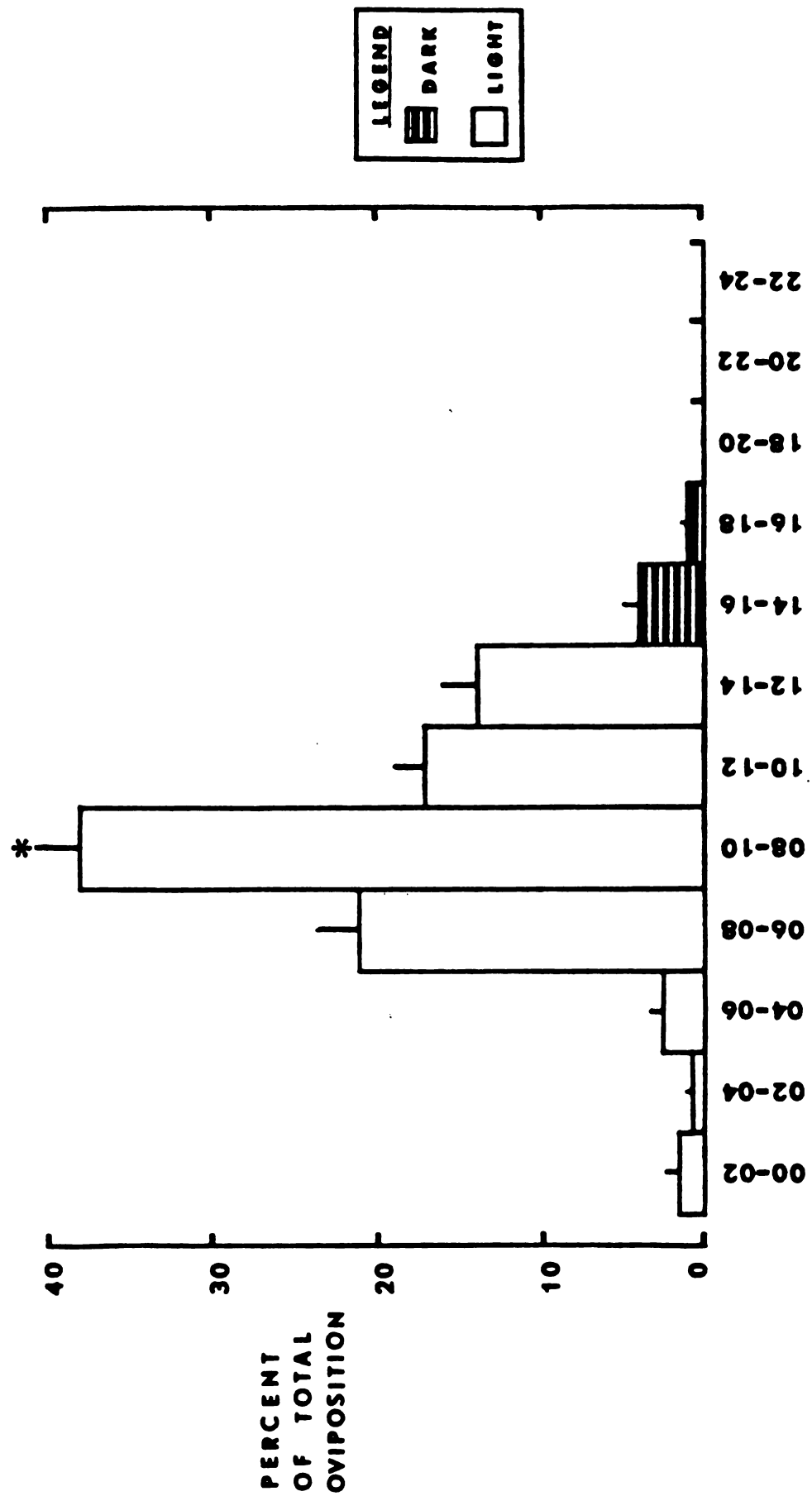



Figure 19. Distribution of percent oviposition of pheasants kept under a 24 hour light-dark cycle (14L:10D). *Mean peak oviposition was significantly ($P \leqslant 0.01$) higher than the mean of the other oviposition times. Bars represent means $\pm$ SEM of 33 observations per two hour interval. Arc sin $\sqrt{\%}$ transformation was used for statistical analysis.

(Woodard et al., 1963; Wolford et al., 1964b), and coturnix (Wilson and Huang, 1962). The 5.0% oviposition that occurred during the dark period represented the total ovipositions occurring during the first 4 hours of the dark phase. There were no ovipositions during the last 6 hours of this cycle. A similar observation was also reported for chicken oviposition times (Tanabe and Nakamura, 1980) but not for other avian species.

Mean peak percentage oviposition (37.9%) occurred between 8 to 10 hours into the light phase or an average 19 hours post "lights-off" from the previous day. The peak oviposition time (OT) interval was significantly ($P \leq 0.01$) different from the other OT intervals. By the end of the peak OT, 64% of the total eggs were already oviposited. For any 8 hour period throughout the 24 hour L:D cycle, the last 8 hours of the light period which started 16 hours post "lights-off" from the previous day had the highest percent oviposition (90.24%). The data obtained in this study indicated that pheasant peak OT occurred later than that reported for chickens, but not as late as for coturnix (Arrington et al., 1962; Wilson and Huang, 1962), but at about the same time as for turkeys (Woodard et al., 1963). This was much earlier than the time reported for ducks (Tanabe and Nakamura, 1980). It appears that in wild birds (turkeys, pheasants, and coturnix), the circadian rhythm of oviposition response is quite different from the chickens.

b) Ahemeral 22 hour L:D cycle

Oviposition of pheasants under the ahemeral 22h L:D cycle (14L:8D) occurred throughout the cycle (Figure 20). The oviposition pattern observed during the light phase of this ahemeral L:D cycle was similar to the oviposition patterns observed under a 24 hour L:D cycle for turkeys (Woodard et al., 1963); while the oviposition patterns observed during the dark phase of the cycle were more similar to the patterns reported for ducks (Tanabe and Nakamura, 1980), than the patterns for any other avian species exposed to a 24 hour L:D cycle. The mean peak oviposition (24%) occurred 12-14 hours into the light phase (Table 15) which was 4 hours later than the mean peak observed under the control 24 hour L:D cycle. The peak OT interval was significantly ($P \leq 0.01$) different from the other OT intervals except for the OT intervals that immediately preceeded and followed the oviposition peak (Figure 20 and Table 15). Of the total ovipositions, 60% had already occurred by the end of the mean peak OT. For any 8 hour period throughout the 22 hour L:D cycle, the last 6 hours of light and the first 2 hours of dark which started 14 hours post "lights-off" from the previous day had the highest percent of total ovipositions (68.9%).

Approximately 60% of the pheasant ovipositions occurred during the light period and 40% during the dark. Mean peak OT occurred an average of 21 hours post "lights-off" from the previous day, thus implicating "sunset" as the predominant

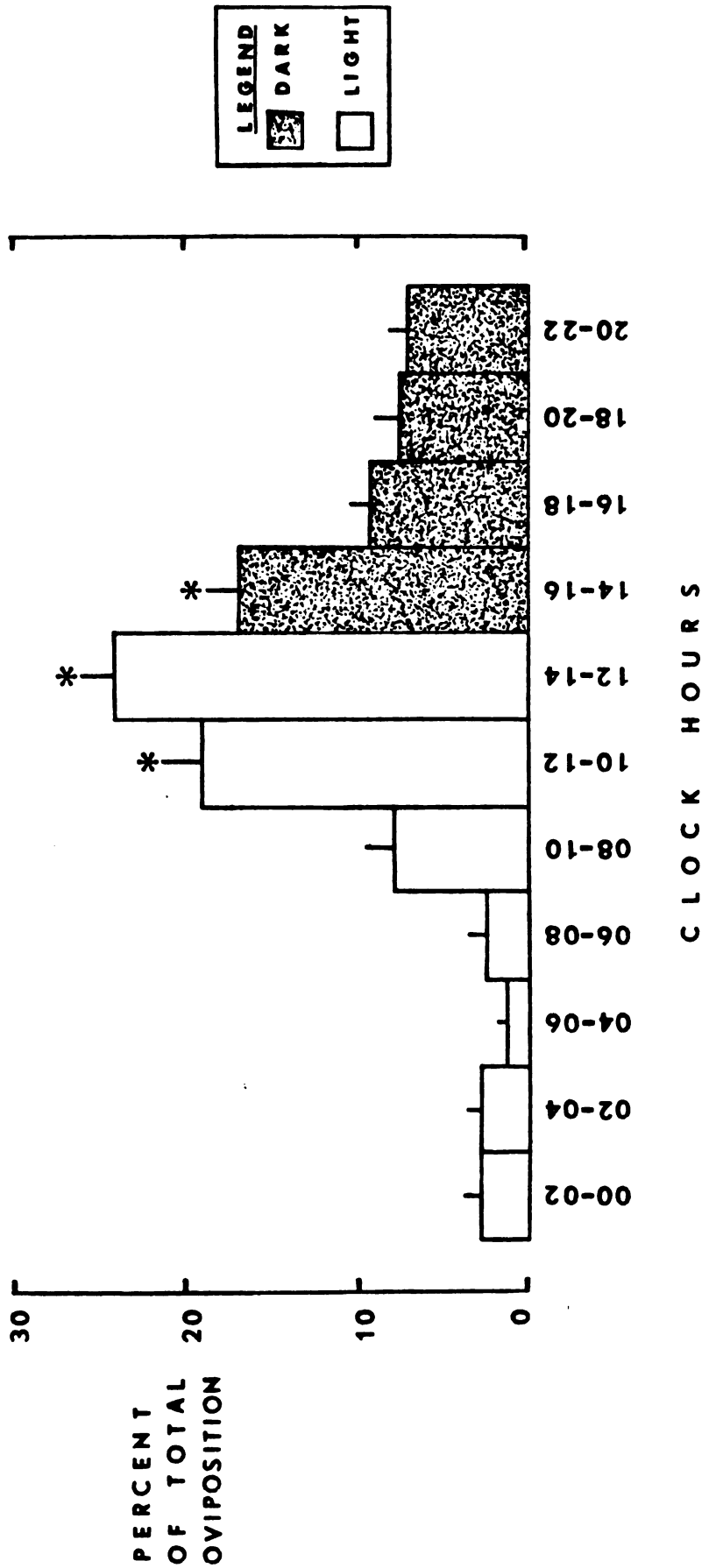



Figure 20. Distribution of percent oviposition of pheasants kept under a 22 hour light-dark cycle (14L:8D). *Mean peak oviposition was significantly ($P \leq 0.01$) higher than the mean of the other oviposition times, except those that also has an asterick. Bars represent means $\pm$ SEM of 34 observations per two hour interval. Arc sin $\sqrt{\%}$ transformation was used for statistical analysis.

phase-setting signal, which was also suggested by Morris (1973). The 2 hours delay in peak OT, compared to the peak OT observed under the 24 hour L:D cycle, represented the 2 hours less darkness for the ahemeral 22 hour L:D cycle. This indicates that the entrainment of OT for pheasants under the 22 hour L:D cycle was occurring later than for those kept under the 24 hour L:D cycle. According to the general entrainment theory, phase activity should be advanced when the L:D cycle is more than 24 hours, and be delayed when less than 24 hours (Pittendrigh and Minis, 1964). This circadian prediction has been shown to work for the oviposition rhythm of chickens (Bhatti and Morris, 1978) and coturnix (Follett and Davies, 1978; Simpson and Follett, 1982), and for coturnix testicular growth rate (Simpson and Follett, 1982). Obviously, this phenomenon also works for the oviposition rhythm of pheasants since peak OT was delayed and 35% less ovipositions occurred in the light compared to the 24 hour L:D cycle.

c) Ahemeral 26 hour L:D cycle

The effect of the ahemeral 26h L:D cycle (14L:12D) on average percentage oviposition distributions at 2-hour intervals of ring-necked pheasants is shown in Figure 21 and Table 15. No oviposition under this L:D cycle was observed to occur between 4 to 8 hours of the dark phase. The circadian rhythm of oviposition observed for pheasants under this L:D cycle was more similar to chickens (Wilson and Huang, 1962; Tanabe and Nakamura, 1980) than any other avian species under the conventional 24 hour L:D cycle. The mean

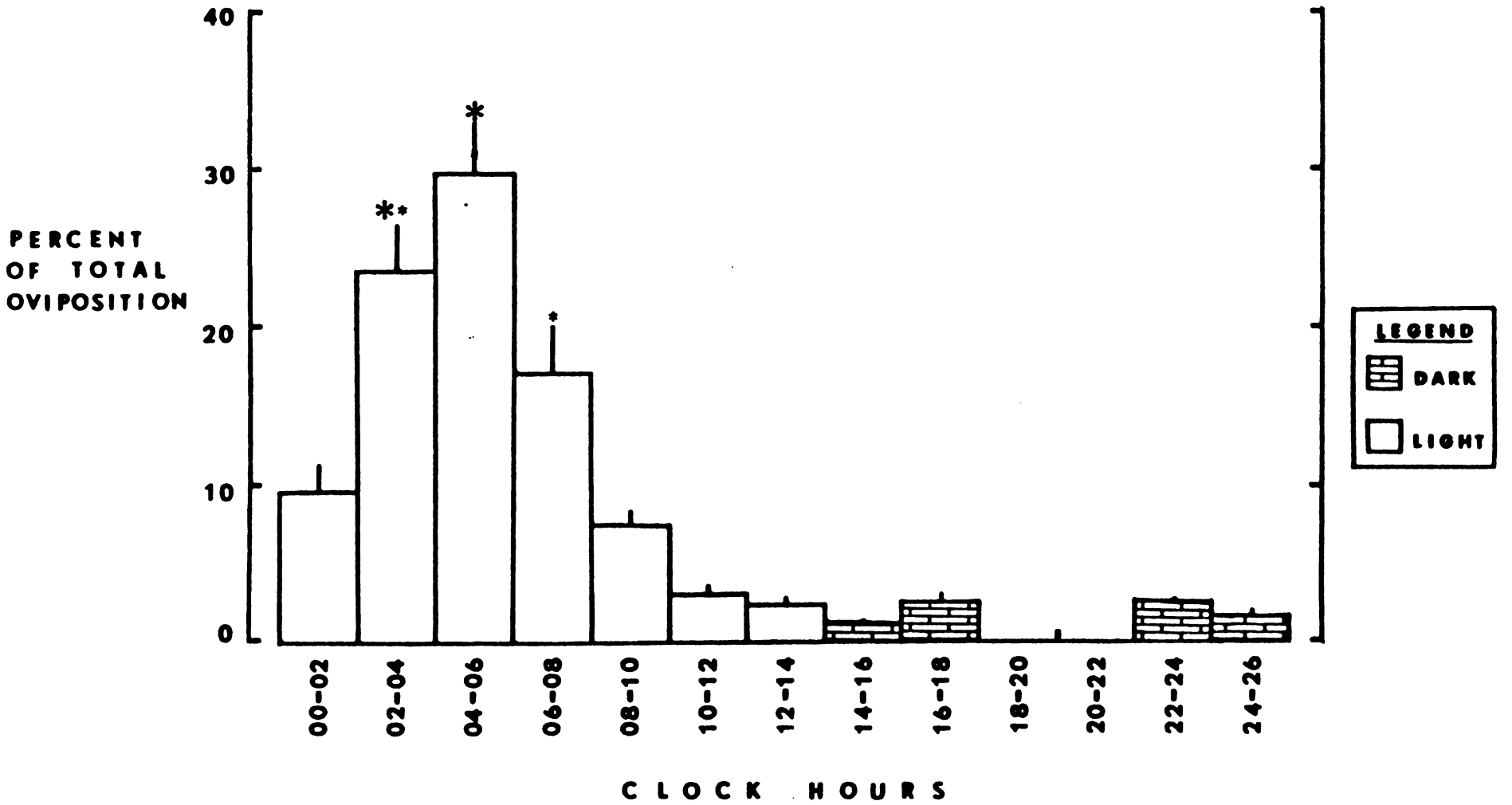



Figure 21. Distribution of percent oviposition of pheasants kept under a 26 hour light-dark cycle (14L:12D). *Mean peak oviposition was significantly ($P \leq 0.01$) higher than the mean of the other oviposition times, except any mean that has the same size asterick as the peak mean. Any other bars having the same size asterick of different size from that of the peak mean is not significantly ($P > 0.01$) different. Bars represent means $\pm$ SEM of 33 observations per two hour period. Arc sin $\sqrt{\%}$ transformation was used for statistical analysis.

peak percent oviposition was 29.7%. This occurred 4 hours earlier (4-6 hours of the light phase) than under the 24 hour L:D cycle. The peak OT interval was significantly ($P \leq 0.05$) different from the other OT intervals except for the OT interval immediately preceeding the oviposition peak. Of the total ovipositions, 62.4% had already occurred by the end of the mean peak OT. The highest percent of total ovipositions (79.6%) for any 8 hour period throughout the 26 hour L:D cycle occurred during the first 8 hours. This started 12 hours post "lights-off" from the previous day.

Approximately 92.1% of the pheasant ovipositions occurred during the light period and 7.9% during the dark. At the beginning of the study it was observed that virtually all ovipositions occurred at least 2 hours prior to darkness. It was only towards the end of egg production that ovipositions started to occur in the dark. Mean peak percent oviposition occurred an average of 17 hours post "lights-off" from the previous day. This indicates that the entrainment of OT for pheasants under the 26 hour L:D cycles was occurring earlier than for those under the 22 and 24 hour L:D cycles, thus also implicate "sunset" as a predominant phase-setting signal as suggested by Morris (1973). The advancement of the phase setting activity of oviposition for the pheasants under the 26 hour L:D cycle which was greater in length than the 24 hour L:D cycle agrees with Ostmann and Biellier (1958) who reported that increasing periods of day length progressively advance time of oviposition to an earlier time of the

day. This also conforms to the general entrainment theory or the circadian prediction by Pittendrigh and Minis (1964). The theory states that phase activity should be advanced when the L:D cycle is more than 24 hours and be delayed when less than 24 hours.

2. Inter light-dark cycle test

In this test, all three L:D cycle treatments taken at any 2-hour interval of the respective cycles, were compared statistically to obtain more information on how the rhythmicity of pheasant oviposition distribution was relatively changing bihourly between cycles as time progressed. The average of the percent oviposition distribution are graphically shown in Figures 22A-L. A table with the averages is also shown (Table 15).

Figures 22A,B, and C represent 2-hour intervals of light within each L:D cycle treatment for the first 6 hours of the light phase. During these intervals, the mean percent oviposition (MPO) for pheasants kept under the ahemeral 26h L:D cycle (14L:12D) was significantly ($P \leq 0.01$) greater compared to the other two L:D cycles. This showed a shift in entrainment of oviposition time due to an increase in the L:D cycle length (Ostmann and Biellier, 1958; Pittendrigh and Minis, 1964; Bhatti and Morris, 1978).

If the entrainment theory (Pittendrigh and Minis, 1964) is correct, then the next largest entrainment of early oviposition in this study should be for the control 24h L:D cycle (14L:10D) since it was the next L:D cycle in hierarachy

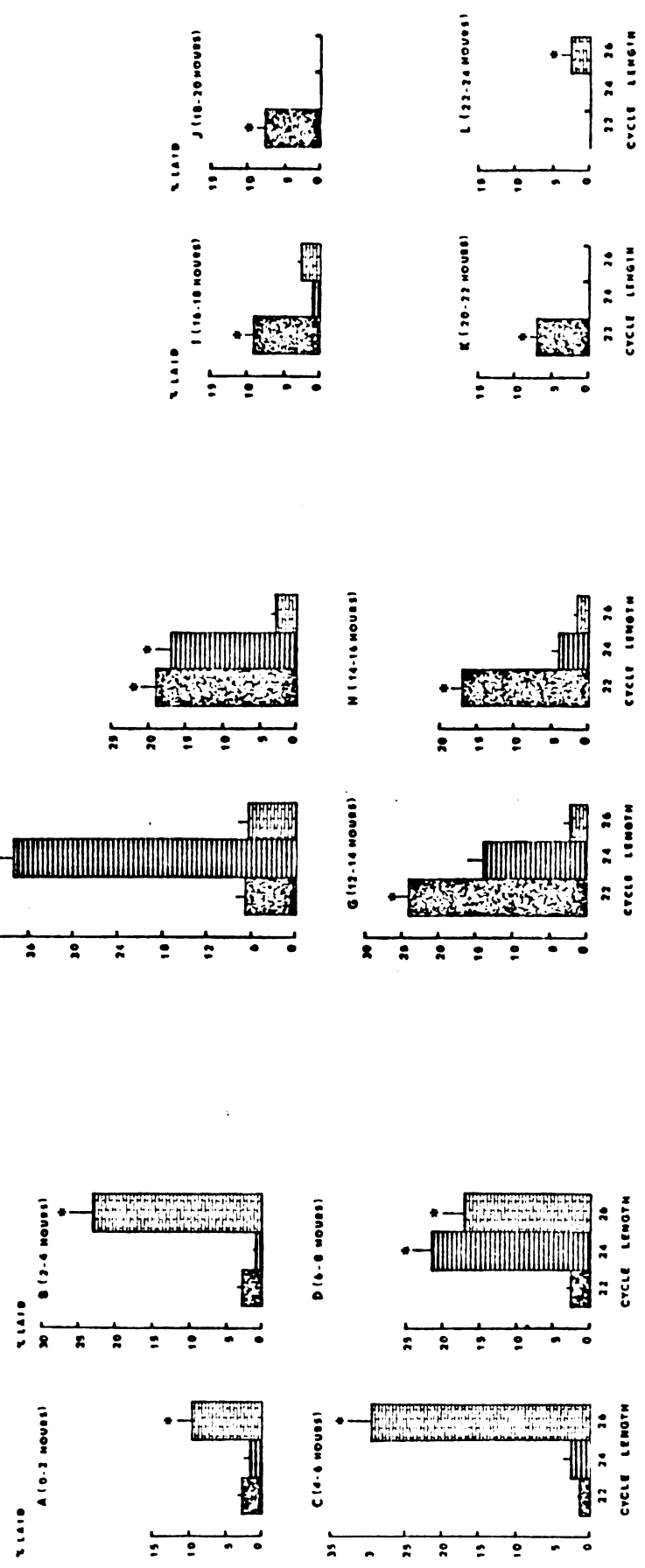


Figure 22 A-L. Distribution of percent oviposition, at 2-hour (h) intervals "after lights on" (ALO), for pheasants kept under 22h (14L:8D), 24h (14L:10D), and 26h (14L:12D) light-dark cycles. The abscissa and the ordinate represent cycle length and percent of total oviposition, respectively. (A) 0-2h; (B) 2-4h; (C) 4-6h; (D) 6-8h; (E) 8-10h; (F) 10-12h; (G) 12-14h; (H) 14-16h; (I) 16-18h; (J) 18-20h; (K) 20-22h; (L) 22-24h. *Mean peak percent oviposition for the light treatment was significantly ($P \leq 0.01$) higher than the mean percent of the other treatment(s) at the particular interval. Bars represent means + SEM of 33-34 observations. Arc sin $\sqrt{\%}$ transformation was used for statistical analysis.

of length to the 26 hour L:D cycle. The proof of this theory can further be seen in Figure 22D for which the MPO under the 24 hour L:D cycle had increased and was greater than the value under the 26 hour L:D cycle. However, these two values were not statistically significant ($P > 0.05$) from each other, but were significantly ($P \leq 0.01$) greater than the value obtained for hens exposed to the 22h L:D cycle (14L:8D). In Figure 22E the MPO for the 24 hour L:D cycle attained its highest value and was significantly ($P \leq 0.01$) different from the other two L:D cycle treatments. Also the means under the 22 and 26 hour L:D cycle increased and decreased, respectively.

Figure 22F also brings out the same transition as Figure 22D. In this case the MPO under the 22 hour L:D cycle was greater than the other two L:D cycles but was only significantly ($P \leq 0.05$) different than the MPO under the 22 hour L:D cycle. The shorter ahemeral cycle length (22 hours) resulted in a majority of the ovipositions occurring after those of the 24 and 26 hour L:D cycles. The entrainment theory (Pittendrigh and Minis, 1964) was also in compliance in Figures 22G,H,I,J, and K. The MPO under the 22 hour L:D cycle was increasing while the values under the other L:D cycles were on the decline or at least significantly ($P \leq 0.01$) greater at all times compared to the other two L:D cycles.

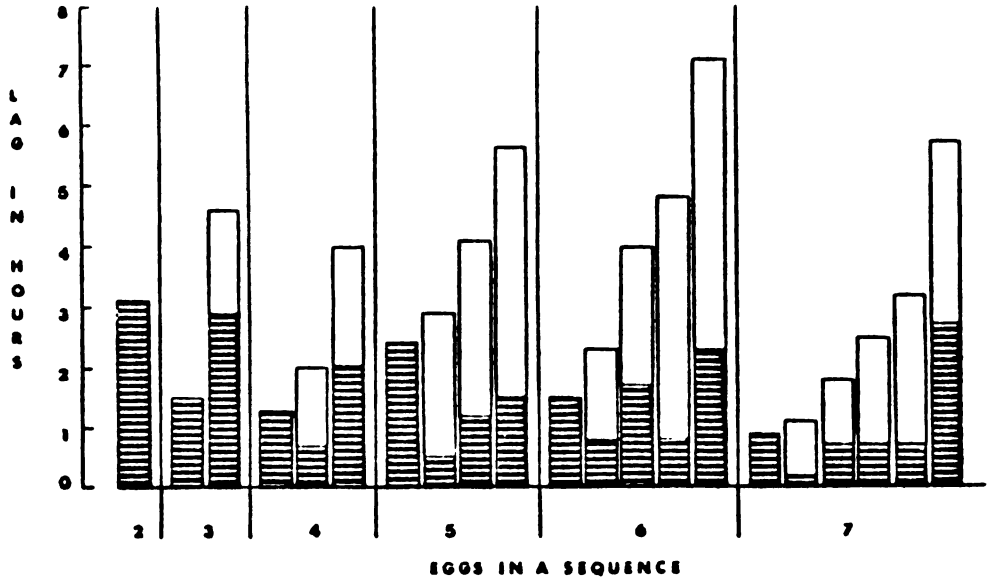
3. Lag time

a) Control 24 hour L:D cycle

Average oviposition lag time for ring-necked pheasant hens reared under the 24h L:D cycle (14L:10D) is shown for various egg sequences in Figures 23A and B. A table with the averages is shown (Table 16). The total or

Figure 23A. Effect of conventional 24 hour (14L:10D) light-dark cycle on oviposition lag in hours between successive eggs in a 2, 3, 4, 5, 6, and 7 egg sequence for pheasants. The "closed" bars in a particular sequence represents the lag between the previous and present oviposition. An "open" bar in a particular sequence represents the total of all previous and present lags.

Figure 23B. Effect of conventional 24 hour (14L:10D) light-dark cycle on oviposition lag in hours between successive eggs in an 8 and 10 egg sequence for pheasants. The "closed" bars in a particular sequence represents the lag between the previous and the present ovipositoin. An "open" bar in a particular sequence represents the total of all previous and present lags.


LAG IN HOURS
0
1
2
3
4
5
6
7
8
2
3
4
5
6
7
EGGS IN A SEQUENCE

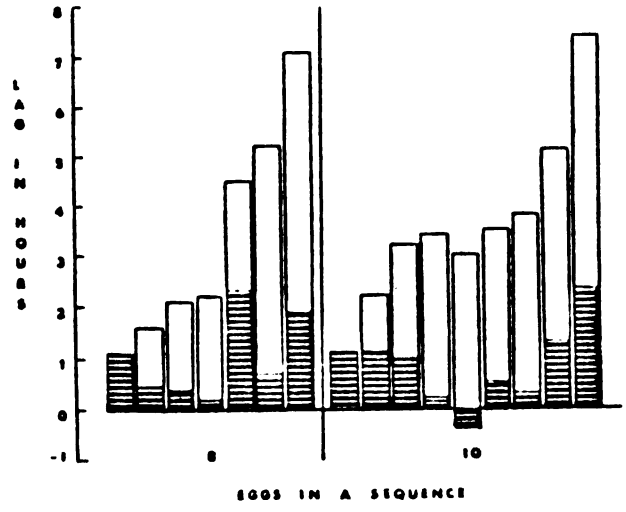

LAG IN HOURS
-1
0
1
2
3
4
5
6
7
8
8
10
EGGS IN A SEQUENCE

Table 16. Changes in lag and egg formation time for pheasants under 22, 24, or 26 hour light-dark cycles.

| Sequence length | Control (24) 14L:10D | | | Ahemeral (22) 14L:8D | | | Ahemeral (26) 14L:12D | | |
|---|---|---|---|---|---|---|---|---|---|
| | TL[1] | AL[2] | n[3] | TL | AL | n | TL | AL | n |
| 2 | 3.1 | 3.1 | 11 | 1.9 | 1.9 | 19 | 5.0 | 5.0 | 14 |
| 3 | 4.4 | 2.2 | 22 | 6.2 | 3.1 | 21 | 8.7 | 4.4 | 10 |
| 4 | 4.0 | 1.3 | 11 | 7.3 | 2.4 | 20 | 8.1 | 2.7 | 8 |
| 5 | 5.6 | 1.4 | 9 | 11.4 | 2.9 | 13 | 13.8 | 3.5 | 6 |
| 6 | 7.1 | 1.4 | 7 | 11.3 | 2.3 | 4 | 15.0 | 3.0 | 7 |
| 7 | 5.9 | 1.0 | 5 | 13.4 | 2.2 | -- | 3.1 | 0.5 | 4 |
| 8 | 7.1 | 1.0 | 5 | 10.8 | 1.5 | 4 | 16.1 | 2.3 | 4 |
| 9 | -- | -- | -- | 7.2 | 0.9 | 2 | -- | -- | -- |
| 10 | 7.4 | 0.8 | 6 | 7.0 | 0.8 | 4 | -- | -- | -- |
| 11 | -- | -- | -- | -- | -- | -- | 16.7 | 1.7 | 2 |
| Avg. overall lag | | 1.5 | | | 1.8 | | | 2.5 | |
| EFT[4] (24 + avg.) | | 25.5 | | | 25.8 | | | 26.5 | |

[1] TL = Total lag hour(s).

[2] AL = Average lag hours(s).

[3] n = Number of observations.

[4] EFT = Egg formation time (hours).

cumulative lag obtained under the 24 hour L:D cycle for any particular sequence did not exceed 7 hours. This was also reported in turkeys (Wolford et al., 1964b) and chickens Sturkie and Mueller, 1976) reared under the conventional 24 hour L:D cycle. The average overall lag obtained under this L:D cycle treatment for pheasants was 1.5 hours. As egg sequence size increases, the average lag time in a sequence decreases. Negative lag was also obtained in one of the larger egg sequences (10) (Figure 23B). This observation was also reported for turkeys (Wolford et al., 1964b) and chickens (Sturkie and Mueller, 1976). It was also noted that the lag time was greater between the initial and terminal ovipositions of a sequence than between the other ovipositions within that sequence.

Generally, the pheasant, like the turkey and chicken, produces a similar oviposition lag time response under the conventional 24 hour L:D cycle. This oviposition lag response of pheasants could be due to the genetic selection of these pheasants for improved egg production.

b) Ahemeral 22 hour L:D cycle

There was a continued increase in total lag time under the ahemeral 22h L:D cycle (14L:8D) as egg sequence increased (Figures 24A and Table 16). This was the established pattern until a sequence size of 7 eggs was obtained, giving a total lag of 13.4 hours. Above the 7 egg sequences, total lag started to decline reaching as low as 7.0 hours for the 10 egg sequences. The average overall lag was 1.8 hours,

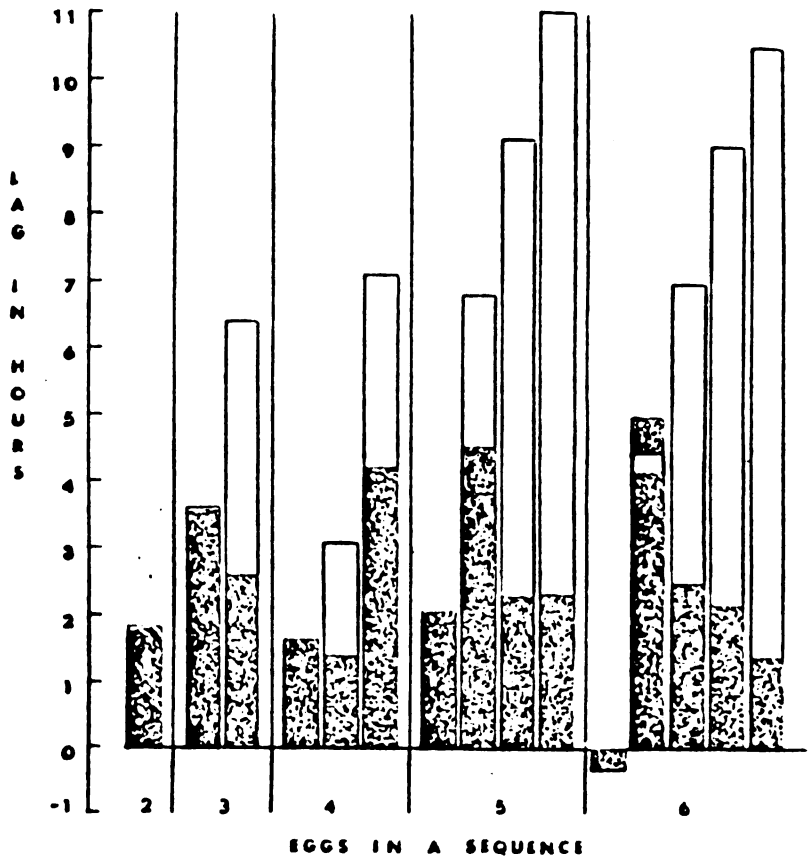



Figure 24A. Effect of ahemeral 22 hour (14L:8D) light-dark cycle on oviposition lag in hours between successive eggs in a 2, 3, 4, 5, and 6 egg sequence for pheasants. The "closed" bars in a particular sequence represents the lag between the previous and present oviposition. An "open" bar in a particular sequence represents the total of all previous and present lags. In a "close" bar with an open space, the total lag is the distance between the abscissa and the beginning of the open area.

representing only 0.3 hours more than under the 24 hour L:D cycle (Table 16). This pattern is not understood and does not agree with the results obtained under the 24 hour L:D cycle used in this study, or with the reports of Wolford et al. (1964b), and Sturkie and Mueller (1975) on oviposition lag time. It is obvious from the data obtained in this study that the ahemeral 22 hour L:D cycle caused a different response for total lag than did the 24 hour L:D cycle (Table 16). This difference should not be surprising since the rhythm of oviposition was also affected by the ahemeral 22 hour L:D cycle.

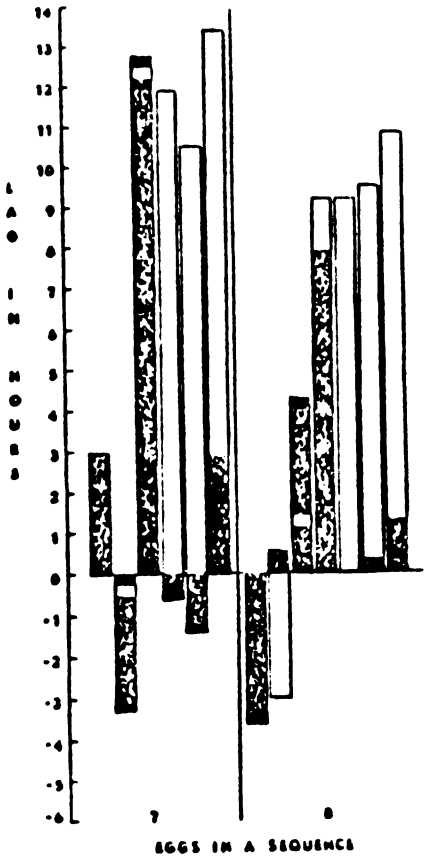
The discontinuation of the trend to increase total lag as egg sequence size increased, at the 8 egg sequence, was due to the negative lag values that were produced (Figures 24B and C). This indicates that ovulation was taking place progressively earlier each day. Thus, the short ahemeral L:D cycle could be turning on the release of the ovulatory hormone, LH, at shorter intervals each day by stimulating some controlling mechanism (receptor) in the brain of the pheasant.

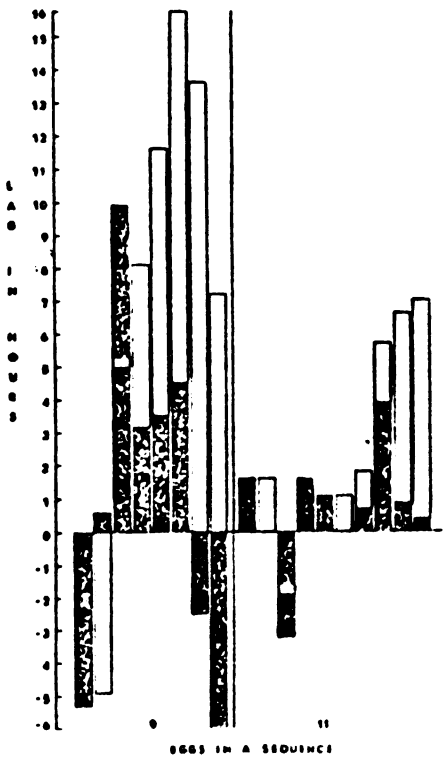
c) Ahemeral 26 hour L:D cycle

Total lag time under ahemeral 26h L:D cycle (14L:12D) continues to increase as egg sequence increases, except at the 7 egg sequence (Table 16). This was unlike the ahemeral 22 hour L:D cycle for which the continued increase was only to the 7 egg sequence, but for this L:D cycle treatment the increase in total lag was for all sequences. The decrease in

Figure 24B. Effect of ahemeral 22 hour light-dark cycle on oviposition lag in hours between successive eggs in a 7 and an 8 egg sequence for pheasants. The "closed" bars in a particular sequence represent the lag between the previous and present oviposition. An "opened" bar in a particular sequence represents the total of all the previous and present lags. In a "closed" bar with an open space, the total lag is the distance between the abscissa and the beginning of the open area.

Figure 24C. Effect of ahemeral 22 hour (14L:8D) light-dark cycle on oviposition lag in hours between successive eggs in a 9 and an 11 egg sequence for pheasants. The "closed" bars in a particular sequence represents the lag between the previous and present oviposition. An "opened" bar in a particular sequence represents the total of all the previous and present lags. In a "closed" bar with an open space, the total lag is the distance between the abscissa and the beginning of the open area.


EGGS IN A SEQUENCE



total lag for the 7 egg sequence was due to a large negative lag (Figure 25B). The number of negative lags obtained under this L:D cycle (Figures 25A,B, and C) were less than the number obtained under the 22 hour L:D cycle. The maximum average total lag obtained under the 26 hour L:D cycle treatment was 16.7 hours which was greater than the maximum average total lag obtained under the 24 and 22 hour L:D cycle. The overall average was 2.5 hours (Table 16).

It is obvious that ahemeral L:D cycles affect total lag time in pheasant egg production. The ahemeral 26 hour L:D cycle acts to extend the lag threshold (7 hours) for cessation of oviposition by some unknown mechanism, which probably lies at the pituitary-gonadal axis, such that longer egg sequences may be produced.

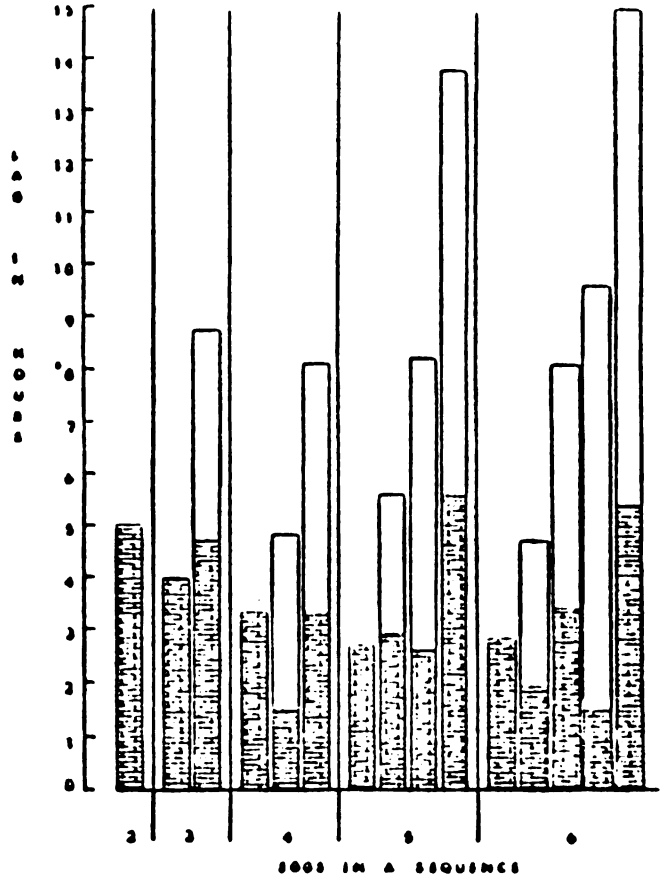
4. Egg formation time and egg sequence length occurrences

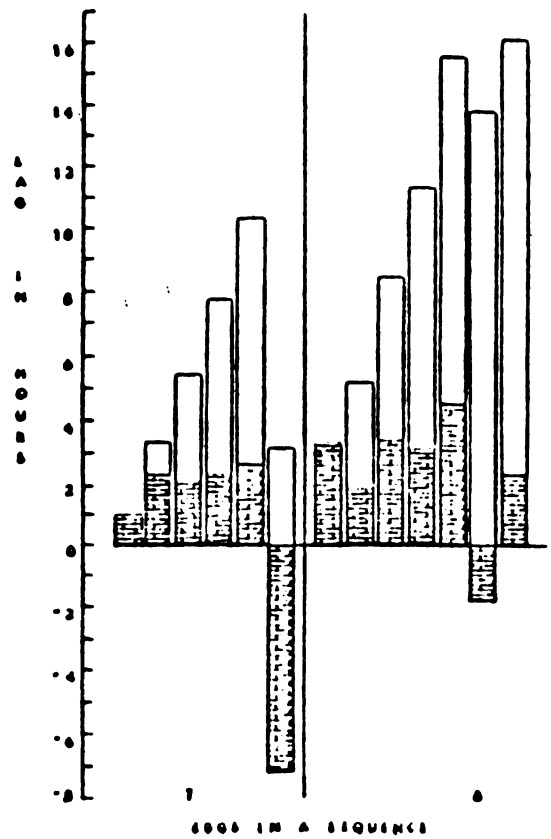
a) Control 24 hour L:D cycle

Egg formation time (EFT) for ring-necked pheasants under the 24h L:D cycle (14L:10D) averaged 25.5 hours (Table 16). This was within the range (24-26 hours) reported for chickens and coturnix under a similar L:D cycle (Atwood, 1929; Warren and Scott, 1936; Arrington et al., 1962; Woodard and Mather, 1964; Morris, 1973; Tucker and Ringer, 1982). As the egg sequence length was increased, the average lag time decreased from 3.1 hours for 2 egg sequences to 0.8 hours for 10 egg sequences (Table 16). This represents a range of EFT from 24.8 to 27.1 hours. This meant that the intra-clutch intervals also decreased for pheasants as egg sequence size increases. A similar situation was also observed in chickens reared under a 24 hour L:D cycle (Heywang, 1938).

Figure 25A. Effect of ahemeral 26 hour (14L:12D) light-dark cycle on oviposition lag in hours between successive eggs in a 2, 3, 4, 5, and 6 egg sequence for pheasants. The "closed" bars in a particular sequence represents the lag between the previous and the present oviposition. An "open" bar in a particular sequence represents the total of all previous and present lags.

Figure 25B. Effect of ahemeral 26 hour (14L:12D) light dark cycle on oviposition lag in hours between successive eggs in a 7 and an 8 egg sequence for pheasants. The "closed" bars in a particular sequence represents the lag between the previous and the present oviposition. An "open" bar in a particular sequence represents the total of all previous and present lags.





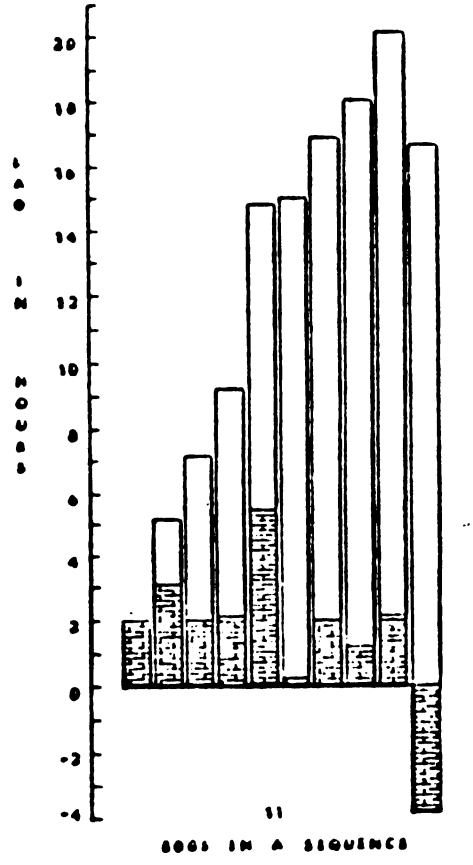


Figure 25C. Effect of ahemeral 26 hour (14L:12D) light-dark cycle on oviposition lag in an 11 egg sequence for pheasants. The "closed" bars in the sequence represents the lag between the previous and the present oviposition. The "open" bar in the sequence represents the total of all previous and present lags.

The greatest occurrence of any sequence length was for the 1 egg sequence which was observed to occur 141 times (Table 17). An egg sequence length as long as 71 was obtained under the 24 hour L:D cycle. The total number of sequences obtained was 605. This resulted in an average of 4.6 eggs per sequence. According to Tucker and Ringer (1982), if the length of L:D cycles were synchronized with the length of the time of follicular maturation, longer egg laying sequences would be obtained. Based upon the average EFT (25.5 hours) under this L:D cycle, it was obvious that the 24 hour L:D cycle was out of synchrony, by 1.5 hour, with the time of follicular maturation for the pheasants used in this experiment. Thus, the maximum potential of egg sequence size was not realized from pheasants reared under the 24 hour L:D cycle.

b) Ahemeral 22 hour L:D cycle

Ring-necked pheasants kept under the ahemeral 22h L:D cycle (14L:8D) had an average EFT of 25.8 hours (Table 16). This was similar to the average EFT for ring-necked pheasants kept under the control 24 hour L:D cycle. A minimum time is probably required for EFT, thus the pheasant hens kept under the 22 hour L:D cycle treatment required the same time for EFT as those hens under the 24 hour L:D cycle.

Similar to the 24 hour L:D cycle, as the sequence size increased under the 22 hour L:D cycle the average oviposition lag time decreased from 3.1 hours for 3 egg sequences to 0.8 hours for 10 egg sequences (Table 16). This represented a

Table 17. The number of occurrences of each egg sequence length for pheasants under 22, 24, or 26 hour light-dark cycles.

| Sequence length | Number of occurrences Light-dark cycle | | |
|---|---|---|---|
| | 24 (14L:10D) | 22 (14L:8D) | 26 (14L:12D) |
| 1 | 141 | 218 | 85 |
| 2 | 130 | 156 | 50 |
| 3 | 95 | 146 | 37 |
| 4 | 54 | 104 | 29 |
| 5 | 48 | 72 | 22 |
| 6 | 38 | 47 | 25 |
| 7 | 11 | 34 | 13 |
| 8 | 17 | 26 | 5 |
| 9 | 19 | 16 | 14 |
| 10 | 8 | 6 | 5 |
| 11-20 | 30 | 19 | 42 |
| 21-30 | 7 | 0 | 13 |
| 31-40 | 2 | 0 | 6 |
| 41-50 | 2 | 0 | 4 |
| 51-60 | 1 | 0 | 2 |
| 61-76 | 2 | 0 | 7 |
| 81 | 0 | 0 | 1 |
| 84 | 0 | 0 | 1 |
| Total eggs/ Total sequences | 2785/605 | 2919/844 | 3031/361 |
| Avg. no. eggs/ sequence | 4.6 | 3.5 | 8.4 |

range of EFT from 24.8 to 27.1 hours. Intra-clutch intervals were observed to decrease for pheasants under the 22 hour L:D cycle as egg sequence size increased. This was also true for the pheasants under the 24 hour L:D cycle in this study and for chickens reared under a 24 hour L:D cycle (Heywag, 1938).

A greater incidence of shorter egg sequence length occurred for hens exposed to the 22 hour L:D cycle than compared to those hens exposed to the 24 hour L:D cycle (Table 17). There were no egg sequences beyond the 20 egg sequence. The total number of egg sequences obtained was 844, a 40% increase over the total obtained under the 24 hour L:D cycle. A large majority of these were smaller sequences which consequently resulted in an average of 3.5 eggs per sequence. This was 1.1 egg/sequence less than the egg sequence average for hens under the 24 hour L:D cycle. The shorter egg sequence size obtained was probably due to the follicular maturation time (25.8 hours) being out of synchrony with the 22 hour L:D cycle (Tucker and Ringer, 1982). This was off by 3.8 hours. Hens under the 22 hour L:D cycle tend to produce a higher percent hen-day egg production compared to those hens under the 24 hour L:D cycle (Figure 9) because of the higher incidence of short egg sequences and also the higher incidence of hens producing 2 eggs per day.

c) Ahemeral 26 hour L:D cycle

The ring-necked pheasants, exposed to the ahemeral 26h L:D cycle (14L:12D) average egg formation time was 26.5 hours (Table 16). It is obvious that the egg formation time or the follicular maturation time was in synchrony with the 26 hour L:D cycle than with the 22 or 24 hour L:D cycle previously discussed. This was not surprising since Morris (1973) reported mean intra-clutch interval or egg formation time to increase and be of a similar length to the L:D cycle when the L:D cycle length was increased above 24 hours.

Similar to the 22 and 24 hour L:D cycles, average lag tended to decrease as egg sequence length increases, ranging from 0.4 hours to 5.0 hours (Table 17). This represented a larger range in egg formation time (24.5-29.0 hours) compared to the ranges reported under the other L:D cycles in this experiment. The 1 egg sequences obtained from hens under the 26 hour L:D cycle occurred less than under the other treatments. There was also a higher incidence of longer egg sequences; a length as long as 84 eggs was obtained. The number of sequences that occurred under the 26 hour L:D cycle was 361 which gave an average of 8.4 eggs per sequence. This showed relative success in improving egg production, via the use of a L:D cycle length that was closely synchronized with the time of follicular maturation resulting in longer sequences. Consequently, percent hen-day egg production was greater under the 26 hour L:D cycle than under the 22 and 24 hour L:D cycles (Figure 9). Although eggs oviposited under the 26 hour

L:D cycle spent an average of 0.7 hours longer in the oviduct, there was no improvement in egg weight (Table 12) or egg specific gravity (Appendix F, Table F1). This was inconsistent with the report of Morris (1973). This author stated that increases in egg weight was due to increases in albumen and shell deposition due to the extra oviducal term of the egg when hens are reared under ahemeral L:D cycles.

## H. Progesterone and LH Rhythms During Pheasant Ovulatory Cycle

### 1. Control 24 hour L:D cycle

The LH and progesterone rhythms, taken at 3-hour intervals over a period of time during the ovulatory cycle of ring-necked pheasants kept under the 24h L:D cycle (14L:12D), is shown in Figure 26. The average values are shown in Table 18. The progesterone value started to exceed the LH value at approximately 18 hours prior to ovulation (Figure 26). At 6-9 hours prior to ovulation, the surge values of LH (7.5 ng/ml) and progrsterone (8.2 ng/ml) were attained. This was also reported in chickens kept under a 24 hour L:D cycle (Williams and Sharp, 1978; Johnson and van Tienhoven, 1980; Tanabe and Nakamura, 1980). The surge value obtained for progesterone is similar to the surge value (8.0 ng/ml) reported for turkeys (Sharp et al., 1981) but not to the surge values 3-6 ng/ml) reported for chickens (Furr et al., 1973; Etches and Cunningham, 1976; Follett and Davies, 1978; Tanaka and Kamiyoshi, 1980) or coturnix (Tanabe and Nakamura, 1980; Gulati et al., 1981). The LH surge value shown in Table 18

Table 18. Pheasants plasma LH and progesterone levels (ng/ml) during the ovulatory cycle under the control 24 hour light-dark cycle (14L:10D).

| Hours before ovulation | n | Progesterone | n | LH |
|---|---|---|---|---|
| 21-24 | 7 | 1.67 ± 0.83[a]* | 8 | 3.39 ± 0.47 * |
| 18-21 | 5 | 1.24 ± 0.42 * | 7 | 2.79 ± 0.34 * |
| 15-18 | 4 | 3.23 ± 0.99 | 4 | 2.68 ± 0.72 * |
| 12-15 | 5 | 3.54 ± 1.77 | 6 | 2.05 ± 0.25 * |
| 9-12 | 9 | 4.29 ± 1.1 | 5 | 2.42 ± 0.39 * |
| 6- 9 | 8 | 8.28 ± 1.8 | 7 | 7.54 ± 2.32 |
| 3- 6 | 10 | 6.76 ± 1.4 | 7 | 3.63 ± 0.88 * |
| 0- 3 | 9 | 0.85 ± 0.23 * | 7 | 2.51 ± 0.26 * |

[a] Mean ± SEM.

* Significantly ($P < 0.05$) different from the highest mean within the column.

n = Number of observations.

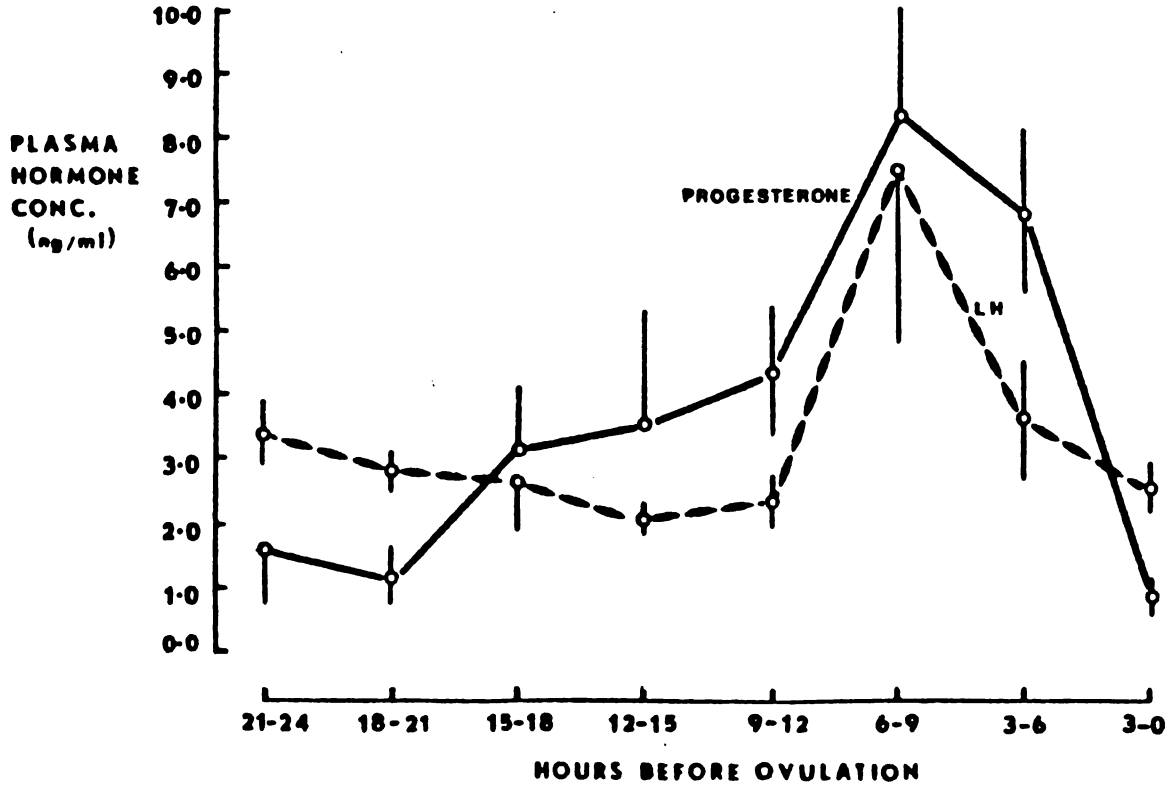



Figure 26. Changes in plasma progesterone and LH concentrations during the ovulatory cycle of the pheasant. The birds were kept under a 24h (14L:10D) conventional light-dark cycle. Each point represents the mean ± SEM.

is similar to that reported in coturnix (Gulati et al., 1981), ducks (Tanabe and Nakamura, 1980), and chickens (Johnson and van Tienhoven, 1980; White and Etches, 1984a). Other reports on LH studies in avian species indicated that the surge value of LH can be of lower levels (Mashaly et al., 1976; Follett and Davies, 1978; Tanabe and Nakamura, 1980; Sharp et al., 1981).

The LH mean surge value was significantly ($P \leq 0.05$) different from all the other LH mean values for the various intervals prior to ovulation (Table 18). This was not the case for the progesterone surge value, which was only significantly ($P \leq 0.05$) different from the mean basal values at the beginning and end of the ovulatory cycle.

At ovulation, the progesterone value was lower than the value for LH. It is therefore believed that the cascade of events, leading to ovulation, that have been reported for chickens (Williams and Sharp, 1978; Sharp, 1980), occurred in ring-necked pheasants under the 24 hour L:D cycle. Although LH and progesterone surged simultaneously, the progesterone surge was maintained for a longer time than for the LH. Thus, progesterone could be playing an extended role in ovulation, probably stimulating the synthesis of an enzyme (collagenase) involved in the rupture of the follicle as suggested by Doi et al. (1980) and tanabe and Nakamura (1980).

It is of interest to note that during the ovulatory cycle LH values starts to decline at 24 hours prior to ovulation to its nadir value at 12-15 hours before ovulation occurs. The

LH nadir value which was immediately followed by the LH surge, has been suggested as a possible change in feedback sensitivity of the hypothalamus or pituitary gland to the gonadal steroids, although major changes in the positive feedback mechanism to progesterone have not been observed (Wilson and Sharp, 1975; Etches and Cunningham, 1976; White and Etches, 1984a).

2. Ahemeral 22 hour L:D cycle

Starting at 18 hours prior to ovulation, the plasma progesterone levels (Figure 27) for the ring-necked pheasants exposed to the ahemeral 22h L:D cycle (14L:8D) started to increase. A similar observation was made for plasma progesterone levels under the 24 hour L:D cycle. This increase continued at a rapid pace, and the level was maintained above 4.7 ng/ml prior to the surge time. The surge value, 6.9 ng/ml (Table 19), under this L:D cycle was lower than the surge value under the 24 hour L:D cycle, but was within the range 6-8 ng/ml) reported for chickens (Tanabe and Nakamura, 1980) and turkeys (Opel and Arcos, 1978; Sharp et al., 1981). Progesterone surge time occurred 3-6 hours prior to ovulation, which was 3 hours later than under the 24 hour L:D cycle. The surge in progesterone has also been reported to occur later than 6 hours prior to ovulation for chickens when L:D cycles were less than 24 hours (Liou and Biellier, 1979; Liou et al., 1980) or equal to 24 hours (Furr et al., 1973; Follett and Davies, 1978; Tanabe and Nakamura, 1980). The progesterone surge was only significantly ($P \leq 0.05$) different from the

Table 19. Pheasant plasma LH and progesterone levels (ng/ml) during the ovulatory cycle under an ahemeral 22 hour light-dark cycle (14L:8D).

| Hours before ovulation | n | Progesterone | n | LH[1] |
|---|---|---|---|---|
| 21-24 | 12 | 0.90 ± 0.3[a] * | 12 | 2.56 ± 0.29 |
| 18-21 | 6 | 1.15 ± 0.9 * | 3 | 2.77 ± 0.76 |
| 15-18 | 4 | 4.7 ± 0.5 | 6 | 2.25 ± 0.42 |
| 12-15 | 5 | 5.4 ± 1.2 | 3 | 1.43 ± 0.79 |
| 9-12 | 4 | 5.9 ± 1.9 | 4 | 4.08 ± 0.69 |
| 6- 9 | 6 | 5.5 ± 1.2 | 8 | 4.13 ± 0.97 |
| 3- 6 | 7 | 6.9 ± 1.7 | 7 | 1.99 ± 0.34 |
| 0- 3 | 6 | 0.5 ± 0.3 * | 7 | 2.30 ± 0.38 |

[1] Means were not significantly ($P > 0.05$) different from the mean with the highest value within the column.

[a] Mean ± SEM.

* Significantly ($P \leq 0.05$) different from the highest mean within the column.

n = Number of observations.

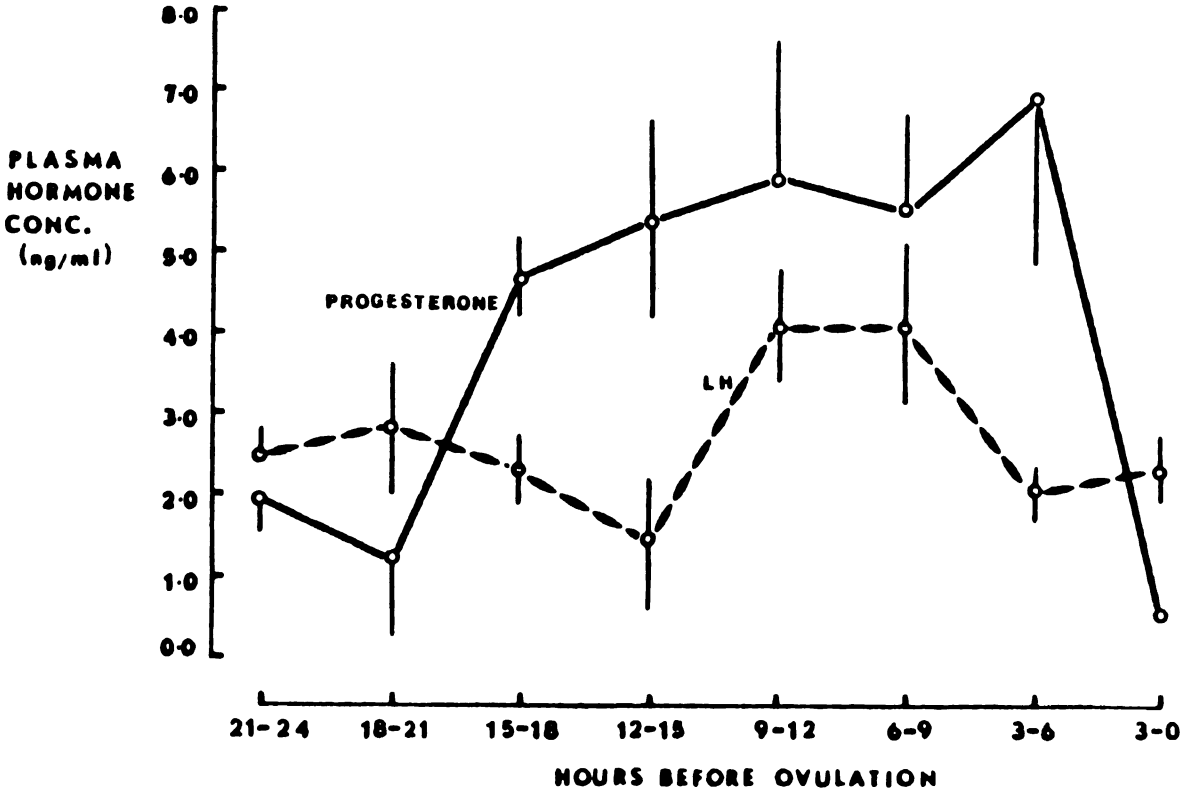



Figure 27. Changes in plasma progesterone and LH concentrations during the ovulatory cycle of the pheasant. The birds were kept under a 22h (14L:8D) ahemeral light-dark cycle. Each point represents the mean ± SEM.

mean basal values at the beginning and end of the ovulatory (Table 19). This was also the case for the controls previously discussed.

Plasma LH levels did not start to increase until 12-15 hours prior to ovulation. There was a plateau of LH levels (approximately 4.1 ng/ml) for 6 hours between the 9-12 to 6-9 hour intervals prior to ovulation. This surge in plasma LH level was not significantly ($P > 0.05$) different from the mean LH values at the other intervals prior to ovulation (Table 19). The shift in oviposition time for pheasants kept under the 22 hour L:D cycle (Figure 20) was due to the change in phase of LH. However, LH surge remained fixed relative to ovulation (Figure 27). Although the surge of LH and progesterone did not occur simultaneously, the LH surge did occur prior to the progesterone surge which is consistent with other reports in avian species (Furr et al., 1973; Follett and Davies, 1978; Tanabe and Nakamura, 1980). The cascade of events leading to ovulation, in which a small increase in LH will result in increases in progesterone which in turn will cause LH to surge (Williams and Sharp, 1978; Sharp, 1980) were also observed for pheasants kept under the 22 hour L:D cycle.

3. Ahemeral 26 hour L:D cycle

The initial rise, starting at 18 hours prior to ovulation, in plasma progesterone for ring-necked pheasants exposed to the ahemeral 26h L:D cycle (14L:12D) (Figure 28) was also observed for ring-necked pheasants exposed to the 22 and 24 hour L:D cycle. For the remaining intervals prior to

ovulation the rise in progesterone continued, but the pattern was less rapid compared to the pattern observed under the 24 hour L:D cycle. The mean surge value (9.54 ng/ml) which occurred 3-6 hours prior to ovulation was significantly ($P \leq 0.05$) different from the mean values at the other intervals during the ovulatory cycle except the two intervals immediately preceeding the surge (Table 20). There were no significant ($P > 0.05$) differences between the LH mean values during the pheasant ovulatory cycle under the ahemeral 26 hour L:D cycle (Table 20). However LH surge was obtained between the 9-12 and the 6-9 hour intervals prior to ovulation. White and Etches (1984a) using an ahemeral 28 hour L:D cycle in chickens, also reported a surge LH time of 6 hours prior to ovulation. Similar to the shift in oviposition time under the 22 hour L:D cycle (Figure 20), the shift observed under the 26 hour L:D cycle (Figure 21) was due to the change in phase of LH; however, the LH surge remained fixed relative to ovulation (Figure 28). This is consistent with the report of Abdelrazik et al. (1983) who indicated that the LH surge under a long ahemeral L:D cycle (30 hours) remained fixed relative to ovulation.

The cascade of events, leading to ovulation, that were previously discussed for the 22 and 26 hour L:D cycles were also observed under the ahemeral 26 hour L:D cycle.

Under the three L:D cycle treatments used in this study, the data on the pack cell volume (PCV) (Table 21) indicated that hemodilution was not a factor due to repeated sampling

Table 20. Pheasant plasma LH and progesterone levels (ng/ml) during the ovulatory cycle under an ahemeral 26 hour light-dark cycle (14L:12D).

| Hours before ovulation | n | Progesterone | | n | LH[1] |
|---|---|---|---|---|---|
| 21-24 | 13 | 1.72 ± 0.76[a] | * | 15 | 3.25 ± 0.52 |
| 18-21 | 9 | 1.51 ± 0.49 | * | 7 | 2.59 ± 0.33 |
| 15-18 | 4 | 2.30 ± 0.95 | * | 3 | 2.87 ± 0.37 |
| 12-15 | 9 | 5.62 ± 1.02 | * | 9 | 2.67 ± 0.44 |
| 9-12 | 9 | 6.14 ± 1.62 | | 9 | 4.58 ± 1.12 |
| 6- 9 | 9 | 6.69 ± 1.48 | | 9 | 4.67 ± 0.59 |
| 3- 6 | 7 | 9.54 ± 1.41 | | 9 | 4.43 ± 0.67 |
| 0- 3 | 9 | 1.49 ± 0.44 | * | 9 | 2.70 ± 0.39 |

[1] Means were not significantly ($P > 0.05$) different from the mean with the highest value within the column.

[a] Mean ± SEM.

* Significantly ($P \leq 0.05$) different from the highest mean within the column.

n = Number of observations.

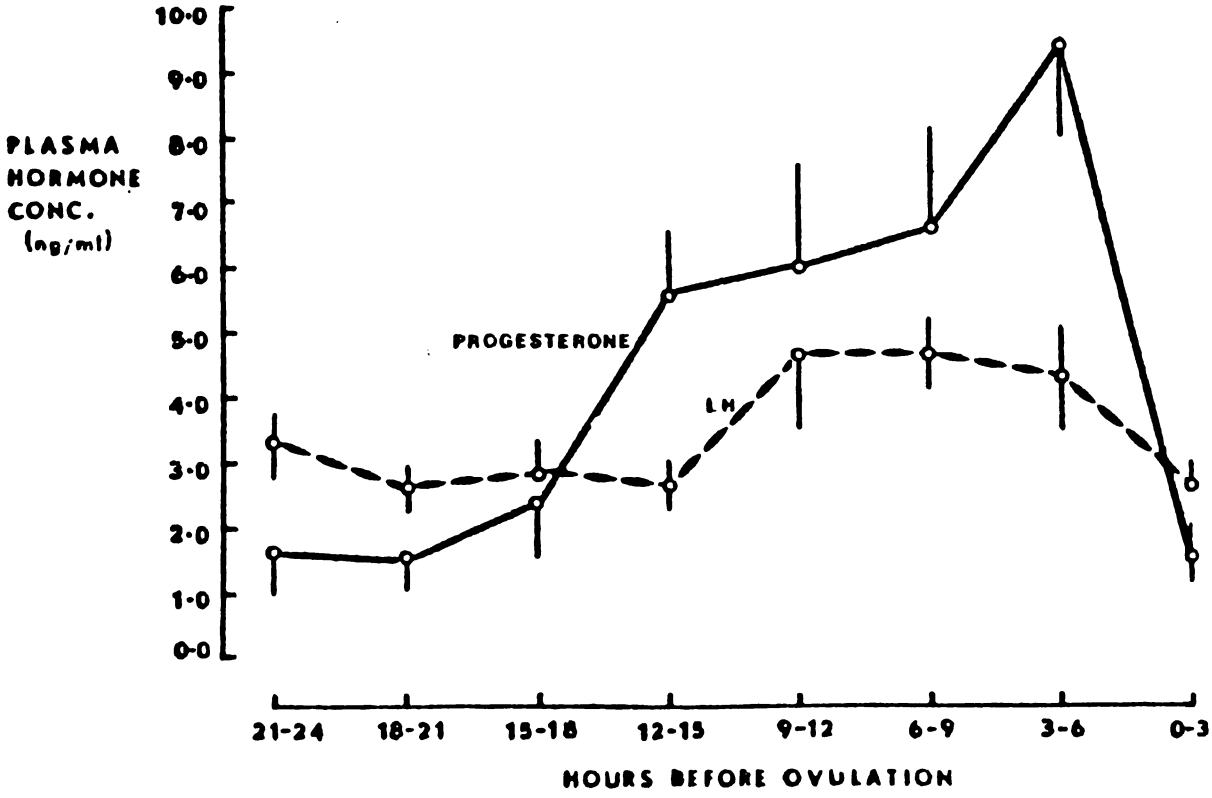



Figure 28. Changes in plasma progesterone and LH concentrations during the ovulatory cycle of the pheasant. The birds were kept under a 26h (14L:12D) ahemeral light-dark cycle. Each point represents the mean ± SEM.

Table 21. The average pack cell volume (Pcv) measured at each blood sampling time during the pheasant ovulatory cycle.

| Light treatment | Hours prior to ovulation | | | | | | | | Avg. |
|---|---|---|---|---|---|---|---|---|---|
| | 21-24 | 18-21 | 15-18 | 12-15 | 9-12 | 6-9 | 3-6 | 0-3 | |
| 22 hour cycle (14L:8D) | 33 | 34 | 34 | 32 | 32 | 30 | 33 | 34 | 32.8 |
| 24 hour cycle (14L:10D) | 32 | 36 | 34 | 35 | 33 | 34 | 28 | 32 | 33.0 |
| 26 hour cycle (14L:12D) | 34 | 31 | 33 | 31 | 32 | 34 | 31 | 34 | 32.5 |
| Overall Average | 33.0 | 33.7 | 33.7 | 32.7 | 32.3 | 32.7 | 30.7 | 33.3 | 32.8 |

(White and Etches, 1984b). All values on PCV were similar to each other and were consistent with the average (34.0% PCV) reported for other female pheasants by Bond and Gilbert (1958, cited by Sturkie and Griminger, 1976).

Generally, progesterone values throughout the pheasants ovulatory cycle seem to be higher than for other avian species, but LH values were always within the expected range. Progesterone and LH rhythms for pheasants kept under the 24 hour L:D cycle followed a similar pattern to that of other avian species, with a simultaneous surge time at 6-9 hours prior to ovulation. The use of the ahemeral 22 and 26 hour L:D cycles resulted in a shift in LH initial rise, but not progesterone, such that the LH rise began 3 hours earlier than that of progesterone. Regardless of the light treatment, LH nadir value occurred 12-15 hours prior to ovulation and also immediately preceding the LH surge. Shifts in oviposition time for the two ahemeral L:D cycles was due to a change in phase of LH with the surge remaining fixed at approximately 6 hours prior to ovulation. A cascade of events involving LH and progesterone leading to ovulation was observed under the three L:D cycle treatments. Also, hemodilution, due to repeated withdrawal of blood, did not occur and therefore, was not a factor in this experiment.

# CHAPTER 6

## SUMMARY AND CONCLUSIONS

### A. Summary

This experiment was conducted to evaluate the effect of ahemeral L:D cycles, 22 (14L:8D) and 26 (14L:12D) hours on female pheasant reproduction during the reproductive period. The effects of these L:D cycles on feed intake and body weight changes of male and female ring-necked pheasants were also examined. All comparisons were made with a conventional 24h L:D cycle (14L:10D).

The data indicated that:

1. The greatest increase (6.6%) in percent hen-day egg production was obtained under the ahemeral 26 hour L:D cycle, but this was not significant ($P > 0.05$).

2. Ahemeral 22 and 26 hour L:D cycles had no significant ($P > 0.05$) effect on egg weight compared to the control cycle. However, egg weight was significantly ($P \leq 0.05$) greater under the 22 hour L:D cycle than under the 26 hour L:D cycle. The DNR strain produced significantly ($P \leq 0.05$) greater egg weights compared to the MSU strain.

3. Ahemeral 22 hour L:D cycle significantly ($P \leq 0.05$) decreased egg shell thickness and egg specific gravity over the control cycle. Egg shell thickness and egg specific gravity for the DNR strain was significantly ($P \leq 0.05$) increased compared to the MSU strain's. There was a correlation coefficient of 0.73 between these two parameters.

4. Neither strain nor light treatments had any significant ($P > 0.05$) effect on egg mass, on percent cracked or percent shell-less eggs, or on percent dead embryos or pipped eggs.

5. Percent fertility obtained under the ahemeral 26 hour L:D cycle was significantly ($P \leq 0.05$) greater than the percent fertility obtained under the 22 and 24 hour L:D cycles. There were no significant ($P > 0.05$) differences in percent fertility between strains.

6. Neither ahemeral L:D cycle nor strain significantly ($P > 0.05$) affected percent hatchability. The highest average percent hatchability (73.6%) was obtained under the 26 hour L:D cycle. This was approximately 10% more than was obtained under the 22 and 24 hour L:D cycles.

7. Most oviposition times occurred later under a 22 hour L:D cycle and earlier under a 26 hour L:D cycle than observed under the 24 hour L:D cycle.

8. Total lag times were greater under the 22 and 26 hour L:D cycles than under the 24 hour L:D cycle.

9. Egg formation times under the 24, 22, and 26 hour L:D cycles were approximately 25.5, 25.8, and 26.5 hours, respectively.

10. LH and progesterone surge occurred approximately 6-9 hours prior to ovulation. The surge of both hormones under the 24 hour L:D cycle occurred simultaneously.

11. Shifts in oviposition times under the ahemeral 22 and 26 hour L:D cycles were due to a change in the phase of LH

with the surge remaining fixed at approximately 6 hours prior to ovulation.

12. Female pheasants consumed an average of 14 g/b/d more feed than the male pheasants. However, there were no significant ($P > 0.05$) differences in feed intake between light treatment or strains for males or females.

13. There were no significant ($P > 0.05$) differences in body weight between light treatments for male or female pheasants. The DNR strain's body weight was significantly ($P \leq 0.05$) greater than the MSU strain's. The decrease in body weight for both male and female pheasants was due to reduced feed intake and the use of body fat (in the case of the female) for yolk synthesis.

## B. Conclusions

Ahemeral L:D cycles did not significantly affect pheasant egg production, but there was a trend for the hens kept under the longer ahemeral L:D cycle (26 hours) to improve egg production and lay in longer sequneces of eggs; thus, using a L:D cycle longer than 26 hours (example, 28 hours) might result in significant improvement in egg production for ring-necked pheasants.

The ahemeral L:D cycles used in this experiment affected reproduction in that, the rhythm of oviposition, oviposition lag time, and egg formation time were altered compared to the results obtained under the control L:D cycle. The significance

of these changes in pheasant reproduction cannot be interpreted at this time.

Based upon the hormone data, it appears that LH surge during the pheasant ovulatory cycle remains fixed relative to the time of ovulation, regardless of the L:D cycle used, although oviposition rhythm was altered.

# APPENDIXES

# APPENDIX A

## TABLES OF PHEASANT RATIONS

Table A1. Pheasant starter ration fed to chicks from one day to six weeks of age.

| Ingredient | Percent |
|---|---|
| Corn | 46.35 |
| Soybean meal, 49% | 39.40 |
| Alfalfa, 17% | 3.00 |
| Fish meal, 60% | 2.50 |
| Meat and bone meal, 50% | 3.00 |
| Whey, dried | 2.00 |
| Salt | 0.25 |
| Dicalcium phosphate | 1.50 |
| Limestone | 1.25 |
| Premix (5004)[1] | 0.75 |

[1] See footnote, Table 3.

CALCULATED ANALYSIS

| | |
|---|---|
| Crude protein ..... | 28.00 |
| Fat ..... | 2.61 |
| Fiber ..... | 3.32 |
| Calcium ..... | 1.47 |
| Phosphorus, available ..... | 0.70 |
| M.E., cal/lb. ..... | 1241.00 |

Table A2. Pheasant grower ration fed to chicks from six weeks to 13 weeks of age.

| Ingredients | Percent |
|---|---|
| Corn | 54.50 |
| Soybean meal, 49% | 25.50 |
| Wheat middlings | 7.50 |
| Alfalfa, 17% | 3.00 |
| Fish meal, 60% | 2.50 |
| Meat and bone meal, 50% | 3.00 |
| Salt | 0.25 |
| Dicalcium phosphate | 1.50 |
| Limestone | 1.50 |
| Premix (5004)[1] | 0.75 |

[1] See footnote, Table 3.

CALCULATED ANALYSIS

| | |
|---|---|
| Crude protein ..... | 22.00 |
| Fat ..... | 3.00 |
| Fiber ..... | 3.64 |
| Calcium ..... | 1.43 |
| Phosphorus, available ..... | 0.63 |
| M.E., cal/lb. ..... | 1269.00 |

Table A3. Pheasant flight ration fed to chicks from 13 weeks to time of stimulating light.

| Ingredient | Percent |
|---|---|
| Corn | 55.40 |
| Soybean meal, 44% | 14.10 |
| Oats | 10.00 |
| Wheat middlings | 10.00 |
| Alfalfa, 14% | 3.75 |
| Meat and bone meal, 50% | 3.00 |
| Salt | 2.50 |
| Dicalcium phosphate | 1.50 |
| Limestone | 1.50 |
| Premix (5004)[1] | 0.50 |

[1] See footnote, Table 3.

CALCULATED ANALYSIS

| | |
|---|---|
| Crude Protein ..... | 16.00 |
| Fat ..... | 3.51 |
| Fiber ..... | 5.30 |
| Calcium ..... | 1.30 |
| Phosphorus, available ..... | 0.55 |
| M.E., cal/lb. ..... | 1259.00 |

# APPENDIX B

## CAGE LAYOUT

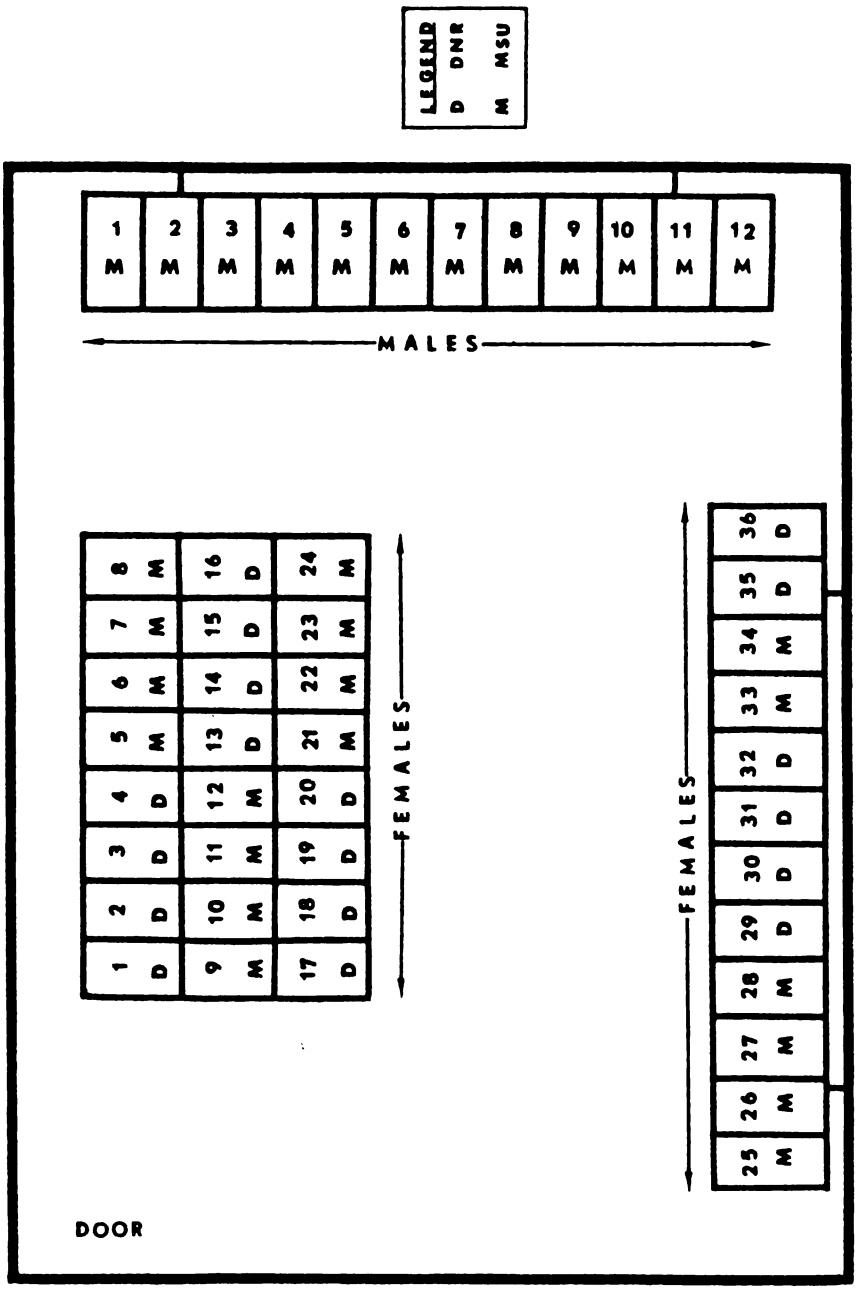



Figure B1. Experimental layout of cages in each light-dark cycle treatment for male and female pheasants.

# APPENDIX C

## PROCEDURES FOR THE DETERMINATION OF SPERM CELL CONCENTRATION

1. The sperm cell count was obtained by using a bright-line hemacytometer[8] and a light microscope.
2. Samples were prepared by filling the tip of a RBC pipette to the 0.5 mark with semen by capillary action.
3. A 0.085% saline + 2% formalin solution was used to dilute the semen to the pipette's 101 mark. This gives a dilution of 200. The formalin immobolizes the spermatozoa, thus facilitating easy counting.
4. The samples counted were done in replicates. Each, consisting of 5 squares or counting chambers (1 x 1 mm), was located on opposite sides of the hemacytometer. Each chamber had a depth of 1 mm and consisted of 16 small squares, thus there was a total of 80 squares.
5. A cover slip was placed over each replicate.
6. From the pipette, a small amount of sample was released at one side of each cover slip, from which the sample spreads, covering all the counting chambers.
7. The sperm cell counts used at any point on the standard curve was the average of the two replicates for a particular sample.
8. The following formula was used for the calculations:

$$\text{Sperm cell/cu.mm} = \frac{\text{Number of cells counted x dilution x 4000}}{\text{Number of small squares counted}}$$

$$= \frac{\text{Number counted x 200 x 4000}}{80}$$

$$= \text{Number of cells counted x 10,000}$$

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[8] American Optical Corporation, Buffalo, NY 14215

Concentration of sperm cells inseminated per volume = Number of sperm cells/cu.mm x number of cu.mm/cc (ml) x volume.

# APPENDIX D

## PROCEDURES FOR BLOOD SAMPLE COLLECTION

1. Blood samples were obtained from pheasant hens by the use of a sterile 5 cc syringe[9], attached to a 22 gauge, 3.81 hypodermic needle[10].
2. Prior to sampling both the syringe and the hypodermic needle were flushed with heparin (4 mg/ml in physiological saline) in order to prevent blood clotting.
3. At each sampling, approximately 4 ml of blood was obtained via cardiac puncture.
4. From each sample of blood, a hematocrit determination was made.
5. The remainder of the blood sample was centrifuged with a Dynac centrifuge[11] at 2000 rpm for 20 minutes.
6. At the completion of the centrifugation process, the plasma was decanted into three separate portions, each to a separate screw cap vial[12], 12 x 35 mm (½ dram), for storage at -20°C until the time of assay.

The blood samples obtained from an individual hen were not within a 24 hour period, but over several egg sequences. This was done in order to avoid hemodilution, which may occur due to the removal of large volumes of blood at very frequent

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[9] Division of Becton, Dickerson, and Co., Rutherford, NJ 07070

[10] Division of Becton, Dickerson, and Co., Rutherford, NJ 07070

[11] Division of Becton, Dickerson, and Co., Rutherford, NJ 07070

[12] VWR Scientific INcorporation, 800 East Fabyan Parkway, Batavia, IL 60510

intervals, and stress which may occur due to sampling via cardiac puncture. The design of sampling was similar to that of Gulati et al. (1981) in Japanese quail and Wilson et al. (1983) in chickens. Samples were taken only on a day when the hen lays. The samples were only considered to be valid if the hen laid an egg on the day following sampling. This precaution was necessary in order to relate hormone concentration to the time of ovulation.

# APPENDIX E

## MODELS AND STATISTICAL ANALYSIS TESTS

A. The models used for the two-way ANOVA.

1. Female Model

$$Yijkl = M+Ai+Bj+(AB)ij+C(ij)k+(AP)il+(BP)jl+(ABP)ijl+E(ijkl)$$

2. Male Model

$$Yikl = M+Ai+C(_i)k+Pl+(AP)il+E(ikl)$$

Where:

Yijkl = Is the variable response for time l for birds k from strain j, receiving light i.

Yikl = Is the variable response for time l for bird k receiving light l.

M = Represents the population mean.

Ai = Represents the fixed effect of the ith level of light (3 levels).

Bj = Represents the fixed effect of the jth level of strain (2 levels).

(AB)ij = Represents the effect of the interaction of light and strain.

C(i)k = Represents the random effect of birds within light (Error I). Contributes to error appropriate for measuring the effect of light.

C(ij)k = Represents the random effect of birds within light and strain (Error I). Contributes to error appropriate for measuring the effect of light and strain.

Pl = Represents the fixed effect of the lth level of time.

(AP)il = Represents the effect of the interaction of light and time.

(BP)jl = Represents the effect of the interaction of strain and time.

(ABP)ijl = Represents the effect of the interaction of light, strain, and time.

E(ikl) = Represents the residual error (Error 2). It contributes to error by measuring all the effects relating to time. It is also the random effect of all unspecified variables.

E(ijkl) = Same as E(ikl) above.

B. Test for Significant differences of treatments.

1. F-test (Table A.5, from Gill, 1978c).

F-value = $MS_t/MS_E$

If the F-value is greater than $f\alpha$, $V_1$, $V_2$, then there are significant differences between treatment means.

$MS_t$ = Mean sum of the squares of the treatments.

$MS_E$ = Mean sum of the squares of the error.

$V_1$ = Number of treatments - 1 (t-1).

$V_2$ = Number of observations - number of treatments (n-t).

2. Treatment comparisons.

a) Bonferroni t-statistics (Table A.10. from Gill, 1978c).

$$t_B = \frac{\bar{X}_1 - \bar{X}_2}{\sqrt{MS_E(\frac{1}{r_1} + \frac{1}{r_2})}}$$

If $t_B$ is greater than $t_B\alpha/2,M,V$ there is a significant difference between the two means being compared.

M = Number of comparisons - 1.

r = Number of replications per treatment or for each mean being compared.

V = Number of observations - number of comparisons

$\bar{X}$ = Treatment mean.

b) Dunnett's t-statistics (Table A.9.1 from Gill, 1978c).

$$t_D = \frac{\bar{Y}_1 - \bar{Y}_2}{\sqrt{MS_E(\frac{1}{r_1} + \frac{1}{r_2})}}$$

If $t_D$ is greater than $t_{D\alpha/2,V,M}$ there is a significant difference between the two treatment means being compared.

M = Number of observations - number of comparisons.

r = Number of replication per treatment or for each mean being compared.

V = Number of comparisons - 1.

$\bar{X}$ = Treatment mean.

C. Standard error of difference (SED) between means (repeated measurements; split plot analysis, see Gill, 1978b).

1. Between the two treatment means within a period.

$$SED = \sqrt{MS_{E_1}\left(\frac{1}{r_1} + \frac{1}{r_2}\right)}$$

2. Between two period means within a treatment.

$$SED = \sqrt{MS_{E_2}\left(\frac{1}{r_1} + \frac{1}{r_2}\right)}$$

D. Standard error of mean (SEM) for the one-way analysis (used for the oviposition and hormone data).

$$SEM = \frac{\text{Standard deviation (SD)}}{\sqrt{n}}$$

$$= \frac{\sqrt{\frac{\Sigma(X-\bar{X})^2}{n-1}}}{\sqrt{n}}$$

n = Number of observations.

E. Coefficient of variation (CV).

1. Intra-assay

CV = SD of the assay quality control values ÷ mean of the assay quality control values.

2. Inter-assay

CV = SD of the quality control averages between the assays ÷ overall quality control mean for the assays.

# APPENDIX F

## TABLES OF THE AVERAGES OF SOME OF THE PARAMETERS MEASURED

Table F1. Average egg specific gravity in two strains of pheasants, under 22, 24, or 26 hour light-dark cycles at 28 day intervals[1,2,3].

| Strain | Period | Light treatment 22 (14L:9D) | 24 (14L:10D) | 26 (14L:12D) | Avg. |
|---|---|---|---|---|---|
| DNR | 1 | 1.0863 | 1.0888 | 1.0899 | 1.0883 |
| | 2 | 1.0843 | 1.0863 | 1.0875 | 1.0860 |
| | 3 | 1.0793 | 1.0811 | 1.0834 | 1.0813 |
| | 4 | 1.0748 | 1.0774 | 1.0772 | 1.0765 |
| | 5 | 1.0728 | 1.0758 | 1.0797 | 1.0758 |
| | Avg. | 1.0795 | 1.0819 | 1.0833 | 1.0816[a] |
| MSU | 1 | 1.0827 | 1.0885 | 1.0858 | 1.0857 |
| | 2 | 1.0797 | 1.0834 | 1.0832 | 1.0821 |
| | 3 | 1.0764 | 1.0791 | 1.0776 | 1.0777 |
| | 4 | 1.0723 | 1.0756 | 1.0734 | 1.0738 |
| | 5 | 1.0683 | 1.0743 | 1.0721 | 1.0716 |
| | Avg. | 1.0759 | 1.0802 | 1.0784 | 1.0782[b] |
| Overall Average | | 1.0777[c] | 1.0810[d] | 1.0809[d] | |

[a,b] Means with different symbols within a column differ significantly ($P \leq 0.05$).

[c,d] Means with different symbols within a row differ significantly ($P \leq 0.05$).

[1] The standard error of difference (SED) (repeated measurements; split plot design, see Gill, 1978b) between any two light treatment means within a period is $\pm$ 0.0022. The SED between any two period means within a treatment is $\pm$ 0.0011.

[2] The SED between two strain means within a period is $\pm$ 0.0018. The SED between any two period means within a strain is $\pm$ 0.0009.

[3] Means of 18 birds per strain or 36 birds per light treatment.

Table F2. Average egg shell thickness (mm) in two strains of pheasants under 22, 24, or 26 hour light-dark cycles at 28 day interval[1,2,3].

| Strain | Period | Light treatment 22 (14L:9D) | 24 (14L:10D) | 26 (14L:12D) | Avg. |
|---|---|---|---|---|---|
| DNR | 1 | 0.309 | 0.318 | 0.321 | 0.316 |
| | 2 | 0.307 | 0.311 | 0.313 | 0.310 |
| | 3 | 0.291 | 0.304 | 0.306 | 0.301 |
| | 4 | 0.285 | 0.297 | 0.284 | 0.289 |
| | 5 | 0.278 | 0.289 | 0.294 | 0.287 |
| | Avg. | 0.294 | 0.304 | 0.304 | 0.301[a] |
| MSU | 1 | 0.286 | 0.319 | 0.307 | 0.304 |
| | 2 | 0.284 | 0.297 | 0.294 | 0.292 |
| | 3 | 0.276 | 0.296 | 0.297 | 0.287 |
| | 4 | 0.269 | 0.296 | 0.276 | 0.277 |
| | 5 | 0.261 | 0.279 | 0.262 | 0.267 |
| | Avg. | 0.275 | 0.296 | 0.295 | 0.285[b] |
| Overall Average | | 0.285[c] | 0.300[d] | 0.294[c,d] | |

[a,b] Means with different symbol within a column differ significantly ($P \leq 0.05$).

[c,d] Means with different symbols within a row differ significantly ($P \leq 0.05$).

[1] The standard error of difference (SED) (repeated measurements; split plot design, see Gill, 1978b) between any two light treatment means within a period is $\pm$ 0.0101 mm. The SED between any two period means within a treatment is $\pm$ 0.0048 mm.

[2] The SED between two strain means within a period is $\pm$ 0.0083 mm. The SED between any two period means within a strain is $\pm$ 0.0039 mm.

[3] Mean of 18 birds per strain or 36 birds per light treatment.

Table F3. Average percent fertility in two strains of pheasants under 22, 24, or 26 hour light-dark cycles at 7 day intervals[1,2,3].

| Strain | Period | Light treatment 22 (14L:8D) | 24 (14L:10D) | 26 (14L:12L) | Avg. |
|---|---|---|---|---|---|
| DNR | 1 | 90.6 | 79.8 | 84.5 | 84.9 |
| | 2 | 89.4 | 83.3 | 92.6 | 88.4 |
| | 3 | 87.5 | 77.3 | 99.1 | 87.9 |
| | 4 | 82.6 | 70.8 | 87.2 | 80.2 |
| | 5 | 81.9 | 70.2 | 92.5 | 81.5 |
| | 6 | 75.6 | 92.5 | 92.9 | 87.0 |
| | 7 | 79.9 | 76.6 | 81.3 | 79.3 |
| | 8 | 74.1 | 81.5 | 88.5 | 81.4 |
| | Avg. | 82.7 | 79.0 | 89.8 | 83.8[a] |
| MSU | 1 | 78.2 | 79.2 | 84.6 | 80.3 |
| | 2 | 87.7 | 91.4 | 95.6 | 91.7 |
| | 3 | 81.6 | 80.7 | 96.8 | 86.4 |
| | 4 | 81.6 | 77.9 | 87.7 | 82.4 |
| | 5 | 77.7 | 86.5 | 90.4 | 84.9 |
| | 6 | 75.5 | 74.7 | 87.8 | 79.3 |
| | 7 | 74.9 | 60.2 | 79.2 | 71.4 |
| | 8 | 72.3 | 71.3 | 87.9 | 77.2 |
| | Avg. | 78.7 | 77.7 | 88.8 | 81.8[a] |
| Overall Average | | 80.7[b] | 78.4[b] | 89.3[c] | |

[a] Means with the same symbol within a column do not differ significantly ($P > 0.05$).

[b,c] Means with different symbols within a row differ significantly ($P < 0.05$).

[1] The standard error of difference (SED) (repeated measurements; split plot design, see Gill, 1978b) between any two light treatment means within a period is ± 9.2%. The SED between any two light treatment means within a treatment is ± 5.2%.

[2] The SED between two strain means within a period is ± 7.5%. The SED between any two period means within a strain is ± 4.2%.

[3] Mean of 19 birds per strain or 36 birds per light treatment.

Table F4. Average percent hatchability[1] in two strains of pheasants under 22, 24, or 26 hour light-dark cycles at 7 day intervals[2,3,4].

| Strain | Period | Light treatment 22 (14L:8D) | 24 (14L:10D) | 26 (14L:12D) | Avg. |
|---|---|---|---|---|---|
| DNR | 1 | 64.5 | 56.9 | 71.4 | 64.3 |
| | 2 | 64.4 | 74.8 | 73.2 | 70.8 |
| | 3 | 77.6 | 76.1 | 75.4 | 76.4 |
| | 4 | 68.9 | 65.4 | 84.9 | 73.1 |
| | 5 | 77.5 | 64.4 | 79.5 | 73.8 |
| | 6 | 66.5 | 57.2 | 74.4 | 66.0 |
| | 7 | 74.7 | 64.6 | 80.1 | 73.2 |
| | 8 | 44.9 | 71.6 | 73.3 | 63.3 |
| | Avg. | 67.4 | 66.4 | 76.5 | 70.1[a] |
| MSU | 1 | 55.3 | 51.1 | 66.7 | 57.7 |
| | 2 | 63.2 | 73.2 | 65.7 | 66.6 |
| | 3 | 68.8 | 57.8 | 81.9 | 69.5 |
| | 4 | 56.8 | 52.7 | 67.9 | 59.1 |
| | 5 | 55.3 | 70.7 | 76.3 | 67.4 |
| | 6 | 67.2 | 61.9 | 67.6 | 65.6 |
| | 7 | 69.8 | 58.6 | 72.1 | 66.8 |
| | 8 | 45.4 | 53.4 | 67.5 | 55.4 |
| | Avg. | 60.2 | 59.9 | 70.7 | 63.6[a] |
| Overall Average | | 63.8[b] | 63.2[b] | 73.6[b] | |

[a] Means with the same symbol within a column do not differ significantly ($P > 0.05$).

[b] Means with the same symbol within a row do not differ significantly ($P > 0.05$).

[1] Of fertile eggs.

[2] The standard error of difference (SED) (repeated measurements; split plot design, see Gill, 1978b) between any two light treatment means within a period is ± 12.9%. The SED between any two light treatment means within a treatment is ± 7.6%.

[3] The SED between two strain means within a period is ± 10.2%. The SED between any two period means within a strain is ± 6.2%.

[4] Mean of 18 birds per strain or 36 birds per light treatment.

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