To the Graduate Council:

I am submitting herewith a dissertation written by Peter Osei entitled "Chick Growth and Metabolic Status as Affected by Photoperiod and Exogenous Melatonin." I have examined the final copy of this dissertation for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Animal Science.

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CHICK GROWTH AND METABOLIC STATUS AS AFFECTED BY PHOTOPERIOD AND EXOGENOUS MELATONIN

A Dissertation

Presented for the

Doctor of Philosophy

Degree

The University of Tennessee, Knoxville

Peter Osei

December 1987

ACKNOWLEDGEMENTS

With great indebtedness, the author wishes to acknowledge the invaluable contributions made by several important people in the preparation of this dissertation. Any credit resulting therefrom is equally shared with them. However, he wishes to be solely responsible for any shortfalls.

Dr. K. R. Robbins, my major professor, whose constructive criticism, encouragement, and absolute hospitality at all times were extremely essential for the successful completion of this program.

Dr. J. K. Miller, Dr. H. V. Shirley, Dr. H. Eiler, and Dr. S. L. Melton, my graduate committee members, for their critique of the research plans and review of the manuscript.

Mr. George McGee for his suggestions and assistance in data collection.

The staff of the university poultry farm for the care of the chickens during the experiments.

Eddie Jarboe, Linda Miller, Kevin Roberson, Hammed Olanrewaju, Sou Fei Chin, and others who in many diverse ways contributed to the successful completion of this work.

I would particularly like to acknowledge the great importance of the unwavering love and support of my parents, Anthony and Gladys, and my best friend, Ama Benson.

ABSTRACT

Series of experiments utilizing broiler chicks were performed to determine the role of the pineal gland and light in chick growth and metabolic status. Chicks were pinealectomized, sham operated on, or left intact. Other groups of chicks were also treated with 15 ppm melatonin. The lighting schedules employed were (1) diurnal light/dark 16L:8D and (2) constant light or 24L:0D. The parameters measured were body weight gain, feed consumption and feed efficiency, energy retention, efficiency of energy utilization for gain, rate of hepatic lipogenesis, jejunal glucose absorption, as well as plasma triiodothyronine (T_3) and throxine (T_4) levels.

Light treatment and dietary melatonin affected feed intake only slightly. However, gain, gain/feed ratio, and energy retention were significantly lower in birds housed under constant light. Dietary melatonin increased gain, gain/feed ratio, and energy retention by an average of 19%. The gain response to melatonin was significantly less when birds were housed under constant light; but there was not a significant light x melatonin interaction for either gain/feed ratio or energy retention. Pinealectomized birds grew significantly more slowly and less efficiently and were little affected by light treatment. However, the response of pinealectomized birds to dietary melatonin was of the same magnitude as either unoperated or sham operated birds.

Constant light significantly depressed and dietary melatonin significantly increased liver lipogenesis, jejunal glucose uptake, and plasma T_3 and T_4 concentrations.

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CHAPTER I

INTRODUCTION

In all modern day vertebrates examined, with the possible exception of the hagfish and crocodiles, pineal organs are known to be present (Oksche, 1971). Such widespread occurrence gives an indication of a rather long and continuing evolution, the early origin of which is hinted at by the existence of pineal systems in the most primitive living fishes and by indications of such systems in even more ancient fossil forms (Edinger, 1956). Far from appearing evolutionary regressive, pineal organs are and have been about as common in vertebrate brains as pituitary glands.

Within the nearly universal distribution of pineal organs in vertebrate brain, however, there has evolved an almost bizarre range of morphological diversity. Nonetheless, the synchrony of the pineal's metabolic activities to light:dark cycles is one which is common to all vertebrates examined thus far.

Comparative studies have clearly demonstrated the fact that, evolutionarily, the pineal gland has undergone the transition from being a photoreceptive organ in lower vertebrates to being glandular in mammals.

In recent years, the concept that the pineal organ of lower forms also functions as an important component of neuroendocrinology has gained support (Reiter, 1973). According to this concept, after the transduction of photic energy into the neural impulse in the eye, the

neural signals are conveyed to the pineal gland by means of a complex network. In the pineal the signal is again transduced, this time into a chemical agent or hormone which is then secreted to eventually influence the functioning of other organs.

Since its discovery, numerous functions have been attributed to the pineal gland, many of which are not clearly understood. The relationship of the pineal gland to body growth and metabolism has been studied in several species with inconsistent results.

Growth of an animal is a summation of a multitude of various factors acting in synergism. Exogenous factors such as food supply, photoperiod, olfactory stimuli, temperature, etc., probably exert their effect on growth by modifying endogenous factors. Such factors include varied phenomenon as genetic make-up, endocrine and exocrine secretions, as well as neurophysiological and neuroendocrine events. It stands to reason that if one alters exogenous stimuli which results in a growth change, one must then closely examine the endogenous change or changes which may have occurred resulting in the abnormal growth and metabolism. This approach calls for the examination of the relationship between the pineal gland, growth and metabolic status, and other factors such as photoperiod and other hormones.

The field of pineal physiology is littered with dead as well as dying hypotheses, and concepts based on inadequate evidence are fantasy, but it is from efforts to test such fantasy that new facts emerge.

The objectives of this study were to determine the role of pineal and light in chick growth and physiological and metabolic status.

Experiments were conducted to obtain information regarding the effects of light, pinealectomy, and melatonin on body weight gains and efficiency; energy retention and efficiency of energy utilization; rate of in vivo lipogenesis; rate of jejunal glucose absorption; and triidothyronine (T_3) and thyroxine (T_4) levels in blood plasma.

CHAPTER II

LITERATURE REVIEW

The pineal gland has fascinated philosophers and scientists for centuries and the enormous scientific literature which has accumulated over recent years is testimony to its mysteries. The greatest impetus for the study of the gland has come from observations relating it to the control of the reproductive system.

Despite the rich heritage of the pineal association with reproduction, the early scientific work was less conclusive for the exact nature of pineal control of the gonads. Enormous evidence was in favor of an inhibitory action, even though reports of both stimulatory influence or no influence at all were available. Nevertheless, many scientists relegated the pineal to the position of a vestige of a visual system in lower vertebrates with an unknown function at best in mammals. Thus, the organ which was once acclaimed by philosophers and wise men as seat of the mind (Herophilus) and seat of the soul (Descartes) became the Job of the scientific world. Plagued by anecdotes relating to philosophical tenets, the pineal recurrently evoked severe despair among many scientists who attempted to elucidate the latent function of this organ.

Today, indisputable evidence has been garnered and the null hypothesis that the pineal has no significant functional activity or role is heavily contradicted.

Pineal As An Endocrine Organ

There are two major means of demonstrating glandular function of an organ: 1) produce a deficiency state by excising the gland; or 2) exaggerate the functional state by administering an extract of glands from the same or different animals. When these procedures were applied to the pineal about a half-century ago, the results were hardly spectacular. Extirpation experiments were decidedly inconclusive and extracts provided little or no information. Even though some of the experimental work was well done, most was uncritical. This, then, was the uncertain status in 1954 when Kitay and Altschule made their critical appraisal of 1762 published reports. They cautiously concluded that the available laboratory and clinical evidence justified the presumption that the pineal is a functional gland even if its physiological role remained undefined.

This landmark study had a resounding effect on the field. There was evident proof that the pineal was susceptible to critical study in laboratory animals using standard experimental procedure. Most important, it opened up the prospect of obtaining what was so desperately needed--some firm, indisputable evidence and the dispelling of long-standing confusion.

In reference to the endocrine capabilities of the pineal organ, a large number of compounds have been considered to be the mediators of the hormonal effects. Among these are indoles, peptides (Vaughan et al., 1976; Pevet 1983) and the unknown antigonadotropic peptides (Benson and Ebels, 1981; Ebels, 1983). Even though the peptides have received increasing attention recently, the indoles, especially

melatonin, remain the pineal hormones that have been studied most extensively in the past two decades.

Several methodologies have been used to demonstrate that the pineal organ is involved in indole metabolism. Oguri et al. (1968) first reported that when trout (salmo gairdneri) were injected with radiolabelled 5-hydroxytryptophan, the precursor to serotonin, the uptake rate expressed as ratio of radioactivity in organs (mg) to that in blood (µl) was greater in the pineal organ than in several other organs sampled. Uptake of 5-hydroxytryptophan and serotonin has since been examined radioautographically in the trout (Hafeez and Zerihun, 1976) and a cyclostome (Meiniel, 1979).

The indoleamine serotonin is also found to be present in rat pineal glands (Wurtman et al., 1968) in concentrations 10-100 times greater than in those brain areas known to have high serotonin levels, i.e., diencephalon and midbrain. Human as well as monkey pineals also have high levels of serotonin (Wurtman et al., 1968). Quay (1963) demonstrated that rat pineal serotonin content varies with daily photoperiod as well as with the estrous cycle. Pineal serotonin falls with the onset of dark and is particularly low on the nights of diestrus and proestrus. The daytime peak was 9 times higher than the nighttime. The role of serotonin as a precursor for melatonin (via N-acetylserotonin) and the diurnal inverse relationship between serotonin content and the melatonin-forming enzyme hydroxyindole-o-methyl transferase (HIOMT) activity were also elucidated. It was shown that the serotonin rhythm was endogenous (persisted after blinding or continuous dark) whereas the HIOMT rhythm followed that of

norepinephrine, being exogenous (extinguished by deprivation of light). In avian species, pineal serotonin has been detected by fluorometry (Quay, 1966) by bioassay (Hedlund and Ralph, 1967), and by fluorescence histochemistry (Fuxe and Ljunggren, 1965). The amounts extractable vary among various species; the pigeon is reported to have a maximum of about 250ng in its pineal (Quay, 1966) whereas the Japanese quail's pineal has at most only about 1.4ng (Hedlund and Ralph, 1967). An enzyme, 5-hydroxytryptophan decarboxylase, which forms serotonin from 5-hydroxytryptophan, also has been detected in the pineal of quail (Snyder and Axelrod, 1964).

Owman (1971) pointed out that only a small amount of pineal serotonin is directly engaged in the synthesis of melatonin and that a considerable proportion of the serotonin may thus have other roles in the gland. He reviewed the several endocrine organs (gastric mucosa, thyroid C cells, parathyroid gland) that secrete peptidic hormones and also store high amounts of biogenic amines such as serotonin. He considered it reasonable to assume that the high concentration of serotonin in the pineal gland reflects the presence of some yet-unidentified protein or polypeptide hormone that would perhaps help to explain several pineal effects that cannot be attributed readily to the pineal indoles. The amine could probably change membrane permeability and allow the release of peptidic hormone. This view definitely permits an active role for pineal serotonin even if not as a classic hormonal messenger.

The greatest bulk of the literature on epiphyseal compounds seeks to characterize melatonin as the principal humoral agent of the pineal

gland. The compound was first isolated and identified by Lerner and co-workers in 1959. In the pineal, 5 hydroxylation followed by decarboxylation converts tryptophan to 5-hydroxytryptamine (serotonin). Serotonin is N-acetylated by serotonin N acetyltransferase to form N-acetylserotonin, which is then o-methylated by hydroxyindole-o-methyltransferase (HIOMT) producing melatonin (Wurtman et al., 1968; Axelrod, 1974).

The contention that melatonin is a hormone was based on circumstantial evidence (Wurtman et al., 1968) until the demonstration and quantification of melatonin in the chicken serum by the Rana pipiens tadpole bioassay and gas chromatography-mass spectrometry (Pelham et al., 1972). Subsequently, circulating melatonin was found in all the animals studied by bioassay, radioimmunoassay, and gas chromatography-mass spectrometry (Lynch and Wurtman, 1981; Rollag, 1981).

By the use of the tadpole bioassay Ralph and Lynch in 1970 demonstrated that pinealectomy abolished the bioassayable melatonin in the circulation of chickens (Pelham et al., 1972) and rats (Pang and Ralph, 1975). These findings together with the parallel patterns of diurnal rhythms of pineal and serum melatonin give an indication that circulating melatonin in birds and mammals may originate primarily or exclusively from the pineal gland (Pang and Ralph, 1975). The significant reduction in serum melatonin after pinealectomy was supported by other studies (Ozaki and Lynch, 1976; Gern et al., 1978; Yu et al., 1981; Bittman et al., 1983), but the question whether or not

serum melatonin originates exclusively from the pineal gland remains controversial.

Using bioassay and radioimmunoassay, detectable levels of melatonin were reported in pinealectomized animals by some investigators (Kennaway et al., 1977; Gern et al., 1978; Yu et al., 1981). Since melatonin has been demonstrated by bioassay, histoimmunochemistry, and radioimmunoassay in some extrapineal sites such as the gut, retina Harderian gland, and brain (Brown et al., 1981; Pang et al., 1983; Reiter et al., 1981), it was suggested that serum melatonin may be partly contributed by these tissues. Levels of serum melatonin were reported to increase 1 day after the removal of the retina by bilateral enucleation (Tang and Pang, 1982) or to remain unchanged several weeks after operation (Pang, 1974) in rats and chickens. The lack of any decrease in serum melatonin levels in the blinded animals suggests that the retina does not contribute significantly to the circulating melatonin concentration. In addition, similar levels of serum melatonin were observed in bilateral enucleated rats and rats that were blinded and had had Harderian glands removed. This suggests that any contribution from the Harderian gland to the melatonin in the circulation would be minimal (Tang and Pang, 1982). The contribution of circulating melatonin by extrapineal sites was a subject of dispute by the findings of Lewy and co-workers who, employing a very specific and sensitive gas chromatography-negative chemical ionization mass spectrometry assay, found that pinealectomy abolished serum melatonin in rats (Lewy et al., 1980a) and humans (Lewy, 1983). Tetsuo et al. (1982) also reported that more than 95% of

urinary excretion of 6-hydroxymelatonin disappeared after pinealectomy in Rhesus monkeys. These findings suggest that serum melatonin is secreted exclusively by the pineal gland.

One of the major sites of action of pineal melatonin is the central nervous system (Pang et al., 1976; Tretini et al., 1979; Brown et al. 1983). Since the pineal is closely connected to other parts of the brain, some investigators (Quay, 1970; Anton-Tay, 1971) have postulated that pineal melatonin is secreted into the cerebrospinal fluid (CSF). This postulation is supported by the fact that if the pineal secretes melatonin into the systemic circulation, the level of melatonin would be greatly diluted before it reaches the central nervous system. The finding that more ^{3}H-melatonin was taken up by the rat brain when ^{3}H-melatonin was given intraventricularly as opposed to intravenously (Anton-Tay and Wurtman, 1969) also provided some circumstantial evidence for the above speculation. However, present evidence indicates that secretion of pineal melatonin into the systemic circulation is more likely.

Oksche (1971) suggested that pineal-ventricular communication might exist in vertebrates but morphological studies failed to demonstrate any direct communication between the third ventricle and the pineal in birds (Quay, 1965; Ariens, 1971). In mammals, anatomical and vascular considerations also suggest that the pineal gland cannot secrete melatonin into the CSF under any known pathway (Ariens, 1971). On the other hand, secretory granules that were believed to unload their contents into the perivascular cavity were demonstrated by electron microscopy in birds (Bischoff, 1969) and mammals (Ariens,

1971). The above histological studies show that pineal melatonin may be secreted into the systemic circulation before it reaches the brain or CSF.

Brown et al. (1979) compared the daytime melatonin concentrations of human CSF samples originating in the lumbar sac or basal cistern and found no gradient between the two groups of CSF studied. This gave an indication that CSF melatonin arose from the circulation. Other investigators (Arendt et al., 1977; Vaughan et al., 1978; Tan and Khoo, 1981) also suggested that pineal melatonin is secreted into the circulation after they showed that levels of melatonin were higher in the serum than in the ventricular and/or lumbar CSF.

Two further observations lend additional support to the secretion to blood hypothesis. Using a new melatonin radioummunoassay, Withyachumnarnkul and Knigge (1980) compared melatonin concentrations in CSF, peripheral plasma, and plasma from the confluens sinuum of rats. It was discovered that melatonin concentration in the plasma of the confluens sinuum was about eight times that of the peripheral plasma and over eight times that of the CSF. Moreover, the darkness-induced increases in levels of plasma melatonin were not accompanied by parallel increases in the indole levels in CSF. These authors promptly suggested that melatonin was secreted into the systemic circulation in rats (Withyachumnarnkul and Knigge, 1980). The suggestion that the pineal normally secretes melatonin (Anton-Tay, 1971) is teleologically sound but it is based on thin and circumstantial evidence. A more likely route of pineal melatonin is via the systemic circulation.

Melatonin in the circulation is hydroxylated on position 6 followed by conjugation with sulfate (70%) and glucuronic acid (6%) in the liver (Kopin et al., 1961). The remaining melatonin is excreted into the urine directly (Ozaki and Lynch, 1976) or converted into other metabolites such as 5-methoxyindoleacetic acid (Kopin et al., 1961).

The half life ($T_{1/2}$) of ^{3}H-melatonin from the whole mouse (Kopin et al., 1961) or rat plasma (Ozaki et al., 1976) was found to be 3-4 min for the first 10 min following injection but 30-35 min about 40 min after melatonin administration. When ^{3}H-melatonin or unlabeled melatonin was infused into the carotid artery of unanesthetized rats and blood samples were collected from the jugular vein, a $T_{1/2}$ of 23 min was obtained for the ^{3}H-melatonin experiment vs 17 min for the unlabeled indole (Gibbs and Vriend, 1981).

Using a bolus intravenous injection of unlabeled melatonin and a two compartment open model of analysis (Gibaldi and Perrier, 1982), the distribution and disappearance of melatonin were studied (Chan et al., 1984). An initial distribution phase with a $T_{1/2}$ of 3 min and a terminal elimination phase with a $T_{1/2}$ of 19 min were obtained. The biological $T_{1/2}$ of melatonin at midlight and middark were similar, but the volumes of distribution and metabolic clearance rate were greater at middark. It was demonstrated that the volume of distribution increased with higher levels of melatonin and the middark value was significantly greater than the midlight value. This clearly suggests an increased tissue uptake of melatonin in the dark period.

Light and Pineal Activity

The pineal region of many lower vertebrates contains highly specialized cells capable of functioning as photoreceptors (Kelly, 1962). Like the cells of the mammalian retina, these elements respond directly to environmental lighting by generating nervous impulses which are then carried to the brain by the pineal nerves. With evolution, the pineal of higher forms has lost its photoreceptors and has been transformed into a compact glandular structure which no longer directly transmits sensory information to the brain. However, certain of the biochemical functions of these forms continue to be regulated by environmental illumination via an indirect neural route.

Environmental lighting is a major regulatory factor of indole amine synthesis and regulation in the pineal gland. Studies in the sixties on pineal serotonin content (Quay, 1963) and HIOMT activity (Axelrod et al., 1965) give an indication that different lighting schedules have marked influences on pineal melatonin metabolism. Serotonin is at a maximum near the beginning of the light phase in a conventional 24-hour photoperiod and at a minimum near the middle of the dark phase in both the pigeon (Quay, 1966) and Japanese quail (Hedlund and Ralph, 1967). This cycle can be phase-shifted by altering the phasing of the imposed diurnal photoperiod (Quay, 1966). Pineal HIOMT activity of young Japanese quail undergoing sexual maturity varies diurnally, being more active in the dark phase and less in the light; this cycle tends to be sustained for 3 days in continuous light (Sayler and Wolfson, 1969). The phasing of HIOMT activity in the quail is consistent with its observed melatonin cycle (Ralph et al., 1967).

Using a fluorometric assay, Quay (1963) showed a rhythm of melatonin in the rat pineal with high levels in the dark period and low levels in the light period. Subsequently, diurnal rhythms of pineal melatonin were demonstrated in all other animals studied (Lynch and Wurtman, 1981; Pang et al., 1983; Reiter, 1983). The diurnal rhythms of pineal melatonin correlate well with the rhythms of pineal N-acetyl serotonin contents (Pang et al., 1983) and NAT (Binkley et al., 1979) and HIOMT activities (Axelrod et al., 1965). Parallel melatonin rhythm with peak levels in the dark and low levels in the light have also been observed in the circulation of the mammals and birds studied.

The pattern of pineal melatonin in the dark period varies among species. Reiter (1983) summarized results in rodents and categorized the nocturnal melatonin patterns in pineals into three classes: 1) the rhythm peak late in the dark period; 2) the peak level near the middle of the dark period; and 3) peak levels prolonged during the majority of the dark period.

In mammals, information on ambient lighting is transmitted to the pineal gland through a neural pathway with the optic nerve, optic chiasma, suprachiasmatic nucleus, paraventricular nucleus, median forebrain bundle reticular formation, intermedio-lateral cell column, superior cervical ganglion, nervi-conarii, and the sympathetic nervous input as a sequence of the essential elements (Moore et al., 1967; Wurtman et al., 1968; Klein et al., 1983). Studies in the rat pineal gland suggest that in the dark spontaneous sympathetic impulses release norepinephrine in the pineal gland (Klein et al., 1970; Shein, 1971). Norepinephrine increases cyclic AMP activity, which in turn activates

NAT and melatonin synthesis and release by the pineal gland (Klein et al., 1970; Shein, 1971). However, investigations in Syrian hamsters, chipmunks, and humans have demonstrated that adrenergic stimulation does not increase or decrease pineal or serum melatonin levels (Vaughan et al., 1976; Lipton et al., 1982; Reiter et al., 1982). Moreover, the hamster pineal exhibited no diurnal variation in norepinephrine contents (Morgan et al., 1976). Thus, factors other than norepinephrine may play a part in the control of diurnal melatonin synthesis and secretion (Lipton et al., 1982).

Ralph et al. (1974) found that pineal and serum melatonin in rats and chickens persisted in constant darkness, with high levels during the active phase and low levels during the inactive phase. It was suggested that light acted as an agent for an endogenous oscillator which in turn regulated the melatonin rhythm through the autonomic innervation of the pineal (Ralph et al., 1974). In a series of investigations, Klein and Moore (1979) showed that the suprachiasmatic nucleus is probably the endogenous oscillator of pineal rhythm.

In the chicken, the retina is not essential for the regulation of pineal rhythms by light (MacBride and Ralph, 1972; Ralph et al., 1975). It was suggested that if the chicken pineal is not a direct photoreceptor, an extraretinal photoreceptor might regulate the pineal through some unknown neural or humoral factor (Ralph et al., 1974). When chicken pineals were autotransplanted into the anterior chamber at 3-7 days of age and the birds were studied in adulthood, there was a diurnal rhythm of serum melatonin with detectable levels of serum melatonin at middark but nondetectable levels at midlight. This

suggests that normal neural input is not essential for the melatonin rhythm in the serum and, thus, possibly the melatonin rhythm in the pineal. Moreover, when the autotransplanted pineal was deprived of light by masking the eye with the pineal transplant and leaving the other eye untouched, melatonin in the serum increased to the nighttime level even if the animal was under illumination. These experiments demonstrated that, under the above in vivo conditions, the chicken pineal is directly photoreceptive. Considering the findings around the same period (Ralph et al., 1971, 1974; Macbride and Ralph, 1972), it was suggested that a dual control of chicken pineal rhythms may be operating (Pang, 1974).

> The first control is environmental lighting which the chicken pineal can detect directly. The second controlling influence is derived from an unknown endogenous oscillator which sends its message to the pineal through the superior cervical ganglia. This endogenous oscillator is also responsible for the synchronization of the locomotor activity of the animal. An alternative explanation would be that the endogenous oscillator controls the locomotor activity of the animal while the pineal rhythms are in turn controlled by the animal's locomotor activity through the superior cervical ganglion (Pang, 1974).

The above hypothesis is interesting but requires additional experimentation. The direct photoreceptive ability of the chicken pineal was later demonstrated clearly under in vitro conditions (Binkley et al., 1979; Deguchi, 1979) where the rhythmicity of pineal enzymes was found to coordinate well with photic manipulation. Recent evidence has also demonstrated that in birds such as sparrows and quails, the suprachiasmatic nuclei are important for the circadian rhythms of pineal indole metabolism (Ebihara and Kawamura, 1981; Simpson and Follett, 1981; Takahashi and Menaker, 1982) and may be the

endogenous oscillators proposed (Ralph et al., 1975). Moreover, the chicken pineal appears to contain its own circadian oscillators. Rhythms of melatonin and NAT were demonstrated in the chicken pineal under constant darkness in vitro (Deguchi, 1979; Cassone and Menaker, 1983). However, photic and neuronal inputs remain important for the chicken pineal gland because 1) in vitro rhythms of melatonin lasted only for 2 to 4 cycles under continuous darkness; and 2) superior cervical ganglionectomized hens did not maintain persistent melatonin in the pineal in constant darkness (Cassone and Menaker, 1983). Thus, the above findings are consistent with the dual control hypothesis.

Environmental illumination not only synchronizes melatonin rhythms in the pineal glands of birds and mammals through the direct and/or indirect neural input, its intensity and duration also affect the levels of pineal and serum melatonin. Brainard et al. (1983) demonstrated that pineal NAT and melatonin in Syrian hamsters were suppressed by irradiances of cool white fluorescent light. The ED_{50} for pineal melatonin was found to be as low as 0.058 $\mu W/cm^2$. However, for animals that are used to high intensity of light in the daytime, a much higher intensity of light (>400 $\mu W\ cm^2$) is required to reduce the nocturnal rise in pineal melatonin production (Vaughan et al., 1976; Lewy et al., 1980b; Hurlburt et al., 1982). The above observations suggest a species difference in the amount of retinal input required for the activation of the neural substrate responsible for the light inhibition of pineal melatonin production and secretion. Alternatively, the sensitivity of the neural substrate to the retinal input may vary among different species.

The effect of change in the duration of light on pineal and serum melatonin also varies among different species. Brown et al. (1981) reported no shift in the diurnal rhythms of serum melatonin as a function of the length of the dark period in rats and hamsters. Circulating melatonin levels of hamsters in 1.5L:22.5D were lower than those of hamsters in 12L:12D. In contrast, a slight shift in the diurnal rhythm of pineal melatonin was observed by Tamarkin et al. (1979) in Syrian hamsters kept in short photoperiod. Other species such as Siberian hamster, white-footed mouse, and ewe showed prominent changes in the amplitude and/or duration of pineal and serum melatonin rhythms after alteration of photoperiods under laboratory or natural environmental conditions (Goldman et al., 1981; Petterborg et al., 1981; Lynch et al., 1982; Bittman et al., 1983; Kennaway et al., 1977). Thus, it appears that in some species light only participates in the entrainment of the endogenous oscillator responsible for the synchronization of the pineal melatonin rhythm. In such cases, light input within the normal range of intensity does not play an important part in the regulation of the amplitude and duration of the pineal and serum melatonin rhythms. On the other hand, in another group of animals, the same amount of light appears to be able to entrain as well as to affect the amplitude and duration of the rhythms of pineal and serum melatonin. Animals that exhibit responses somewhere between the two extremes postulated above are anticipated.

Pineal-Thyroid Interaction

Thyroid hormones are necessary for normal growth and development of animal species. Although thyroid hormones have been extensively investigated, little is known of how their effects are exerted (Bernal and Refetoff, 1977). Investigators have speculated that slightly hypothyroid or hyperthyroid conditions could alter the growth of normal healthy animals (Falconer and Draper, 1967). Selection for change in body size may exert its effects, in part by altering endocrine function (Fenton, 1960; Chai, 1970). Selection for different growth parameters in swine has altered plasma thyroid hormone concentrations (Bakke and Tveit, 1977). Chai (1970) has shown that selection for thyroid gland activity resulted in a positive correlated response in body size of mice. Recently investigators implicated thyroid hormone in the control of factors necessary for growth (Gaspard et al., 1978).

Hormone secretion by the thyroid gland is regulated by the pituitary glycoprotein, thyroid stimulating hormone (TSH). Circulating levels of thyroxine (T_4) and triiodothyronine (T_3) may also be influenced by factors such as the concentration of hormone-binding proteins in the serum, the degree of tissue uptake, and the rate of metabolic clearance. Experimental studies testing effects of the pineal gland on thyroid function have been designed primarily with the intention of testing whether the pineal gland influences secretion of hormones by the thyroid.

The control system regulating thyroid hormone secretion is usually described as consisting of three major components: the thyroid gland, the pituitary, and the hypothalamus. Stores of thyroid hormones in the

form of thyroglobulin in the thyroid follicles and in the form of T_4 and T_3 bound to plasma-binding proteins ensure an adequate supply of free hormone available for uptake and utilization by tissues. In physiological conditions of thyroid hormone excess, feedback inhibition of TSH secretion by T_4 tends to restore hormone concentrations to original levels. In physiological conditions of thyroid hormone deficiency, increased TSH secretion occurs. Although it is generally accepted that the pituitary is the major site of action for feedback inhibition by T4, there are also reports that the hypothalamus is sensitive to feedback signals of thyroid hormones (Kajihara and Kendall, 1969; Knigge and Joseph, 1971). Central nervous system induced alterations is TSH secretion are mediated primarily by thyrotropin releasing hormones (TRH), a hormone also necessary for maintenance of tonic levels of TSH secretion (Greer, 1952; Bowers et al., 1970). Studies of pineal-thyroid interactions have raised the question of which component, if any, of the control system is influenced by the pineal.

Ambivalent results concerning the connection between the pineal gland and the thyroid are characteristic. The apparent complexity of the supposed relationship is confusing and, in general, has delayed firm conclusions. A great variety of means whereby the pineal could control thyroid metabolism have been proposed but none definitely proved.

Surgical removal of the pineal gland has been reported to cause enlargement of the thyroid gland in several avian and mammalian species (Thieblot, 1965; Reiter, 1971). In mice, pinealectomy leads to

a slight but long-term enlargement of the thyroid gland (Houssay et al., 1966; Lombard des Gouttes, 1967). Thyroid hypertrophy after pinealectomy in rats, especially in males, seems to be somewhat more pronounced. Scepovic (1963) and Pazo et al. (1968) noted a highly significant accelerated growth of the thyroid glands of pinealectomized rats over those of control animals. Associated with the hypertrophy, Scepovic (1963) recorded a conspicuous hyperplastic response as well, whereas Pazo et al. (1968) could not differentiate, on the basis of the histological appearance of the thyroid tissue, glands of pinealectomized rats from those of sham-operated controls.

Other investigators, however, found no effect of pinealectomy on thyroid weight, iodine uptake by the thyroid, or thyroid secretion rate (Bick et al., 1969). Furthermore, Rowe et al. (1970) found no effect of pinealectomy on plasma TSH levels 28 days after pinealectomy. More recently, Brammer et al. (1979) concluded, from a study of the neuroendocrine-thyroid system of control and pinealectomized rats, that pinealectomy did not significantly influence pituitary or serum TSH, serum T_4 or T_3, or hypothalamic content of TRH.

The effect of pinealectomy on TSH levels of male rats was studied more carefully by Niles et al. (1979). They estimated TSH levels at several different times throughout the daily light cycle; furthermore, they measured plasma TSH levels in rats under both a conventional 12-h light and 12-h dark (12L/12D) lighting schedule and in rats under a 1L/23D lighting regime. The effects of pinealectomy were found to depend on light-cycle time in animals kept under short photoperiod for 72 days; the greatest difference was found 3h after the 1-h daily light

period. Statistically, this was supported by a highly significant interaction effect of cycle time and treatment (pinealectomy vs controls) in the analysis of variance. Thus, by controlling adequately for circadian variations in hormone levels, Niles et al. (1979) were able to demonstrate significant effects of pinealectomy on plasma TSH rhythms in rats. In contrast to other reports, Klandolf et al. (1978) reported higher T_4 concentrations in control than in pinealectomized chickens during darkness. They concluded the pineal gland was stimulatory to T_4 secretion but not to T_3 production.

The isolation and characterization of melatonin (Lerner et al., 1958, 1959) have led to experiments testing its effects on a variety of hormones (Cardinali, 1981). Houssay et al. (1966) reported that melatonin administration prevented the thyroid hypertrophy obtained in male mice after pinealectomy but had no effect on the size of the thyroid glands of sham operated controls. Results of this experiment suggested that melatonin administration was also found to reduce the thyroid weight of pinealectomized and ovariectomized female rats (DeFronzo and Roth, 1972).

Administration of melatonin into rats subcutaneously, intraperitoneally, or intraventricularly has been reported to inhibit iodine uptake by the thyroid gland (Baschieri et al., 1963; Reiter et al., 1965; Deprospo et al., 1968). DeProspo et al. (1969) suggested that endogenous melatonin produced in the pineal gland and regulated by environmental lighting schedules was responsible for inhibition of iodine uptake by the thyroid glands of rats. They found that rats kept in constant darkness had iodine uptake values of less than 50% of

iodine uptake values of rats kept under 12L/12D laboratory lighting conditions. Another interesting concept suggested by DeProspo et al. (1968) was that the effect of melatonin on iodine uptake by the thyroid gland might depend on the time of day of administration. This prediction was based on the findings of Fiske and Huppert (1968) that administration could prevent the diurnal rhythm of serotonin in the pineal gland. The prediction was supported by later research on the effects of melatonin on the neuroendocrine-thyroid and neuroendocrine-gonadal axis of hamsters (Tamarkin et al., 1976; Vriend et al., 1977).

Administration of melatonin has been reported to inhibit thyroid hormone secretion rate (TSR) in young male and female rats (Ishibashi et al., 1966; Singh and Turner, 1972). The melatonin-induced inhibition of TSR has been reported to be age dependent. Other evidence for an inhibitory effect of melatonin on the neuroendocrine-thyroid axis of rats comes from the study of Niles et al. (1979). They found that rats bearing melatonin antibodies by active immunization had plasma TSH levels that were significantly higher than controls.

Although some of the studies of melatonin administration on the thyroid system provide evidence for an inhibitory effect, there are two reports which are notable exceptions (Panda and Turner, 1968; Gordon et al., 1980). A frequently quoted paper by Panda and Turner (1968) reported increased TSH after melatonin injections. Gordon et al. (1980) also reported significantly increased iodine uptake by the thyroid and significantly increased plasma TSH in young rats treated with melatonin. These investigators used a dose of melatonin equal to that used in the earlier studies; a difference between this study and

the earlier ones is that Gordon et al. (1980) injected their rats for 21 days with melatonin, about twice as long as the periods of administration in the other studies. It is perhaps understandable that different protocols of melatonin administration could result in opposite effects of thyroid hormone secretion.

Melatonin has also been found to be counterinhibitory to circulating thyroid hormone levels when administered via the drinking water (Zucker and Stephan, 1973). When administered to blind hamsters, melatonin in the drinking water was found to prevent the inhibition of T_4 and free thyroxin index (FTI) obtained after orbital enucleation. Counterinhibitory effects of melatonin on testes weights were also found in this experiment. The counterinhibitory effects of melatonin were reported to be dose dependent between 1 of 80 µg melatonin per ml of drinking water (Vriend and Gibbs, 1983). Significant counterinhibitory effects on T_4 and FTI were obtained with 10 µg/ml of melatonin in the drinking water. Significant counterinhibitory effects on testes weights were found with as little as 1 µg/ml. Restoration of T_4 levels, FTIs, and testes weight to normal or near normal levels was obtained with the 80 µg/ml dose.

The counterinhibitory action of melatonin in various experiments raises the question of what the physiological action of melatonin is. These experiments suggest that the natural action of melatonin may be a little more complex than simply inhibitory or stimulatory. The counterinhibitory actions of melatonin give an indication that eventually this substance may be useful in management of clinical hypothalamic hypogonadism and clinical hypothalamic hypothyroidism.

Pineal Gland and Growth

An understanding of the factors which govern the growth and development of animals is fundamental to any attempt to influence the productivity of farm animals, not only in meat characteristics but also in reproductive function and lactation. Arbitrary attempts to influence animal performance which ignore the underlying physiological principle involved have only a limited chance of success and allow no scope for deviation from the confines of the original work. As it becomes increasingly important to improve the efficiency of livestock production and to direct tissue development further away from what might be regarded as its natural form, it also becomes important to establish the physiological limitations to growth and to the development of the body tissues.

Growth of an animal is a summation of a multitude of exogenous and endogenous factors acting in synergism. It is obvious that if one alters an exogenous stimulus which results in a growth change, one must then closely examine the endogenous change or changes that may have occurred in the animal resulting in the abnormal growth. This relatively straightforward and oversimplified approach quite accurately describes the present status and the future objectives of investigators involved in examining the relationship between the pineal gland, growth and metabolic status, and other factors such as photoperiod as well as other hormones. The relationship of the pineal gland to body growth and metabolism has been studied in several species with variable results.

It has been known for at least 30 years that growth of rats housed in total darkness or blinded (Browman, 1940) was stunted compared with rats kept under usual laboratory conditions (12-16 hours of light daily). Badertscher (1924) reported that in cockerels, pinealectomy retarded body growth during the first 2 to 3 months post surgery, after which they attained body weights similar to controls. In contrast to these findings, Malm et al. (1959) reported that pinealectomy stimulated the growth of rats for up to 14 weeks post surgery. Cogburn and Harrison (1977) have reported that pinealectomized cockerels had depressed body weights for up to 7 weeks post surgery, which is consistent with the work of Badertscher (1924) and in contrast with that of Malm et al. (1959) with rats. Singh and Turner (1967) reported that melatonin injections stimulated the growth of juvenile cockerels. McKeown et al. (1975) gave melatonin injections to pigeons which resulted in elevated growth hormone levels, suggesting that the pineal may influence the release of growth hormone.

Phettplace and Nockels (1985) have given the indication that melatonin administration increases the growth and feed efficiency of leghorn cockerels. Weight gains were increased linearly with increasing melatonin levels in the diet. The level of triiodothyronine in the serum was also found to increase.

Energy Utilization and Body Gain

In chickens, poor feed utilization has been associated with increased heat production and metabolic rate (Pym and Farrell, 1977; Morrison and Leeson, 1978) while efficiency of feed utilization has

been well correlated with body growth and body weight (Fox and Bohren, 1954; Proudman et al., 1970). Nevertheless, selection for increased growth rate can be separated from selection for better feed efficiency (Pym and Farrell, 1977), although the metabolic modifications associated with such responses remain unknown.

Using lines of chickens selected for 8 week body weight (Siegel, 1962), it was found that when pair-fed, high weight lines had better feed efficiency than low weight line birds (Siegel and Wisman, 1966). However, the advantage disappeared when the birds were fed ad libitum, possibly because of an overconsumption of feed by the high weight line birds. When dietary energy levels were changed, the two lines differed in their ability to adjust caloric intake; the high weight line consumed disproportionately more calories.

Quantitative analyses of feeding behavior in broilers and young layers showed that broilers consumed significantly more feed than layers and ate more meals but spent significantly less time in feed activity (Masic et al., 1974). Barbato et al. (1980) obtained similar results when comparing the high and low weight lines. In a related study, Cherry and Siegel (1979) demonstrated that food was digested and cleared at a faster rate from the high weight line and this occurred in a proportionately small gut.

As chickens age, the rate of weekly proportionate increases in body weight declines (Marks, 1979). Hence, the relative amount of energy required for growth per unit metabolic size also declines. This, together with evidence that suggests that the modern, rapid-growing broiler lacks well-defined appetite regulatory mechanisms (Lacy

et al., 1983), suggests that the modern broiler is unable to adjust energy consumption for changes in the relative requirements for growth. Hence, as the relative amount of energy required for growth declines, the excess energy consumed may result in the increased rate of daily fat accretion as indicated by Robbins and Ballew (1984).

The apparent inability of the broiler to regulate energy consumption to meet energy needs appears not, however, to be the sole factor responsible for excessive fat deposition. For example, Robbins (1981) determined that a heavy, rapidly growing broiler breed was 15% more efficient in retaining ingested energy than a lighter, slower growing breed. This observed higher efficiency may be due to a lower maintenance energy requirement, a higher efficiency of energy utilization above maintenance, or both. The higher efficiency observed in a rapidly growing breed of chicken is similar to results which were obtained with mice. Canolty and Koong (1976) found that selection for rapid post weaning growth rate in mice resulted in a great increase in efficiency of energy utilization. This was not accompanied by significant changes in maintenance energy requirements but was due primarily to a higher efficiency of energy utilization for gain. Similarly, Deb et al. (1976) found that obese Zucker rats were twice as efficient as their lean counterparts in retaining ingested energy. Again, this higher efficiency was due to a higher efficiency of energy utilization for gain and not the result of a lower maintenance energy requirement. However, Lin et al. (1979) determined that a major contributing factor to the higher energetic efficiency of genetically obese vs lean mice is a much lower maintenance requirement.

Studies involving anabolic and catabolic responses to nutritional manipulations have provided valuable information regarding lipid metabolism in chickens and other avian species. Shapira et al. (1978) observed different levels of lipogenic enzyme activities in the liver and adipose tissues of heavy and light breeds of chickens in response to forced feeding. Correlated lipogenic responses to several selection criteria, all of which influenced body weight, were recently reported (Hood and Pym, 1982). It has also been demonstrated that the difference in fat content between fat line (FL) and lean line (LL) chickens was not due to differences in feed intake but to differences in metabolic utilization of energy (Leclercq and Saadoun, 1982). A higher insulin secretion was observed in the FL chickens compared to LL after a test meal or a glucose load (Simon and Leclercq, 1982), explaining the preferential utilization of energy nutrients for lipid synthesis in the FL. Saadoun and Leclercq (1987) found differences in rates of lipogenesis in both liver and carcass of FL and LL genotypes. FL birds exhibited enhanced hepatic lipogenesis.

Light, Appetite, and Weight Regulation

For species living outside the tropics, the appearance of winter represents a marked adaptational challenge in that extremely cold temperatures occur at a time when food supplies are scarce. The harsh environmental conditions have been termed the ultimate "factors" which have exerted evolutionary pressures for animals to develop mechanisms for conserving energy for survival (Thomson, 1950). Different species have evolved different mechanisms for conserving energy, some of which

involve alterations in eating behavior and body weight. Thus hamsters, for example, store energy in the form of body fat and become obese in the winter (Hoffman et al., 1982; Campbell et al., 1983). Voles, on the other hand, actually conserve energy by losing weight, thus decreasing their energy requirements and continue to forage during the winter months (Dark and Zucker, 1985). They compensate for the thermal insulation lost in becoming leaner by developing a long winter pelage which insulates their bodies as well as the fat they have lost (Dark and Zucker, 1985).

In chickens, Siegel et al. (1961) reported significantly heavier body weights and improved feed conversion efficiency in white leghorn pullets at 8 weeks of age when light was restricted to only 6 hours per day compared with 14 hours light per day. However, Buckland and Hill (1970) found great inconsistencies, with about half the studies showing improved growth on continuous light while the rest indicated that selected intermittent light/dark schedules enhanced feed conversion and maximized growth.

Among humans, there is ample evidence from epidemiological data that seasonal rhythms occur in a variety of human phenomena including the rates of birth, growth, suicide, and death from natural causes (Aschoff, 1981). There is also remarkable evidence dating back to the end of the last century and well reviewed by Attarzadeh (1983) of a seasonal variation in weight in humans with a tendency to increase weight in the fall and winter and to lose it most easily in the spring and summer. This phenomenon can even be observed in growing children, despite the fact that growth in height occurs maximally in the spring

and summer months. These observations have practical applications for the treatment of obesity. For example, a recently conducted study showed that the best results in weight loss programs were observed in the spring and the worst during the winter (Zahorska-Markiewicz, 1980). Humans also reveal seasonal changes in food choice which appear to be independent of the availability of different types of foods (Zifferblatt et al., 1980).

Patients with seasonal affective disorder (SAD) show an exaggerated behavioral response to the seasons which during the fall and winter months reaches such a degree of severity as to impair functioning both at work and in interpersonal relationships. The winter depression is generally accompanied by a tendency to oversleep, overeat, gain weight, and crave carbohydrates (Rosenthal et al., 1984). The symptoms of weight gain and increased appetite are significantly correlated with one another as well as with carbohydrate craving. They are also significantly correlated with hypersomnia, however, suggesting that the regulation of the pattern of sleeping and eating in SAD may be coordinated by neurophysiological systems that vary in an interrelated way.

Whereas temperature extremes as well as scarcity of food have been referred to as the ultimate factors responsible for the evolutionary pressure to develop seasonal rhythms, it is quite vivid that these factors come too late in the season to serve as effective triggers for initiating the seasonal energy conservation changes in weight and metabolism seen in certain animals. This calls for the consideration of other environmental changes and photoperiod; the illuminated

fraction of the 24-h day is by far the most important across the many types of season rhythms studied (Immelmann, 1973). It is understandable that this would be the case, because changes in day length vary most predictably from year to year. Thus, the winter weight gain observed in hamsters can be reproduced in the laboratory by artificially shortening the photoperiod (Hoffmann et al., 1982), as can the winter loss observed in voles (Dark and Zucker, 1985).

Humans, on the other hand, naturally increase the length of their photoperiod by the use of artificial lighting. If the seasonal changes in eating and weight described above are influenced by photoperiod, how is it that they persist despite the use of artificial lighting? The first clue to resolving this paradox came from an observation of Lewy et al. (1980b) that whereas the nocturnal secretion of melatonin by the pineal could be suppressed by ordinary intensity artificial light in animals, it required light of far higher intensity (2500 lux) to suppress nocturnal melatonin secretion in humans. It was this finding that inspired the treatment of patients with SAD by extending their winter photoperiod with bright artificial light (James et al., 1985; Rosenthal et al., 1985).

The indole derivative melatonin, secreted by the pineal gland nocturnally on a circadian basis, is an important neurochemical transducer of photoperiod in animals (Tamarkin et al., 1985). Photoperiodic information is conveyed by way of the retina, retinohypothalamic track, supra chiasmatic nuclei, and superior cervical ganglia to the pineal gland where it influences the timing of melatonin secretion. The resulting pattern of both endogenous

production and photoperiod may influence the seasonal behavioral response. The role of melatonin in the regulation of seasonal rhythms has been established more clearly for reproductive behavior than for metabolism. Wade and Bartness (1984), however, have shown that in Syrian hamsters housed in long photoperiods daily injections of melatonin in the late afternoon increase body weight and fat content, an effect that is also seen if the photoperiod is shortened.

In view of the fact that light has an effect on growth and the pineal activity is affected by light, this study was undertaken to investigate the role of the pineal in body weight and metabolism. Attempts were made to determine how the pineal might elicit this function. Various experiments were carried out to find out how light and the pineal influenced body weight and several metabolic parameters associated with growth and energetic efficiency.

CHAPTER III

MATERIALS AND METHODS

Animals and General Management

Hubbard crossbred feather-sexed broiler chicks were used in experiments 1-2; Peterson crossbred feather-sexed chicks were used in experiments 3-5. Fertile eggs were obtained from breeder flocks maintained at the University of Tennessee and hatched in the university hatchery. One-day post hatching chicks were sexed and all morbid, unthrifty chicks were discarded. The remaining chicks were individually wingbanded and randomly distributed to treatment groups.

Chickens were housed in electrically heated battery brooders kept in light proof, heated, and ventilated rooms. The rooms were maintained at 28°C. Lighting schedule varied within and among experiments. However, light intensity during the photophase was constant and averaged 14 lux at bird level. All chicks were started in the appropriate photoperiod at 1 or 2 days posthatching. Experiments lasted 3 to 4 weeks in duration.

Except for experiment 3, feed and water were provided ad libitum. Body weight gain, feed consumption, and mortality were measured in each experiment. All diets were stored at 25°C.

Melatonin was obtained from Sigma Chemical Company (St. Louis, MO).

Experiment 1

This experiment was conducted to determine what effects, if any, dietary melatonin has on rate of gain, feed efficiency, and body composition of broiler chicks. Independent experimental variables were sex, dietary energy level, and the presence or absence of dietary melatonin. The treatment design was a 2x2x2 factorial; the experimental design was completely randomized. The composition of the basal diets is presented in Table 1. Melatonin was added at a level of 15 mg/kg diet; all birds were housed under a 16h light:8h dark (16L:8D) photoperiod. Experimental diets were fed from day 1 to day 21 post-hatching to triplicate groups of 10 chicks each per treatment.

On day 21 all birds were weighed; two chicks from each replicate group with body weights nearest the mean weight of that group were fasted for 24h, then killed by cervical dislocation for body composition analysis. The whole uneviscerated carcass of each chick (6 per treatment) was autoclaved in 1 volume of tap water and homogenized. A ca 500 ml volume of homogenate was then dried for 72 to 96h in a forced-air drying oven set at 60°C. The dried material was finely ground, subsampled, and analyzed for dry matter, ether extract, and protein per Association of Official Analytical Chemists (AOAC, 1970). Body composition data were expressed on a fresh tissue basis.

Data were statistically analyzed by analysis of variance using the GLM procedure of SAS (1982). The significance of the main effects of sex, melatonin, and dietary energy level and each of the 2- and the 3-way interactions were measured for each dependent variable.

Table 1. Basal diets.[1]

Ingredient	High Energy %	Low Energy %
Corn starch	31.62	15.17
Dextrose	25.00	25.00
Isolated soy protein	27.35	27.35
Mineral premix[2]	5.37	5.37
Choline chloride	0.20	0.20
Vitamin mix[2]	0.20	0.20
Corn Oil	3.00	3.00
DL-methionine	0.60	0.60
Cellulose	6.66	23.22
	100.00	100.00

[1]High energy diet was formulated to contain 3300 kcal/kg while the low energy diet contained 2700 kcal/kg. Both diets contained 23% CP.

[2]Baker et al., 1979.

Experiment 2

Because accurate feed consumption data could not be obtained in experiment 1, this growth trial was conducted. For this experiment the high energy diet (Table 1) with and without melatonin was used. Two photoperiods were employed, 16L:8D and 23L:1D, thus resulting in a 2x2 factorial arrangement of treatments (i.e., 2 levels of melatonin x 2 photoperiods). The experimental design was completely randomized. Experimental diets were fed from day 1 to day 28 post hatching to triplicate groups of 6 male chicks each per treatment.

Dependent variables measured were body weight gain, feed consumption, and feed efficiency. Data were analyzed by analysis of variance and significance of melatonin, photoperiod, and their interaction tested.

Experiment 3

This was conducted to determine the effect of pinealectomy on efficiency of energy utilization. All birds were fed the melatonin-free high-energy diet (Table 1). Control (unoperated), sham operated, and pinealectomized chicks were used. Chicks were grown for 3 days, surgery was performed on day 4, and the experiment was begun on day 7 post hatching. The surgical procedures have been described by Osei (1978). All birds were housed under 16L:8D photoperiod.

For each group of chicks (i.e., control, sham operated, and pinealectomized), triplicate groups of 6 chicks each were randomly assigned to each of 3 treatments: ad libitum feed consumption, 67% of ad libitum, and 33% of ad libitum. Groups receiving restricted quantities of feed were fed in two installments: at 0800 and 1700 hr.

The amount received was based on the previous day's consumption by their respective ad libitum-fed groups. To accomplish this, experimental groups fed ad libitum started and finished the experiment 1 day prior to the other experimental groups. The feeders employed resulted in small quantities of uningested feed by those groups fed restricted amounts. Uningested feed was therefore weighed daily, and feed consumption data were corrected.

At the beginning of each experiment, 6 chickens of each group with body weights near the mean of the experimental birds were selected for initial carcass analysis. Upon terminating the experiment, each experimental group was weighed and killed by cervical dislocation. The feathered carcass of each chicken was then ground through a Hobart meat grinder. The ground carcasses were pooled within experimental groups, thoroughly mixed and a ca. 500 sample was taken. The representative sample was dried to greater than 95% dry matter at 100°C, then finely ground in a Wiley mill for analysis. Each sample was analyzed in duplicate for total gross energy using a bomb calorimeter.

Metabolic body size ($kg^{\cdot 75}$) was calculated as the average body weight during the 14-day experimental period (Lin et al., 1979). The change in body energy per kilogram body $weight^{\cdot 75}$ was regressed on energy intake per kilogram body $weight^{\cdot 75}$ with adequate fits obtained using the simple linear equation. Maintenance energy requirements were estimated from the fitted regression equation ($y = a + bx$) as $-a/b$ which represents energy intake at zero body energy gain. The variances of the estimated maintenance energy requirements were approximated as $(-a/b)^2 \times (V(a)/a^2 \quad V(b)/b^2 - 2\,CV(a,b)/axb)$. The variances (V) of a

and b and their covariance (CV) were determined by multiplying the inverse of the sums of squares and crossproducts matrix from regression by the error mean square from the analysis of variance of body energy gain per kg metabolic weight. The slopes of the regression lines indicated the efficiency with which dietary energy, above that required for maintenance, was utilized for body energy gain.

Experiment 4

This was conducted to determine the effects of pinealectomy, light, and dietary melatonin on the rate of gain, feed consumption, feed efficiency, and energy retention capabilities. Unoperated control, sham operated, and pinealectomized chicks were fed the high energy diet (Table 1) with and without melatonin. Two lighting schedules were employed, 16L:8D and constant light (CL), thus resulting in a 3x2x2 factorial arrangement of treatments (i.e., 3 surgical procedures x 2 levels of melatonin x 2 lighting regimes). The experimental design was completely randomized. Experimental diets were fed from day 7 to day 21 post hatching to triplicate groups of 6 male chicks each per treatment. All birds were weighed periodically and the feed consumption also measured. Surgery was performed on day 4 and the birds allowed 3 days to recuperate.

Upon terminating the experiment, birds were weighed and killed by cervical dislocation. The feathered carcass of each chicken was then ground through a Hobart meat grinder. The ground carcasses were pooled for each replicate within the various experimental groups, thoroughly mixed and a ca. 500g sample was taken. The representative sample was then dried for 72h in a forced-air drying oven set at 60°C. The dried

material was finely ground in a Wiley mill for analysis. Each sample was analyzed in duplicate for total gross energy using a bomb calorimeter. Total gross energy of the carcass as expressed as a fraction of the metabolizable energy consumed to obtain percent energy retention.

Dependent variables for experiment 4 were body weight gain, feed consumption, feed efficiency, and percent energy retention. Data were analyzed by analysis of variance and significance of melatonin, photoperiod and their interaction tested.

Experiment 5

This study was undertaken to obtain information regarding the effect of light treatment and dietary melatonin on rate of gain. Feed consumption, feed efficiency, and selected metabolic parameters viz. jejunal glucose transport, rate of hepatic lipogenesis, and plasma T_3 and T_4 levels.

The experimental group of 160 male chicks were fed the high energy diet (Table 1) with and without melatonin. Two lighting schedules were employed, 16L:8D and CL, thus resulting in a 2x2 factorial arrangement of treatments (i.e., 2 levels of melatonin x 2 photoperiods). The experimental design was completely randomized. Experimental diets were fed from day 1 to day 29 post hatching to 5 replicate groups of 8 chicks in each treatment. All birds were periodically weighed and feed consumption measured.

Upon terminating the experiment, blood samples were drawn from 2 birds in each replicate group and plasma T_3 and total T_4 determined. In vivo hepatic lipogenesis was conducted on 2 birds from each of 5

replicate groups of all treatments. Two additional birds were also selected from the various replicates within the treatment, killed by cervical dislocation, and the rate of jejunal glucose uptake determined.

Plasma T_3 and T_4 were determined using RIA kits provided by Travenol-Genentech. Blood samples were collected from chicks at 0900 hrs using a heparinized needle and syringe by cardiac venipuncture into NH_4-heparinized sample tubes (Sarstedt). Red blood cells were removed by centrifugation at 200xg for 5 minutes and the plasma samples were cooled in ice before storage. Samples were stored at -20°C until needed.

For the assay of T_3, 50 ul of plasma was used. To each anti-T_3 coated tube including standards and samples was added 1.0 ml of tracer-buffer reagent except for total tubes. The tubes were vortexed gently and incubated for 1 hour in a 37±2°C water bath after which all except those containing only tracer-buffer were aspirated. All tubes were counted in a gamma counter for 1 minute with the window suitably adjusted for Iodine-125. A standard curve was prepared from the standards and the unknown values were obtained from the standard curve by interpolation.

Ten l of plasma was used for the T_4 assay. To the bottom of the appropriate anti-T_4 coated tubes were added either 10 l of T_4 serum standard or 10 l of each sample. One ml of tracer-buffer reagent was added to each tube. The tubes were gently vortexed and incubated at 25°C for 45 minutes. After incubation, tubes were aspirated and counted in a gamma counter for 1 minute. The T_4 concentrations in the

plasma samples were determined from a standard curve prepared from five serum standards. All assays (both T_3 and T_4) were conducted in triplicate.

Jejunal glucose absorption was determined using the method of Walker et al. (1981). Two chicks from each of 5 replicates within the experimental groups were killed, laparotomized, and a section of the jejunum immediately distal to the pancreatic ducts was excised, freed of mesentery tissue, and flushed with isosmotic incubation medium [.08M Tris-HCl buffer (pH 7.4) contained NaCl (120 mM), K_2HPO4 (3 mM), $MgCl_2$ (1 mM), $CaCl_2$ (1 mM)]. Narrow (about 6 mm) cross-sectional rings of tissue were cut from the proximal jejunum and 3 rings were randomly added to each of 4 test tubes containing 4.0 ml of the incubation medium.

The medium was held at 40°C in a water bath and constantly aerated with small bubbles of an O_2:CO_2 mixture (95:5). Following a 1-minute period of temperature equilibration, 1.0 ml of .01 M glucose solution (certified grade glucose, Fisher Scientific Company, Fairlawn, NJ) was added to each tube, yielding a concentration of .002 M glucose in the incubation medium. Four replicate samples from each of the 2 birds were incubated for 15 minutes. The control consisted of medium without tissues. After incubation, tissues and media were separated; the tissues were dried to a constant weight and weighed.

The colorimetric method of Somogy (1945) was used as a assay for glucose. Uptake of glucose by the tissue samples was calculated as the difference between micromoles of glucose in the control tubes and that remaining in each tube which had contained intestinal tissue. Data

were put in the form of uptake of glucose (micromoles per gram dry weight of tissue) for each incubated tube. The mean amount of glucose uptake and tissue weight in the 4 samples from each bird was calculated.

In vivo hepatic lipogenesis was determined according to the method of Saadoun and Leclercq (1987). Two fully fed birds from each of 5 replicate groups of 4 treatments were injected intraperitoneally (IP) with 1.54 m C_i tritiated water in 1 ml saline solution. Ten minutes post injection, the birds were killed by cervical dislocation and the livers were removed.

Liver lipids were extracted in a chloroform-methanol mixture (2:1). About 5 g of liver was homogenized in 100 ml solvent and filtered. The residue was reextracted in an equal volume of the mixture. The extract was washed 3 times with distilled water. The aqueous phase was then removed and methanol added to the organic phase. The solvent was evaporated and the lipids remaining at the bottom of the flask were saponified in about 40 ml KOH dissolved in ethanol. Saponification lasted for 10 min at 40°C. The soap solution was acidified with 40 ml of 6N HCl. Fatty acids were then collected quantitatively by washing this solution 4 times with 50 ml hexane.

Ten ml of hexane was placed in a scintillation vial and 10 ml of counting solution (Biofluor) added. Counting was done in a beta counter for 1 minute. Rate of in vivo liver lipogenesis was determined as dpm / tissue / 10 min.

Dependent variables for experiment 5 were body weight gain, feed consumption, feed efficiency, liver lipogenesis, jejunal glucose

uptake, and plasma T_4 and T_3. Data were statistically analyzed by analysis of variance using the GLM procedure of SAS (1982). The significance of the main effects of melatonin and light as well as their interaction were tested.

CHAPTER IV

RESULTS

Experiment 1

The results of experiment 1 are shown in Table 2 with the corresponding analysis of variance (ANOVA) in Table 8 (Appendix). Energy level affected body weight gains in both males and females. Birds fed the high energy diet gained weights which were significantly higher than those on the low energy diet ($P < .04$). The effect of melatonin on body weight gain was also significant in both sexes ($P < .04$). Chicks given the melatonin diet grew significantly faster ($P < .04$) when fed either the low or high energy diet though they gained more with the high energy diet.

Sex did not affect body weight as both females and males gained almost equal weights at a different energy level and with or without dietary melatonin. The interaction of energy source x melatonin was not significant ($P < .05$). Neither the melatonin x sex interaction nor the three-way interaction of melatonin, sex, and energy level was significant ($P < .05$).

There were differences in carcass crude protein dry matter and fat but such differences were only slight and did not follow any pattern among the various treatments.

Feed intake data for this experiment were unavailable due to early mortality among many of the replicate groups.

Table 2. Effects of dietary energy level, sex, and dietary melatonin on growth, body fat, protein, and dry matter of chicks.[1]

Energy	Melatonin (15 mg/kg)	Gain[2] (g)		Carcass Composition					
				Dry Matter (%)		Crude Protein (%)		Ether Extract	
		Males	Females	Males	Females	Males	Females	Males	Females
2700 Kcal/kg	without	259	257	25.3	27.6	15.6	15.0	3.8	5.4
2700 Kcal/kg	added	282	279	25.2	26.4	15.9	14.7	3.5	4.2
3300 Kcal/kg	without	302	319	28.5	27.6	15.5	14.5	7.4	5.9
3300 Kcal/kg	added	344	341	28.5	28.2	16.0	14.0	6.1	7.5

[1]Data are means of triplicate groups of 10 chicks each fed the experimental diet from day 1 to day 21 post hatching.

[2]The effects of melatonin and energy level were significant ($P < .05$).

Experiment 2

The results of experiment 2 are presented in Table 3 with the corresponding ANOVA in Table 9 (Appendix). Body weight gain was affected by photoperiod. Birds that were grown under 16L:8D gained faster than those grown under 23L:1D photoperiod (552 g vs 534 g). There was about 4% increase in gain and this approached significance ($P < .10$). Birds raised under 23L:1D, however, consumed more feed than those under 16L:8D (883 g vs 843 g). The difference was not significant. Photoperiod also affected feed efficiency of the chicks. Birds grown under 16L:8D had higher gain/feed ratios than their counterparts raised under 23L:1D photoperiod. There was an 8.25% increase in efficiency.

Melatonin administration affected body weight gain under both photoperiods. Birds grown under 16L:8D and fed the melatonin diet exhibited about 8% increased gain which approached significance ($P < .10$). The situation was similar with birds grown under 23L:1D. Melatonin administration resulted in a 10% increase in body weight gain. Chicks grown under both photoperiods and given the melatonin diet consumed more feed than their counterparts without melatonin (883 g vs 841 g). Feed efficiency was also affected by melatonin. Under a 16L:8D photoperiod, melatonin-fed chicks had a 5.3% increase in feed efficiency. Birds raised under 23L:1D photoperiod and fed the melatonin diet also showed about 3% increase in feed efficiency.

Experiment 3

The results of experiment 3 are presented in Tables 4 and 5 with the corresponding ANOVA in Table 10 (Appendix). Feed intake was

Table 3. Effects of photoperiod and dietary melatonin on body weight gain, feed consumption, and feed efficiency.[1,2]

Treatment		Variable		
Photoperiod	Melatonin (mg/kg diet)	Gain (g)	Feed (g)	Gain/Feed (g/kg)
16L:8D	0	532	834	639
16L:8D	15	573	853	673
23L:1D	0	509	849	599
23L:1D	15	560	913	613

[1]Data are means of triplicate groups of 6 male chicks each fed the experimental diets from day 1 to day 28 post hatching.

[2]For each variable the effect of photoperiod and melatonin was not significant ($P < .10$).

Table 4. Effect of pinealectomy on body weight gain, carcass energy gain, and percent energy retention.[1]

Surgical Procedure	Feeding Treatment	Variable			
		Gain[2] (g)	Feed (g)	Carcass Energy Gain (Kcal)	Percent[2] Energy Retention
Unoperated control	Ad libitum feed intake	403	608	707	35.2
	67% of ad libitum	225	407	451	33.6
	33% of ad libitum	134	200	229	34.7
	Ave.	254	405	462	34.5
Sham operated	Ad libitum feed intake	388	583	656	34.1
	67% of ad libitum	207	390	422	32.8
	33% of ad libitum	127	192	221	34.9
	Ave.	241	388	433	33.9
Pinealectomized	Ad libitum feed intake	311	571	475	25.2
	67% of ad libitum	171	382	297	23.6
	33% of ad libitum	97	188	170	27.4
	Ave.	193	380	314	25.4

[1]Data are means of triplicate groups of 6 male chicks each fed the allotted diet from day 7 to day 21 post hatching.

[2]The main effect of pinealectomy was significant ($P < .01$).

Table 5. Effect of pinealectomy on maintenance energy requirements and efficiency of energy utilization above maintenance.[1]

Surgical Procedure	Regression Equation[2,3,4]	Maintenance Energy[5] Requirement ± Standard Error (Kcal ME/kg $BW^{.75}$/day)
Unoperated control	Y = -88.68 + .4651x (16.996) (.0245) r^2 = .984	188 ± 31.8
Sham operated	Y = -86.65 + .4413x (21.32) (.033) r^2 = .964	186 ± 27.0
Pinealectomized	Y = -41.53 + .2487x (16.195) (.0255) r^2 = .931	167 ± 60.9

[1]Data are means of triplicate groups of 6 male chicks each fed the allotted diet from day 7 to day 21 post hatching. Surgery was performed on day 4.

[2]Y = Energy gain (Kcal/kg $BW^{.75}$/14 days); x = energy consumed (Kcal/kg $BW^{.75}$/14 days).

[3]Values in parentheses are the standard errors of the parameter estimates.

[4]The main effect of pinealectomy on efficiency of dietary energy utilization (slopes of regression equation) was significant ($P < .01$).

[5]The main effect of pinealectomy was not significant ($P < .05$).

affected slightly by pinealectomy. However, gain and energy retention were significantly reduced by pinealectomy. Unoperated control birds gained significantly faster than pinealectomized birds (254 g vs 193 g). The sham operated chicks had gains similar to the unoperated controls (241 g vs 254 g) but significantly different ($P < .01$) from their pinealectomized counterparts. Unoperated control chicks retained an average of 34.7% of ingested metabolizable energy while pinealectomized birds had a percent retention of only 25.4%. The difference was significant ($P < .01$). The value of the sham operated chicks was close to that of the unoperated control (33.9% vs 34.7%) but significantly different from that of pinealectomized chicks ($P < .01$).

Regression equations and estimated maintenance energy requirements for the various surgical groups are shown in Table 5. An indicated by the slopes of the regression lines, pinealectomy significantly affected the efficiency of energy utilization above maintenance. However, differences in maintenance energy requirements among the surgical groups were not significant. Pinealectomized birds were 46% less efficient than unoperated control birds in utilizing energy above maintenance. Sham operated birds were 43% more efficient than pinealectomized ones in the utilization of dietary energy above maintenance. The differences in energy utilization capabilities between the unoperated control and pinealectomized chicks as well as sham operated and pinealectomized were all significant ($P < .01$). The maintenance energy requirements for sham operated control, unoperated control, and pinealectomized birds were found to be different but such differences were not significant ($P < .05$).

Experiment 4

The results of experiment 4 are shown in Table 6 with the corresponding ANOVA in Table 11 (Appendix). Melatonin affected body weight gain, feed consumption, gain/feed ratio, as well as energy retention in both photoperiods and among all surgical groups. Unoperated control birds grown under 16L:8D and fed the melatonin diet exhibited a 23% increased gain and this was significant ($P < .05$). Sham operated and pinealectomized birds also gained faster when fed the melatonin diet and raised under 16L:8D ($P < .05$). The situation was similar with birds grown under CL. All surgical groups had significantly more gain ($P < .05$) when fed the melatonin diet even though the gains were lower when compared to the 16L:8D lighting schedule. For example under CL sham operated, melatonin-fed birds had an 18% improved gain compared to a 20% improved gain under 16L:8D. Feed consumption was also significantly enhanced ($P < .05$) in all melatonin-treated birds irrespective of the lighting schedule under which they were housed.

Melatonin also had an effect on feed efficiency of the chicks in all the surgical groups and under both lighting regimes. Under 16L:8D melatonin-fed, unoperated control chicks had a 15% increase in feed efficiency compared to their counterparts without melatonin. This increase was significant at the .05 level. The case was similar in both pinealectomized and sham operated chicks. Under constant light melatonin administration significantly increased ($P < .05$) feed efficiency in all the surgical groups.

Table 6. Effects of pinealectomy, light treatment, and dietary melatonin on body weight gain, feed consumption, feed efficiency, and energy retention of chicks.[1]

Variable	Surgical Procedure	Light Treatment: 16L:8D, Melatonin: Without	Light Treatment: 16L:8D, Melatonin: Added	Light Treatment: Constant Light, Melatonin: Without	Light Treatment: Constant Light, Melatonin: Added
Gain (g)[2,3,4,5]	Unoperated control	382	469	354	403
	Sham operated	363	434	335	395
	Pinealectomized	290	368	315	349
Feed consumed[2] (g/14 days)	Unoperated control	575	614	589	603
	Sham operated	566	596	564	570
	Pinealectomized	539	575	559	565
Gain/feed[2,3] (g/kg)	Unoperated control	667	765	602	670
	Sham operated	643	727	594	692
	Pinealectomized	535	628	559	620
Energy retention[2,3,4] (% of consumed metabolizable energy)	Unoperated control	34.7	42.6	33.6	39.0
	Sham operated	34.0	41.3	31.8	37.8
	Pinealectomized	27.8	39.6	27.1	36.1

[1]Data are means of 3 groups of chicks each fed the experimental diets from day 7 to day 21 post hatching.

[2]The main effect of melatonin was significant ($P < .05$).

[3]The main effect of pinealectomy was significant ($P < .01$).

[4]The main effect of light treatment was significant ($P < .05$).

[5]The melatonin x light treatment interaction was significant ($P < .01$).

Energy retention was significantly higher ($P < .05$) in all melatonin-fed chicks irrespective of lighting schedule and surgical group. For example, sham operated birds fed the 15 ppm melatonin diet exhibited 21% increase in energy retention when housed under 16L:8D and 18% when housed under CL. Similarly, unoperated control chicks showed 21% increase under 16L:8D and 16% when housed under CL. All the increases were significant ($P < .05$).

Both pinealectomy and light affected all the parameters measured. Feed consumption was only slightly affected but the rest were significantly changed. Unoperated control birds housed under 16L:8D had a significantly higher percentage energy retention than those raised under constant light ($P < .05$). Energy retention in sham operated birds was also significantly affected by lights ($P < .05$). However, there was no effect of light on energy retention for pinealectomized chicks. Body weight gain was significantly affected by light among unoperated and sham operated chicks but not pinealectomized ones. Sham operated birds that were grown under a 16L:8D lighting regime gained faster than those grown under constant light (363 g vs 335 g). There was an 8% increase which was significant ($P < .05$). Unoperated control birds under 16L:8D photoperiod also grew significantly faster ($P < .05$) than their constant light counterparts. Feed/gain ratios were significantly higher ($P < .05$) in both unoperated and sham operated birds grown under 16L:8D as compared to those under constant light. Such difference was not observed in pinealectomized chicks. The gain response to melatonin was significantly less when birds were raised under CL ($P < .01$) but there was not a significant

light x melatonin interaction for feed consumption, gain/feed ratio, or energy retention ($P < .01$).

Pinealectomized birds grew more slowly and less efficiently, were less capable of retaining ingested metabolizable energy, and were little affected by light treatment. However, the response of pinealectomized birds to dietary melatonin was of the same magnitude as either unoperated or sham operated birds.

Experiment 5

The results of experiment 5 are presented in Table 7 with the corresponding ANOVA in Tables 12 and 13 (Appendix). As in previous studies, melatonin significantly increased ($P < .03$) body weight gain and feed consumption as well as feed efficiency. All the other parameters measured were also increased significantly ($P < .03$) by melatonin administration irrespective of lighting regime.

Light also had an effect on all the parameters. Feed consumption was only slightly affected but constant light significantly depressed ($P < .05$) body weight gain, feed efficiency, liver lipogenesis, jejunal glucose uptake, and plasma T_3 and T_4 concentrations. There was not a light x melatonin interaction ($P < .20$) for any variable measured.

The standard curve of the thyroxine (T_4) radioummunoassay was linear with a coefficient of determination (R^2) of .99. Accuracy of the assay was tested by recovery of serially diluted thyroxine added to plasma of known thyroxine concentration. The mean recovery of added T_4 was 96%. The coefficients of variation within and between assays were 3.7 and 4.2% respectively. Crossreactivity of the T_4 antibody with T_3

Table 7. Effects of light treatment and dietary melatonin on growth, feed consumption, feed efficiency, and selected metabolic parameters of chicks.[1]

Variable	Light Treatment			
	16L:8D		Constant Light	
	Melatonin			
	Without	Added	Without	Added
Weight (g) gain[2,3]	511	634	476	525
Feed consumed (g)[2]	838	886	835	860
Gain/feed (g/kg)[2,3]	609	715	570	610
Liver lipogenesis[2,3] (x/o[3] dpm/tissue/10 min)	60.4	144.4	32.5	111.3
Jejunal glucose uptake[2] mol/g dry tissue/15 min)	60.9	105.6	45.1	79.1
Plasma T_4 (ng/ml)[2,3]	15.4	21.0	14.1	17.8
Plasma T_3 (ng/ml)[2,3]	1.86	4.18	1.27	3.05

[1]Data are means of 5 replicate groups of 8 chicks each. Chicks were fed the experimental diets from day 1 to day 29 post hatching.

[2]The main effect of melatonin was significant ($P < .03$).

was less than 2.2% while crossreactivity with melatonin was less than .001%.

The relationship of plasma triiodothyronine (T_3) to counts bound was linear with a coefficient of determination of .99. The mean percentage recovery for the T_3 assay was 96.63. Crossreactivities of T_3 antibody with T_4 and melatonin were less than 2.1% and .001% respectively. The mean coefficients of variation within T_3 radioimmunoassay was 4.1% while that between assays was 4.3%.

CHAPTER V

DISCUSSION

Body Weight

In this study 15 ppm melatonin administration was found to consistently stimulate body weight gain irrespective of the photoperiod under which the birds were housed. Effects of melatonin on body weight gain have been studied. Singh and Turner (1967) reported that melatonin injections stimulated the growth of juvenile cockerels. Wade and Bartness (1984) showed that in Syrian hamsters housed under long photoperiods daily injections of melatonin in the late afternoon increased body weight, an effect that is also seen if the photoperiod is shortened. However, Injidi and Forbes (1983) injected 10 mg melatonin/kg body weight into egg-laying chickens and found a depression in growth.

DeVlaming (1980) found that melatonin treatment stimulated body weight gain as well as somatic growth. The weight gains were increased linearly with various doses of melatonin administration. Pinealectomy reduced the body weight increases, suggesting that the pineal might be involved in the accelerated growth. Similarly, Phettplace and Nockels (1985) have given the indication that melatonin administration increases the growth and feed efficiency of leghorn cockerels. Weight gains were increased linearly with melatonin levels in the diet. The growth response of the chicks to melatonin was light dependent. Melatonin stimulated growth only when the photoperiod was not

continuous. McKeown et al. (1975) gave melatonin injections to pigeons which resulted in an elevated growth hormone level, indicating that melatonin may influence the release of growth hormone.

Other studies have found the melatonin effect on growth to be photoperiod dependent. However, evidence provided by this study indicates that exogenous melatonin modified growth notwithstanding the light schedule. Exogenous melatonin increased feed efficiency and body weight gain when birds were housed under continuous light or under a light:dark cycle. Also, the performance of pinealectomized chicks that were fed melatonin was of equal magnitude as either unoperated control or sham operated birds.

Pinealectomized birds grew slower and less efficiently than their sham operated and unoperated control counterparts. These results are consistent with those of Badertscher (1924), Machemer et al. (1971), and Cogburn and Harrison (1977) who respectively found that pinealectomy in chickens resulted in depression of body weight. In contrast, Malm et al. (1959) reported that pinealectomy stimulated the growth of rats for up to 14 weeks post surgery. Injidi and Forbes (1983) have indicated that pinealectomy enhances body weight gain. On the contrary, Darre et al. (1980) found a depression in body weight of pinealectomized birds and this was without a commensurate change in feed intake.

Results in this study show that while feed consumption of pinealectomized chicks was not significantly different from that of the control, there was a much lower feed efficiency accompanying a significantly reduced gain in body weight. Pinealectomy also resulted

in a lower retention of metabolizable energy. Birds with their pineals removed were little affected by light treatment. However, the response of these birds to dietary melatonin was of the same degree as either their unoperated or sham operated counterparts.

The pineal gland is the major if not the only source of endogenous melatonin (Pelham, 1975). Since melatonin has been found to consistently stimulate growth and the removal of the pineal has an opposite effect, it is logical to speculate that the pineal organ has a role in regulating body weight gain. Administration of melatonin to pinealectomized birds results in a growth response comparable to that of intact birds. Even though other studies (Malm et al., 1959; Injidi and Forbes, 1983) have given the indication that the presence of the pineal is inhibitory to growth, evidence from the present study is clearly in favor of a positive effect of the pineal on growth. The pineal most probably elicits this function with melatonin as a mediating agent.

In this work, birds raised under constant light had significantly lower body weight gains than those raised under 16L:8D. These results are in agreement with those obtained by Siegel et al. (1961), Voitle (1969), and Osei (1978). In contrast, Buckland and Hill (1970) found great inconsistencies with about half the studies showing improved growth on continuous light while the rest indicated that selected intermittent light/dark schedules enhanced feed conversion and maximized growth. The effect of light on feed intake and body weight gain has been investigated in various other studies.

Hoffman and colleagues (1982) in their study of golden hamsters have shown that weight gain may be produced by shortening the photoperiod. Wade (1983) also showed that when housed under 10L:14D hamsters exhibited increased body weight gain, feed efficiency, carcass energy content, as well as percent energy retention.

In humans, there is tremendous evidence that seasonal rhythms occur in a variety of phenomena including growth, suicide, and even death from natural causes (Aschoff, 1981). People with seasonal affective disorder (SAD) exhibit behavioral response to the various seasons, which during the fall as well as winter months reaches a degree of severity to impair personal and social relationships. These patients tend to be anergic and unmotivated during the fall and winter months but exhibit a high degree of enthusiasm during the spring and summer months. The depressed condition among such people in winter is accompanied by overeating, enormous craving for carbohydrates, as well as excessive gain in body weight. The symptoms of weight gain and increased appetite are significantly correlated with one another as well as with carbohydrate craving. This gives an indication that a regulation of the pattern of eating in SAD may be coordinated by neurophysiological systems that vary seasonally in an interrelated manner (Rosenthal et al., 1984).

Temperature and scarcity of food have been implicated in the evolutionary pressure to develop seasonal rhythms, but these factors come too late in a season to be effective triggers for initiating the seasonal energy conservation changes in weight and metabolism seen in humans and animals. Another set of environmental changes have

therefore been considered to serve as triggers for the initiation of seasonal behavior changes. Of other factors under consideration, photoperiod, the illuminated fraction of the 24-hr day, is by far the most important across the types of seasonal rhythms studied (Immelman, 1973). It is logical that this would be the case because changes in day length vary most predictably from year to year. Thus, the winter gain observed in hamsters can be reproduced in the laboratory by artificially shortening the photoperiod (Hoffman et al., 1982). Bright artificial light has been used to extend the winter photoperiod of SAD patients to suppress body weight gain and general sluggishness (Rosenthal et al., 1985).

Ralph et al. (1974) have indicated that pineal and serum melatonin in chickens persists in constant darkness and Pelham et al. (1972) reported that serum melatonin may originate primarily or entirely from the pineal gland. It has been suggested that light acted as an agent for an endogenous oscillator which in turn regulated the melatonin rhythm through the autonomic innervation of the pineal (Klein and Moore, 1979). In a series of experiment, they showed that the suprachiasmatic nucleus is probably the endogenous oscillator under question.

In this study pinealectomy, light, and melatonin administration have all influenced feed efficiency, energy retention, and body weight gain. In view of the fact that melatonin is a product of the pineal and the pineal is also affect by light, it is appropriate to speculate that the pineal and light combine to modify growth. Photoperiodic information is perhaps conveyed by way of the suprachiasmatic nuclei to

the pineal gland where it both suppresses and influences the timing of melatonin secretion. The resulting pattern of melatonin secretion, a product of both endogenous production and photoperiod, may influence some aspects of body metabolism to effect increased weight gain.

Thyroid Activity

Thyroid activity was significantly affected by both the lighting schedule and melatonin administration. Birds fed the 15 ppm melatonin diet had higher levels of plasma T_3 and T_4 hormones. Chickens raised under constant light had a significantly lower plasma T_3 and T_4 than those raised under 16L:8D. These results are consistent with those of Panda and Turner (1968) and Gordon et al. (1980). Klandolf et al. (1978) also reported a higher T_4 concentration in control than in pinealectomized chicks. On the contrary, Deprospo et al. (1969) suggested that endogenous melatonin produced in the pineal gland and regulated by environmental lighting schedules was responsible for inhibition of iodine uptake by the thyroid glands of rats. Another interesting concept postulated by Deprospo et al. (1968) was that the effect of melatonin might depend on the time of day of administration.

Melatonin administration into rats subcutaneously, intraperitoneally, or intraventricularly has been reported to inhibit iodine uptake by the thyroid gland (Baschieri et al., 1963; Reiter et al., 1965). Houssay et al. (1966) found that melatonin administration prevented the thyroid hypertrophy obtained in male mice after pinealectomy. Gordon et al. (1980) used a dose of melatonin equal to that used in earlier studies; a difference between their study and the earlier ones is that they injected the melatonin about twice as long as

the periods of administration in the other studies. This suggests that perhaps different protocols of melatonin administration could result in different effects on thyroid hormone secretion. Singh and Turner (1972) found that the thyroid secretion rate (TSR) responded with a maximal decrease after the injection of a lower dose of melatonin (50 g/100 g body weight) in the female hamster, while a double dose of melatonin was required to obtain the same degree of inhibition in the male rat. According to these data, the TSH-thyroid system of the hamster is more sensitive to melatonin than that of the rat. At the same time, the sex difference also could be taken into account in this experiment. Wurtman (1971) discussed in greater detail the role of species differences concerning the pineal and thyroid action. He pointed out that some species of animals show greater responses to light/dark effects or to melatonin than others.

Investigating changes in blood and pituitary TSH concentrations of melatonin-treated rats of different ages, Panda and Turner (1968) concluded that the regulatory effect of the hypophyseal-pineal is more marked in immature animals than in the adults. Similarly, Relkin (1972) demonstrated that when male rats were pinealectomized at 21 days of age and investigated on the 28th day of life, changes in thyroid activity (plasma and pituitary TSH level, protein bound iodine (PBI) blood level) were characteristic. These findings might be due to age differences. Thus, the apparent contradictions found in the pineal-thyroid literature may be due to such factors as differences in the species of experimental animals, age differences of the employed

animals, circadian and circannual rhythms, and divergent lighting schedules used by the different investigators.

A widely discussed question is the mechanism of action of melatonin by which it influences thyroid activity. Thieblot (1965) showed that administration of melatonin produced marked hyperactivity of the thyroid as evidenced by various histological changes. Panda and Turner (1968) observed an increase in blood TSH content with concomitant hyperplasia and hypertrophy of the thyroid gland in melatonin-treated rats. Similar findings were reported by De Fronzo and Roth (1972). A proportional increase in thyroid DNA and RNA content with significantly increased ^{131}I-uptake were observed in chronically melatonin-treated rats. The possibility cannot be excluded that melatonin might actually have a direct peripheral action on the thyroid gland, thus stimulating the biochemical mechanism of thyroid hormone secretion. At the same time, melatonin might act analogous to that observed for the gonadotrophin system also at the level of the central nervous system, influencing the function of the serotoninergic neurons of the brain system and modifying TSH release.

Thyroid hormones are necessary for normal growth and development but little is known about how their effects are exerted (Bernal and Retetoff, 1977). Recently, investigations have implicated thyroid hormone in the control of factors necessary for growth (Gaspard et al. 1978). Chai (1970) has shown that selection for thyroid activity resulted in a positive correlated response in body size of mice. Results in the present study have indicated that the pineal might influence the thyroid with melatonin as a mediating agent. The thyroid

in turn by way of its secretions might act on some factors necessary for increased body weight gain.

Energy Retention and Utilization

The efficiency of energy retention and usage was affected by both pinealectomy and melatonin administration. Birds with intact pineals had a significantly higher energy retention and also were more efficient in utilizing energy than pinealectomized ones. Melatonin administration also resulted in a significantly enhanced energy retention. Birds fed 15 ppm melatonin also exhibited an increased jejunal glucose absorption and a faster hepatic lipogenesis.

In chickens, poor energy utilization has been associated with increased heat production and metabolic rate (Morrison and Leeson, 1978) whereas efficiency of energy utilization has been well correlated with body growth and body weight gain (Proudman et al., 1970). Masic et al. (1974) found that broilers consumed significantly more feed than layers and ate more meals but spent significantly less time in feeding activity. Similar results were obtained by Barbato et al. (1980) when comparing high and low weight lines. Cherry and Siegel (1979) in a related study demonstrated that food was digested and cleared at a faster rate from a high weight line of chickens.

A heavy, rapidly-growing breed is also more efficient in energy retention (Robbins, 1981). He determined that a heavy-growing broiler breed was 15% more efficient in retaining ingested energy than a lighter, slower-growing breed. The difference in observed efficiency may be due to a lower maintenance energy requirement, a higher efficiency of energy utilization above maintenance, or both. This

higher efficiency has also been observed in mice (Canolty and Koong, 1976). It was shown that selection for rapid post weaning growth rate in mice resulted in a great increase in efficiency of energy utilization. This was not accompanied by significant changes in maintenance energy requirement but was due primarily to a higher efficiency of energy utilization for gain. In this study, maintenance energy requirements were not significantly affected by pinealectomy. However, the efficiency of energy utilization for growth (i.e., energy above maintenance) was significantly lower in pinealectomized birds and higher in melatonin-treated ones. Thus, the higher efficiency of energy retention observed in birds with intact pineals and those treated with melatonin is primarily due to a much higher efficiency of energy utilization above maintenance.

Deb et al. (1976) reported that obese Zucker rats were twice as efficient as their lean counterparts in retaining ingested energy. This higher efficiency was due to a higher efficiency of energy utilization for gain and not the result of a lower maintenance energy requirement. However, Lin et al. (1979) determined that a major factor contributing to the higher energetic efficiency of genetically obese vs lean mice is a much lower maintenance energy requirement. It has also been demonstrated that the differences in weights between fat line (FL) and lean line (LL) chickens was not due to differences in feed intake but to differences in metabolic utilization of energy (Leclercq and Saadoun, 1982). Saadoun and Leclercq (1987) in another study found differences in rates of lipogenesis in both liver and carcass of FL and LL birds. FL chickens exhibited enhanced hepatic lipogenesis.

Heavy breed chickens exhibit better efficiency of energy utilization and retention, increased jejunal glucose absorption, and an enhanced hepatic lipogenesis. All the above seems to account for the increased body weight of the heavy breed. Results in this work have indicated that melatonin and pinealectomy have an effect on these same parameters. Continuous light also tended to have a negative effect. Chickens with intact pineals were more efficient in utilizing and retaining energy while melatonin administration also enhanced energy retention, nutrient absorption, as well as hepatic lipogenesis. The pineal through melatonin may have a direct or indirect influence on these and other aspects of body metabolism in eliciting increased body weight gain.

CHAPTER VI

SUMMARY AND CONCLUSIONS

Attempts were made in this study to determine the role of the pineal gland in chick growth and physiological as well as metabolic status.

Experiments were conducted to obtain information respecting the effects of light, pinealectomy, and melatonin on body weight gain, feed consumption, feed efficiency, energy utilization, and several metabolic parameters associated with energetic efficiency viz. hepatic lipogenesis, jejunal glucose uptake, and plasma T_3 and T_4 levels.

It was found that light and the pineal have effects on body growth and energetic efficiency. Pinealectomy was determined to significantly depress feed efficiency and body weight gain. Birds with intact pineals retained and utilized energy above maintenance much more efficiency than their pinealectomized counterparts. Melatonin administration increased gain, gain/feed, and energy retention by an average of 19%. Light treatment like melatonin affected feed intake only slightly. However, gain, gain/feed ratio, and energy retention were significantly lower in birds housed under constant light. The gain response to melatonin was significantly less when birds were housed under constant light, but there was not a significant light x melatonin interaction for either gain/feed ratio or energy retention. Pinealectomized chicks were little affected by light treatment.

However, the response of pinealectomized birds to dietary melatonin was of the same magnitude as either unoperated or sham operated birds.

Dietary melatonin and light treatment were also found to have effects on all the metabolic parameters measured. Constant light significantly depressed while 15 ppm dietary melatonin significantly increased liver lipogenesis, jejunal glucose uptake, and plasma T_3 and T_4 concentrations.

The experiments described herein have established a positive role for the pineal in body weight gain of chickens. Pinealectomy, light treatment, and melatonin administration influenced feed efficiency, body weight gain, and several metabolic parameters associated with energetic efficiency and growth. Considering the fact that melatonin is a product of the pineal and pinealectomy and melatonin administration have opposite effects, it is conceivable to conclude that the pineal is essential for body weight regulation with melatonin as the mediating agent.

How the pineal elicits this function is unclear but light is definitely involved. Photoperiodic information is perhaps conveyed by way of an endogenous oscillator to the pineal where it both suppresses and influences the timing of melatonin secretion. The consequent pattern of melatonin secretion, which is a product of both endogenous production and photoperiod, may have an influence on several aspects of body metabolism to effect body weight regulation. The null hypothesis, that the pineal has no significant activity or role, is contradicted by evidence in this study.

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APPENDIX

Table 8. Experiment 1 summary of analysis of variance.[a]

		Gain			Dry Matter			Crude Protein			Body Fat		
SOV	df	MS	F	Pr>F	MS	F	Pr>F	MS	F	Pr>F	MS	F	Pr>F
Treatment	1	3518.6	4.25	.0078*	5.37	2.57	.0558	1.6	1.15	.3801	7.07	11.21	.0001
Energy	1	19665.4	23.77	.0002*	24.8	11.86	.003	.48	.35	.565	36.26	57.44	.0001
Melatonin (mel)	1	4401.04	5.32	.0348*	0.17	.08	0.781	.006	.004	.945	0.51	0.81	.382
Sex	1	30.88	NS		2.04	0.98	0.337	9.13	6.5	.021	2.10	3.33	0.086
Energy x mel	1	117.04	NS		1.40	0.67	0.424	0.15	.01	.91	1.17	1.85	.192
Energy x sex	1	126.04	NS		8.17	3.91	0.065	.60	.43	.52	2.22	3.52	.079
Melatonin x sex	1	145.04	NS		0.17	.08	0.781	.96	.69	.42	1.55	2.46	0.136
Energy x mel x sex	1	145.04	NS		0.88	0.42	0.525	.08	.06	.811	5.70	9.04	.0084
Error	16	827.38			2.09			1.39			0.63		

[a]Df = degrees of freedom; MS = mean square; Pr > F indicates the probability that calculated F is greater than tabular F; and NS = not significant.

Table 9. Experiment 2 summary of analysis of variance.[a]

SOV	Df	Gain			Feed Consumption			Feed Efficiency		
		MS	F	Pr>F	MS	F	Pr>F	MS	F	Pr>F
Treatment	3	2196	1.26	.3504	3637	1.12	.3962	.002	1.24	0.3574
Photoperiod	1	216.8	.12	.73	4144	1.28	.291	.0042	2.53	0.150
Melatonin	1	6302	3.62	.0934	5250	1.62	.239	.00168	1.01	0.345
Photox melatonin	1	70.1	.04	.846	1518	0.47	0.51	.0003	0.18	0.680
Error	8	1739			3242			.00167		

[a]Df = degrees of freedom; MS = mean square; Pr > F indicates the probability that calculated F is greater than tabular F.

Table 10. Experiment 3 summary of analysis of variance.[a]

SOV	Df	Gain			Feed Consumption			Carcass Energy Gain			Percent Energy Retention		
		MS	F	Pr>F	MS	F	Pr>F	MS	F	Pr>F	MS	F	Pr>F
Treatment	2	1998	139	.0001	461	6.13	.03	38097	214	.0001	213	184	.0001
Control vs Px	1	2521	175	.0001	888	11.79	.013	63448	357	.0001	330	285	.0001
Control vs Sham	1	1476	102	.0001	34	.46	0.522	12746	71.84	.0001	97	84	.0001
Error	6	14.3			75			177			1.15		

[a]Df = degrees of freedom; MS = mean square; Pr>F indicates the probability that calculated F is greater than tabular F.

Table 11. Experiment 4 summary of analysis of variance.[a]

SOV	Df	Gain			Feed Consumption			Gain/Feed			% Energy Retention		
		MS	F	Pr>F	MS	F	Pr>F	MS	F	Pr>F	MS	F	Pr>F
Treatment	11	7488	66.68	.0001	1308	1.43	.222	.01	10.70	.0001	75	13.49	.0001
Control vs pinealectomy (C1)	1	32120	286.01	.0001	7315	8.01	.0093	.047	38.15	.0001	139	24.98	.0001
Control vs sham (C2)	1	2069	18.43	.0003	110	0.12	.7316	.009	7.25	.0127	7.70	1.38	.2523
Photoperiod	1	5402	48.10	.0001	84	0.09	.7643	.012	9.85	.0045	79	14.6	.0001
C1 x photo	1	4108	36.58	.0001	5	0.01	.9414	.012	9.62	.0049	0.10	0.02	.8900
C2 x photo	1	364	3.25	.0842	572	0.63	.4364	.0001	0.10	.7583	1.94	0.35	.5613
Melatonin	1	35658	317.51	.0001	4511	4.94	.036	.063	50.74	.0001	5.84	1.04	.0001
C1 x melatonin	1	280	2.49	.1273	22	0.02	.878	.0001	0.12	.7331	21.6	3.86	.0612
C2 x melatonin	1	29	0.26	.6136	74	0.08	.778	.0002	0.21	.6475	4.4	0.79	.3839
Photo x melatonin	1	1950	17.37	.0003	1667	1.83	.189	.0007	0.61	.4415	8.44	1.51	.2312
C1 x photo x melatonin	1	8	0.07	.7897	26	0.03	.867	.000006	.01	.9423	0.07	0.01	.1929
C2 x photo x melatonin	1	382	3.41	.0773	11	0.01	.911	.001	.99	.3295	2.33	0.42	.5245
Error	24	112			913			.001			5.59		

[a]Df = degrees of freedom; MS = mean square; Pr>F indicates the probability that calculated F is greater than tabular F.

Table 12. Experiment 5 summary of analysis of variance.[a]

SOV	Df	Gain			Feed Comsumption			Gain/Feed Ratio		
		MS	F	Pr>F	MS	F	Pr>F	MS	F	Pr>F
Treatment	3	22926	440	.0001	2476	1.35	0.2947	.01960	25.86	0.0001
Photoperiod	1	20224	388	.001	972	0.52	0.482	.01972	26.02	0.0001
Melatonin	1	44745	860	.0001	4929	2.68	0.121	.03750	49.48	0.0001
Photo x melatonin	1	3808	73	.0001	1548	0.84	0.372	.0016	2.09	0.167
Error	16	52			1840			.0076		

[a]Df = degrees of freedom; MS = mean square; Pr>F indicates the probability that calculated F is greater than tabular F.

Table 13. Analysis of variance (Experiment 5).[a]

SOV	Df	Jejunal Glucose Absorption			Liver Lipogenesis			Plasma T_4 Conc.			Plasma T_3 Conc.		
		MS	F	Pr>F	MS	F	Pr>F	MS	F	Pr>F	MS	F	Pr>F
Treatment	3	3367	37.99	.0001	13224	240	.0001	46	11	.0003	8	23.5	.0001
Light	1	2255	25.44	.0001	3718	67	.0001	26	6.5	.0213	4.5	13.24	.002
Melatonin	1	7707	89.96	.0001	35870	652	.0001	108	26	.0001	19.3	56.8	.0001
Light x melatonin	1	138	1.56	.2286	85	1.5	.2302	4	.07	.3157	0.16	0.48	.4976
Error	16	88			54			4			0.34		

[a]Df = degrees of freedom; MS = mean square; PR>F indicates the probability that calculated F is greater than tabular F.

VITA

Peter Osei was born July 28, 1955, in Kumasi, Ghana. He graduated from elementary school in that city and obtained his high school diploma in 1977 at Prempeh College. That same year he entered the University of Science and Technology in Kumasi. He graduated with Bachelor of Science honors in Biological Sciences in June 1981 and spent one year as an intern at Max Planck Institute for medical research in Heidelberg, West Germany. In October 1982 he was admitted to the Weizmann Institute of Science, Rehovot, Israel, where he obtained his MS degree in Biochemistry in June 1984. He enrolled in the Graduate School of The University of Tennessee, Knoxville in August 1984 on a research assistantship. He received a Doctor of Philosophy degree in Animal Science with concentration in Nutrition in December 1987.