

PHOTOPERIODIC CONTROL OF PROLACTIN SECRETION IN THE DOMESTIC CHICKEN

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DECLARATION

I hereby declare that this thesis has been composed by myself and has not been submitted for any other degree elsewhere. The work presented herein is my own, and all assistance given to me is acknowledged.

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ABBREVIATIONS

aa	amino acid
ACN	acetonitrile
cpm	counts per minute
DA	dopamine
EDTA	ethylenediaminetetraacetic acid
FSH	follicle stimulating hormone
g	grams
GABA	gamma amino butyric acid
GAP	gonadotrophin associated peptide
GnRH	gonadotrophin releasing hormone
h	hours
HHA	hypothalamo-hypophysial axis
kda	kilodalton
KLH	keyhole limpet haemocyanin
L : D	light : dark
LH	luteinising hormone
M	moles
MBq	megaBecquerel
min	minutes
ml	millilitres
mRNA	messenger ribonucleic acid
N-terminal	amino terminal
ng	nanograms
PHI	peptide histidine isoleucine
PL	placental lactogen
PPD	purified protein derivative
PRC	photoperiodic response curve
PRL	prolactin
sec	seconds
TFA	trifluoroacetic acid
TRH	thyrotropin releasing hormone

VIP

µg

µl

5-HT

vasoactive intestinal

polypeptide

micrograms

microlitres

5-hydroxytryptamine

ABSTRACT

An increase in the concentration of plasma prolactin (PRL) occurs in response to increased daylength in seasonally breeding animals irrespective of whether they are short or long day breeders. In contrast to the wealth of information on the photoperiodic control of PRL secretion in mammals very little information is available in birds. The objective of this thesis is to increase understanding of the photoperiodic control of PRL secretion in birds.

The critical daylength (CDL) required to induce PRL secretion in bantam cockerels reared on short days (8 h light/day) was between 10 and 12 h light/day. Photoperiods of more than 14 h of light were maximally photostimulatory. The CDL was dependent on photoperiodic history because in birds reared on 20 h light/day transfer to photoperiods of 14 h or less resulted in decreased PRL secretion. Transfer from 8 h light/day to a single 20 h long day and back to short days induced an increase in PRL secretion 20 to 22 h after dawn. This increase persisted as a "carry over effect" for 4 days. Changes in ambient temperature or fasting up to 24 h did not affect PRL secretion. This eliminated the possibility that photoperiodically induced changes in PRL secretion could be explained by these factors.

Prolonged exposure of intact male and female turkeys or bantams to 20 h light/day resulted in a depression in plasma PRL. The depression in plasma PRL appeared to be a direct consequence of the development of photorefractoriness and was not the result of a decrease in plasma gonadal steroids, due to ageing or the development of reproductive photorefractoriness. This was deduced from the observation that prolonged exposure of castrated bantam cockerels to 20, 18, 16, and 14 and not to 12 h photoperiods also depressed plasma prolactin indicating the development of photorefractoriness. A 4 h increase in photoperiod did not stimulate PRL release in castrates held for a prolonged period on a 16 h photoperiod but did so in castrates held on 12 h photoperiod. Exposure of photorefractory castrates to short days for 5 weeks dissipated refractoriness and restored the photoperiodic response.

The age at which PRL photoperiodic response first appeared was determined in prepubertal intact male and female bantams reared on 8 h light/day. Sexual maturation occurred at 18 to 20 weeks of age. An increase in PRL secretion was observed after photostimulation in both sexes at 4, 8, 12 and 16 weeks.

Photoinduced PRL release was shown to be mediated by avian PRL releasing hormone, vasoactive intestinal polypeptide, since active immunisation against this neuropeptide blocked the photoinduced PRL release in castrated bantams and intact adult turkey hens.

INTRODUCTION

1.1 General Introduction.

Prolactin (PRL) is the most versatile of hormones produced by the anterior pituitary gland. Its varied actions include the regulation of lactation, gonadal function, parental behaviour, growth, water balance and the immune system (Nicoll, 1974; De Vlaming, 1979; McNeilly, 1986; Neill and Nagy, 1994). The internal and external environmental factors controlling PRL secretion to regulate this diversity of functions are incompletely understood (Ensor, 1975; McNeilly, 1986; Neill and Nagy, 1994). One of the best known environmental factors controlling PRL secretion is daylength. Thus, at non-tropical latitudes, seasonal increases in the concentration of plasma PRL occur in mammals exposed to increasing daylength, irrespective of whether the animal is a short-day or a long-day breeder (Curlewis, 1992). Similarly, in birds breeding at temperate and high latitudes, the concentration of plasma PRL is increased when daylengths increase (e.g. rook, Lincoln *et al.*, 1980; starling, Dawson and Goldsmith, 1982; partridge, Sharp *et al.*, 1986a; ptarmigan, Stokkan *et al.*, 1988; quail, Boswell *et al.*, 1995). In commercial poultry, it is established that PRL secretion increases after photostimulation in the turkey (Burke and Dennison, 1980; Lea and Sharp, 1982; El Halawani, *et al.*, 1996) but there is no information available for the domestic chicken.

The overall aim of this Thesis is to increase understanding of the photoperiodic control of PRL secretion in the domestic chicken.

1.2 Discovery, cellular localisation, structure and functions of PRL.

1.2.1 Discovery of PRL.

The first report of prolactin was by Stricker and Grueter (1928), who showed that injection of crude bovine pituitary extracts into pseudopregnant rabbits induced lactation. It is this function which resulted in the hormone being named prolactin. Riddle and Braucher (1931) subsequently found that bovine pituitary extracts injected into pigeons stimulate production of “milk” by the crop. The first evidence that PRL is involved with the parental behaviour was published by Riddle and colleagues who showed in virgin female rats and laying hens (Riddle *et al.*, 1935, 1942) that injection of the hormone induced pup retrieval and broodiness respectively. PRL was the first pituitary hormone to be prepared to a high degree of purity (White *et al.*, 1937; Li *et al.*, 1941), which greatly facilitated research on its biological activities.

1.2.2 Cellular localisation and structure of PRL.

PRL is produced in cells in the anterior pituitary gland called lactotropes, referring to their role in the control of lactation (Nicoll, 1974). Lactotropes are classified as acidophils, since they stain with acidic dyes, such as orange G, erythrosine and carmosine. Lactotropes have been identified in all vertebrates including amphibians, reptiles and birds where they are localised in the anterior (rostral/ cephalic) region of the pars distalis of the anterior pituitary (Clarke and Bern, 1980; Mikami and Yamada, 1984; Berghman *et al.*, 1992). This regional distribution of lactotropes is absent or much less obvious in the mammalian pituitary (Clarke and Bern, 1980).

PRL is a single-chain polypeptide with a molecular size of 22-26 kDa (Niall *et al.*, 1971). It belongs to the family of hormones including growth hormone and placental lactogen. Chicken and turkey PRLs and most mammalian PRLs have 199 amino acids. Rat, mouse and hamster PRLs are an exception, having 197 amino acids (Sinha, 1995).

Analyses of PRL amino acid sequence homology between chicken and non-avian species demonstrates phylogenetic relationships (Sinha, 1995). The highest homology is with reptiles (alligator, 92%; sea turtle, 86%) and the lowest with teleosts (e.g. salmon, 28%) . Chicken PRL has 79% homology with porcine and equine PRLs, 69-70% with ovine and bovine PRL respectively and 93% homology with turkey PRL (Shimada *et al.*, 1993; Sinha, 1995). Avian and mammalian PRLs have 3 intra-molecular disulphide bridges, while teleost PRL have only 2 disulphide bridges (Shimada *et al.*, 1993). Prolactin exists in several molecular forms derived from alternative splicing of the primary PRL mRNA transcript or by post-translation modification. These are suggested to be responsible for the diverse biological actions of the hormone (Sinha, 1992).

1.2.3 Functions of PRL.

Unlike other pituitary hormones, PRL did not become specialised early in vertebrate phylogeny to regulate a single physiological process (Ben-Jonathan, 1989). Its wide range of functions are summarised in Table. 1.1.

Table 1.1 Functions of PRL.

(Data taken from De Vlaming, 1979; Kikuyama *et al.*, 1986; El Halawani *et al.*, 1988; Horseman and Buntin, 1995; Toyoda *et al.*, 1996).

Vertebrate	Action
Fishes	Osmoregulation Proliferation of melanocytes Growth of seminal vesicles Proliferation of mucous cells Parental care (foam nesting) Reproductive development

Amphibians	<ul style="list-style-type: none"> Protein anabolism (larvae) Tail and gill growth (larvae) Limb regeneration Structural changes accompanying water drive Brain growth (larvae) Cloacal gland development Spermatogenic Proliferation of melanocytes Courtship behaviour Osmoregulation
Reptiles	<ul style="list-style-type: none"> Somatic growth Tail growth Skin sloughing Oviduct development
Birds	<ul style="list-style-type: none"> Proliferation of crop sac mucosa Epidermal hyperplasia in brood patch Incubation behaviour Electrolyte balance Increases body weight and liver size Feather growth Development of female reproductive tract Anti-gonadal stimulates feeding
Mammals	<ul style="list-style-type: none"> Mammary growth Lactation Pseudopregnancy Maternal behaviour Luteotropic Male sex accessory gland development

Protein anabolism
Sebaceous gland growth
Hair growth
Osmoregulation
Erythropoietic
Spermatogenic
Uterine hypertrophy

The many functions of PRL are reflected in the widespread distribution of PRL receptors in many organs including the mammary gland, ovary, uterus, placenta, testis, accessory sex glands, liver, kidney, pancreas, lymphatic tissue, brain and eye (Kelly *et al.*, 1991). The PRL receptor has been cloned in chicken, pigeon and turkey (Tanaka *et al.*, 1992; Chen and Horseman, 1994; Zhou *et al.*, 1996) and as in mammals, is widely distributed in body tissues (Zhou *et al.*, 1996). The avian PRL receptor differs from the mammalian PRL receptor in having two homologous repeat units in the extracellular domain, indicating the potential for two extracellular ligand binding sites (Tanaka *et al.*, 1992).

1.2.4 Avian PRL.

In birds, PRL secreting cells are localised primarily in the cephalic lobe of the anterior pituitary (Hansen and Hansen, 1977; Lopez *et al.*, 1995).

Avian PRL was first purified from chicken pituitary glands (Scanes *et al.*, 1975), with turkey PRL being isolated and purified at a later date (Burke and Papkoff, 1980; Proudman and Corcoran, 1981; Cheng and Etches, 1981). Avian PRL was purified using conventional fractionation techniques, salt precipitation, ion-exchange chromatography, gel filtration and preparative isotachopheresis. The pigeon crop sac bioassay was the most widely used method to evaluate the potency of the purified PRL. The assay involves local injection of PRL into the crop sac of pigeons where it induces

the proliferation of the crop mucosal lining to form the 'crop milk'. The increase in crop sac mucosal weight and/ or thickening is directly related to the potency of the PRL injected (Nicoll,1967).

The first homologous radioimmunoassay for chicken PRL was developed by Scanes *et al.* (1976) using PRL prepared from broiler chicken pituitaries. Unfortunately this assay was not highly specific and was unsuitable for the measurement of PRL in other avian species (Lea and Sharp, 1982). An heterologous radioimmunoassay for PRL (McNeilly *et al.*, 1978), using guinea pig anti-human PRL serum and radiolabelled ovine PRL has been successfully used for a wide range of avian species (e.g. rook, Lincoln *et al.*, 1980; duck, Goldsmith and Williams, 1980; starling, Dawson and Goldsmith, 1983; dove, Lea *et al.*, 1986) although it did not detect an increase in plasma PRL in incubating turkeys (Etches and Cheng, 1982). Homologous radioimmunoassays were subsequently developed for turkey PRL (Burke and Papkoff, 1980; Proudman and Opel, 1981; Etches and Cheng, 1982). All these assays proved to be robust and specific for turkey PRL but of limited value for measuring PRL in non -galliform species. The cloning of chicken PRL cDNA (Hanks *et al.*, 1989a, 1989b, Watahiki *et al.*, 1989, Kumar *et al.*, 1989) made it possible to produce the recombinant-derived chicken PRL (Hanks *et al.*, 1989a) which was used for the development of an homologous chicken PRL radioimmunoassay (Talbot and Sharp, 1994). This assay proved to be more specific than the original chicken PRL assay of Scanes *et al.* (1976) and is suitable for measurement of PRL in some non-galliform species. The cloning and the determination of the nucleotide sequence of turkey PRL (Karatz *et al.*, 1990; Wong *et al.*, 1991) and the production of recombinant turkey PRL (Karatz *et al.*, 1993) has also made it possible to develop a homologous recombinant-derived turkey PRL radioimmunoassay (Guémené *et al.*, 1994).

1.3 PRL secretion and physiological state.

1.3.1 Lactation and parental behaviour in mammals.

The best known function of PRL in mammals is the initiation and maintenance of lactation. Lactogenesis, or the initiation of lactation, usually starts in the last trimester of the pregnancy. One of the hormones involved is PRL which is essential for milk production in rats, rabbits, and women, but plays a lesser role in ruminants once lactation is established (Tucker, 1988). PRL is involved in mammary development and growth, increases mammary gland phospholipid biosynthesis (Rillema *et al.*, 1985) and stimulates the transcription and translation of the casein gene (Matusik and Rosen, 1980). PRL secretion increases within minutes of the onset of suckling and decreases shortly after cessation of the stimulus. Daily surges of PRL release during the first 10 days of pregnancy in rat are involved in the maintenance of corpus luteal function and ensure uninterrupted progesterone production. The daily surges of PRL terminate on day 10 of pregnancy coinciding with increased secretion of placental lactogen, which takes over the role of PRL in maintaining luteal function (Kelly *et al.*, 1976). Plasma PRL levels remain low during the second half of gestation but increase on the day of parturition (Bridges and Goldman, 1975).

In all mammals the increase in plasma PRL at parturition plays an important role in the development of parental behaviour (review Numan, 1994).

1.3.2 Incubation behaviour in birds.

In birds, as in mammals, increased PRL secretion plays an important role in the expression of parental behaviour. A component of avian parental behaviour is incubation behaviour which is associated with increased PRL secretion (duck: Goldsmith and Williams, 1980; canaries: Goldsmith *et al.*, 1984; domestic hen: Lea *et al.*, 1981, Sharp *et al.*, 1988; turkey: El Halawani *et al.*, 1988, El Halawani and Rozenboim, 1993; doves:

Goldsmith *et al.*, 1981, reviews, Lea, 1987; Goldsmith, 1983). Direct evidence that PRL is required for expression of incubation behaviour comes from studies on bantams, where active immunisation against PRL suppresses the behaviour without affecting egg production (March *et al.*, 1994), while passive immunisation with antibodies to PRL releasing hormone, vasoactive intestinal polypeptide (VIP, Section 1.4.1) causes the incubating bantams to desert their nest (Sharp *et al.*, 1989). Similarly in turkeys, active immunisation against VIP prevents incubation behaviour without affecting egg production (El Halawani *et al.*, 1995a) while intra-cranial infusion of PRL induces full incubation behaviour in laying birds (Youngren *et al.*, 1991). In most birds the concentration of plasma PRL increases before the onset of full incubation behaviour, a notable exception being dove, where the PRL concentration increases after the onset of incubation behaviour (Lea, 1987). In the dove it appears that PRL helps to maintain the incubation behaviour but is not essential for its expression (Horseman and Buntin, 1995).

1.3.3 Somatic growth and sexual maturation.

A role for PRL has not been established during somatic growth or sexual maturation. However, in the chicken changes in concentration of plasma PRL occur during somatic growth suggesting functional significance. In the chicken embryo, plasma PRL increases after 13 days of incubation and is high before hatch (Shimada *et al.*, 1991). The concentration of plasma PRL is high in newly hatched chicks, decreases as they become older but increases at the onset of sexual maturation (Harvey *et al.*, 1979a; Sterling *et al.*, 1984). Photoinduced sexual maturation is associated with increased concentrations of plasma PRL in all birds investigated (Section 1.3.4).

1.3.4 Seasonality.

Photoperiod is the major environmental factor controlling the seasonal pattern of PRL secretion at temperate latitudes in mammals and birds, with increasing daylengths stimulating PRL secretion and shortening days having the opposite effect (Pelletier, 1973; Lincoln *et al.*, 1978; Goldsmith, 1983). Continuous or non-photoperiodic seasonal breeders such as cattle may show marked seasonal changes in PRL concentrations which are controlled by the photoperiod (Schams and Reinhardt, 1974; Peters and Tucker, 1978), although in pigs there is no photoperiodic effect on PRL secretion (Revault *et al.*, 1982; Dickman and Hoagland, 1983).

In birds, photoperiodically induced seasonal changes in plasma PRL have been described in several species including the rook (Lincoln *et al.*, 1980), starling (Ebling *et al.*, 1982, Goldsmith and Nicholls, 1984, Dawson and Goldsmith, 1984), grey partridge (Sharp *et al.*, 1986a) and quail (Boswell *et al.*, 1995). The seasonal increase in plasma PRL is associated with increased plasma LH and the full development of the reproductive organs. In many birds this stimulatory effect of long days is lost after a few weeks and is superseded by an inhibitory effect on the reproductive system (Section 1.5.5). The birds are described as becoming 'absolutely photorefractory' having lost the ability to respond to the stimulatory effects of long days on reproductive function. The development of absolute reproductive photorefractoriness is characterised by regression of the gonads and a decrease in plasma gonadotrophins. The onset of absolute reproductive photorefractoriness is also associated with high concentrations of plasma PRL and decreasing LH. After the development of reproductive absolute photorefractoriness and continued exposure to long days, the concentration of plasma PRL decreases, exhibiting photorefractoriness for PRL secretion (e.g. rooks, Lincoln *et al.*, 1980; starlings, Dawson and Goldsmith, 1983; ducks, Sharp *et al.*, 1986b; turkeys, Lien and Siopes, 1989). The association between the development of absolute

reproductive photorefractoriness and the seasonal peak in concentration of plasma PRL suggests a functional relationship. This view is supported by the work showing in birds, that administration of PRL induces gonadal regression (Opel and Proudman, 1980; Buntin and Tesch, 1985). However, the observation that absolute reproductive photorefractoriness is maintained after the development of photorefractoriness for PRL secretion rules out the possibility that increased PRL secretion causes absolute photorefractoriness. This view is supported by the finding that intra-cerebroventricular injection of PRL into breeding starlings held on an 11 h photoperiod (this prevents the development of photorefractoriness, Section 1.5.5) depresses concentrations of plasma gonadotrophins but does not induce the development of reproductive photorefractoriness (Juss and Goldsmith, 1992).

Some birds, including the quail (Robinson and Follett, 1982) and chicken (Sharp, 1993) show another form of reproductive photorefractoriness termed as 'relative photorefractoriness'. This is the condition in which seasonal gonadal regression is induced by the decreasing daylengths in late summer and early autumn while the daylengths are still longer than those which stimulated breeding in the Spring. Birds such as quail which develop 'relative photorefractoriness' remain indefinitely in breeding condition while maintained in summer-like daylengths, and may show a further increase in gonadotrophin secretion if transferred to longer days (Robinson and Follett, 1982; Sharp, 1993). In quail the development of 'relative photorefractoriness' is not associated with a decrease in concentration of plasma LH or PRL (Juss, 1993). Similarly, in the domestic chicken, the development of relative photorefractoriness is not associated with a decrease in plasma LH (Sharp *et al.*, 1992). There is no information available on concentration of plasma PRL in relatively photorefractory domestic chickens.

1.3.5 Feeding.

The secretion of PRL is influenced by the nutritional status (Hall *et al.*, 1986). For example, concentrations of serum PRL are depressed in acutely and chronically starved rats (Xie, 1991). This depression is mediated through an increase in the secretion of hypothalamic dopamine, since pituitary glands from starved rats show no decrease in PRL secretion *in vitro* (Xie, 1991). Similarly, in female pigs, plasma PRL decreases after feed deprivation and increases after refeeding (Hodate *et al.*, 1983).

In birds, administration of PRL increases food intake and/ or body mass (e.g. ducks: Ensor, 1975; sparrow: Yokoyama, 1976; doves: Buntin and Tesch, 1985; quail: Boswell *et al.*, 1995). For this reason it has been suggested that increased plasma PRL induced by photostimulation is responsible for the increased food intake and fat deposition shown by some birds before spring migration. However, measurements of plasma PRL in migratory birds suggest that PRL concentrations are not closely correlated with hyperphagia and fat deposition (Schwabl *et al.*, 1988; Boswell *et al.*, 1995). Furthermore, studies on turkeys failed to support a role for PRL in stimulating food intake, since intra-cerebroventricular injection of PRL in short-day birds failed to stimulate feeding, while peripheral injection of PRL in long day birds depressed food intake (Denbow, 1986). An interaction between nutritional status and the secretion of PRL has been reported for domestic chicken (Harvey *et al.*, 1978). These authors found that plasma PRL decreased after 24 h food withdrawal in 2 week-old cockerels, however, food withdrawal for 72 h did not depress plasma PRL in 6 week-old cockerels. Similarly, in White Leghorn hens, plasma PRL has been reported to decrease after an 11 day fast and to increase after refeeding (Millam and El Halawani, 1986). It is therefore possible that in the domestic chicken, day-to-day changes in concentration of plasma PRL may be related to time elapsed after a nocturnal fast or subsequent feeding.

1.3.6 Ambient temperature.

The secretion of PRL in mammals is influenced by ambient temperature. For example, in cattle, rats and sheep an increase in ambient temperature is associated with an increase in the concentration of serum PRL (Mueller *et al.*, 1974; Smith *et al.*, 1977; Hooley *et al.*, 1979; Schams *et al.*, 1980). Exposure of rats to a gradually decreasing ambient temperature sufficient to induce mild hypothermia causes a fall in plasma PRL at 35 °C, which is reversed when the animal is rewarmed (Okuda *et al.*, 1986). Changes in PRL secretion in response to change in ambient temperature may also depend on the physiological state. For example, acute exposure of non-lactating rats to an ambient temperature of 4 °C has no effect on plasma PRL, while exposure of lactating rats to this temperature causes a depression in serum PRL (Adels *et al.*, 1986).

Evidence that changes in ambient temperature influence concentrations of plasma PRL in birds is equivocal. Transfer of sexually immature cockerels from 24 °C to 45 °C for 30 min or 60 min does not alter concentration of plasma PRL (Harvey *et al.*, 1977). Drenching of cockerels with ice cold water (4 °C) for 1 h significantly increases the plasma PRL concentration but this effect is possibly related to stress rather than temperature (Harvey *et al.*, 1977). In female turkeys, exposure to 10 °C, 24 °C or 30 °C does not affect the rate at which plasma PRL increases after photostimulation (El Halawani *et al.*, 1984b). However, plasma PRL is higher in photostimulated turkeys held at 30 °C than birds held at 18 °C (El Halawani *et al.*, 1984b). It is therefore uncertain whether day-to-day variations in concentrations of plasma PRL in chickens are related to changes in ambient temperature.

1.4 Regulation of PRL secretion by vasoactive intestinal polypeptide, dopamine and steroids.

PRL secretion is controlled by a complex array of stimulatory and inhibitory neuropeptides and neurotransmitters released from the hypothalamus (Fig. 1.1) and by gonadal steroids. In mammals, PRL secretion is predominantly controlled by inhibitory hypothalamic factors. The first indication of such an inhibition was reported in rats, where anterior pituitary glands grafted away from the hypothalamus were shown to retain their luteotropic actions (Desclin, 1950). Later studies showed that disruption of the normal vascular connections between hypothalamus and the pituitary gland and /or transplantation of pituitary to a site distant from the hypothalamus or culture *in vitro* causes a dramatic increase in PRL secretion (Ben-Jonathan *et al.*, 1989; Lamberts and MacLeod, 1990).

In contrast to mammals, in birds, the hypothalamus exerts a principally stimulatory influence on PRL release (Fig. 1.1). Evidence for this initially came from the finding that the secretory activities of the lactotropes, assessed histologically, were not maintained in pituitary glands transplanted to body sites distant from the influence of the hypothalamus (chickens: Ma and Nalbandov, 1963, pigeons: Bayle, 1969; Bayle and Assenmacher, 1965). This was confirmed in studies showing that *in vitro*, avian anterior pituitary glands do not maintain a large output of PRL (pigeons: Tixier-Vidal and Gourdji, 1972; chickens : Bolton *et al.*, 1974). However, as measured by radioimmunoassay, chicken pituitary glands do secrete small quantities of PRL when incubated *in vitro* (Harvey *et al.*, 1982), suggesting minor degree of autonomous secretion. The first direct evidence for a stimulatory role for the hypothalamus in the control of PRL secretion in birds comes from the demonstration that injection of chicken hypothalamic extracts into chickens increases plasma PRL (Harvey *et al.*, 1979b) and

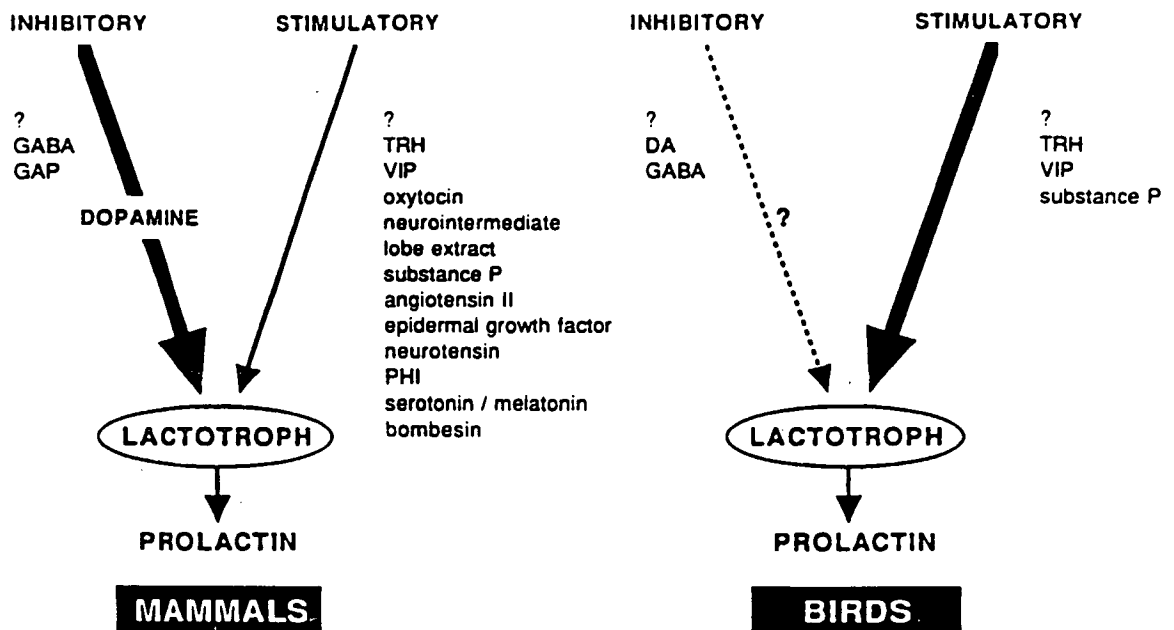


Fig. 1.1 The neuropeptides and or neurotransmitters directly acting on prolactin secreting cells in the anterior pituitary gland of mammals and birds. Note that in mammals the predominant input is inhibitory while in birds it is stimulatory.

GABA (gamma amino butyric acid),
 GAP (gonadotrophin associated peptide),
 TRH (thyrotropin releasing hormone),
 VIP (vasoactive intestinal polypeptide),
 PHI (peptide histidine isoleucine),
 DA (dopamine).

incubation of chicken pituitary glands *in vitro* with chicken hypothalamic extracts results in an increase in immunoreactive PRL in the incubation medium (Hall *et al.*, 1975).

In more recent studies, it has been established that the principal inhibitory and stimulatory factors controlling PRL release in mammals and birds respectively are DA (Section 1.4.2) and VIP (Section 1.4.1). In both birds and mammals gonadal steroids modulate the action of these factors, principally at the level of the anterior pituitary.

1.4.1 Vasoactive intestinal polypeptide (VIP).

VIP is a 28 amino acid neuropeptide originally isolated from the porcine duodenum (Said and Mutt, 1970, 1972) and chicken intestine (Nilsson, 1974). It is found in neurons throughout the nervous system (Said, 1984), including the hypothalamus where it acts as a PRL releasing hormone in both mammals and birds. For example, VIP has been shown *in vivo* to stimulate PRL release in rats (Kato *et al.*, 1978), monkeys (Frawley and Neill, 1981), humans (Conti *et al.*, 1987), ring doves (Lea and Vowles , 1986), chickens (Macnamee *et al.*, 1986) and turkeys (Opel and Proudman, 1988). Vasoactive intestinal polypeptide also stimulates the secretion of PRL from avian pituitary glands *in vitro* demonstrating that it stimulates PRL secretion by acting directly on the anterior pituitary gland rather than by stimulating the release of a PRL releasing factor from the hypothalamus (chicken: Macnamee *et al.*, 1986; Talbot *et al.*, 1991, turkey: Proudman and Opel, 1988; Knapp *et al.*, 1988; El Halawani *et al.*, 1990a; Xu *et al.*, 1996). Further support for this view came from the finding that pre-treatment of cultured pituitary cells with a VIP receptor antagonist significantly depresses the ability of VIP to stimulate PRL secretion (El Halawani *et al.*, 1990b).

Immunocytochemical studies in chickens, turkeys, and doves, demonstrate the presence of VIP- containing neuronal terminals of the hypothalamic median eminence, the site from which peptide is believed to be released to control PRL secretion. These VIP terminals originate from cell bodies located in the medio-dorsal basal hypothalamus

(Mauro *et al.*, 1989; Sharp *et al.*, 1989; Cloues *et al.*, 1990). Further evidence that the basal hypothalamic VIP acts as a PRL releasing factor comes from the studies on incubating chickens and turkeys. A steep increase in plasma PRL at the onset of incubation, correlates with an increase in the amount of hypothalamic VIP (Sharp, *et al.*, 1989; You *et al.*, 1995), VIP mRNA (You *et al.*, 1995) and in the size and number of immunocytochemically localised VIP cell bodies in the basal hypothalamus (Sharp *et al.*, 1989; Mauro *et al.*, 1989; Cloues *et al.*, 1990). Passive immunisation of incubating bantams with VIP-antibodies suppresses plasma PRL and induces nest desertion (Sharp *et al.*, 1989). In turkeys, active immunisation against VIP prevents the development of incubation behaviour and the associated increase in plasma PRL (El Halawani *et al.*, 1995a). Studies on turkeys show that the stimulatory action of VIP on PRL release is mediated by specific VIP receptors in the anterior pituitary gland, the abundance of which increases when the concentration of pituitary PRL is at its highest, in incubating hens (Rozenboim and El Halawani, 1993). Further evidence that VIP is released to stimulate PRL release was obtained in turkey poults hatched from eggs laid by hens actively immunised against VIP. The high VIP antibody titre originating from the maternal egg yolk in these young birds blocked PRL release in response to the administration of VIP or electrical stimulation of the basal hypothalamus (Rozenboim *et al.*, 1996).

The release of VIP from the median eminence is controlled, at least in part, by serotonergic (5-hydroxytryptamine) inputs from unidentified higher neural centres. Systemic administration of serotonin precursors, agonists or uptake blockers stimulate PRL secretion in chickens and turkeys (Rabii, *et al.*, 1984; Hall *et al.*, 1984c; Hargis and Burke, 1984; Macnamee and Sharp, 1989; El Halawani *et al.*, 1995b). Since the anterior pituitary gland does not contain serotonin receptors (Macnamee and Sharp, 1989), the stimulatory action of serotonin on PRL release must be mediated through the release of VIP. This conclusion has been confirmed in turkeys in which active immunisation

against VIP blocks the ability of the serotonin agonist, quipazine, injected intraperitoneally or intra-cerebroventricularly, to stimulate PRL secretion (El Halawani *et al.*, 1995b)

1.4.2 Dopamine (DA).

The secretion of PRL in mammals, but not in birds is predominantly under the inhibitory control of dopamine. However, in birds there is growing evidence for a role for DA in the regulation of PRL secretion. In mammals, PRL secretion is regulated by the tuberoinfundibular dopamine (TIDA) neuronal system, which is located in the arcuate and periventricular nuclei in the hypothalamus, with terminals in the median eminence. DA released from the terminals of the TIDA system into the hypophysial portal blood inhibits the secretion of PRL by the activation of specific DA receptors on the lactotropes of the pituitary gland (Ben-Jonathan *et al.*, 1989, Lamberts and MacLeod, 1990, Neill and Nagy, 1994).

There is no avian equivalent of mammalian TIDA system, although the avian median eminence contains dopaminergic terminals from an unknown source (Sharp and Follett, 1968; Guglielmone and Panzica, 1984; Bailhache and Balthazart, 1993). However, there is evidence in birds that DA plays a minor role in the regulation of PRL release in birds acting at the level of both anterior pituitary gland and hypothalamus (review El Halawani *et al.*, 1988). DA or the DA agonist, apomorphine, inhibits the stimulatory effect of hypothalamic extracts, on PRL secretion by cultured chicken pituitary cells. This effect is partially reversed by the DA receptor antagonists, pimozide (Hall and Chadwick, 1984). Similarly, the DA D₂ receptor agonist (quinpirole), inhibits the VIP-induced PRL secretion from the cultured turkey anterior pituitary cells and the production of PRL mRNA in a dose related fashion, without affecting basal PRL release or basal production of PRL mRNA (Xu *et al.*, 1996).

At the level of the hypothalamus DA appears to exert stimulatory or inhibitory effects on PRL secretion, depending on dose and physiological state. *In vivo*, in non-laying, but not in incubating turkey hens, intra-cerebroventricular administration of DA increases plasma PRL (Hargis and Burke, 1986). Similarly, in pigeons, the intra-cerebroventricular administration of DA agonist stimulates crop sac development suggesting a PRL releasing effect for DA (Nistico *et al.*, 1979). In contrast, the increase in PRL secretion seen after electrical stimulation of the hypothalamus in laying turkeys is inhibited by systemic administration of a DA agonist (El Halawani *et al.*, 1991). Intra-cerebroventricular DA injection studies in turkey suggests that the stimulatory and inhibitory effects of DA on PRL secretion depends upon dose administered. Thus infusion of a high dose (500 nmol/min) inhibits PRL secretion while infusion of a low dose (10 nmol/min) stimulates PRL secretion (Youngren *et al.*, 1995). The stimulatory and inhibitory actions of DA on PRL secretion are mediated by multiple DA receptors (Youngren *et al.*, 1996). Blockage of D₁ DA receptors by antagonists and or immunisation against chicken VIP blocks the stimulatory effect of intra-cerebroventricular infusion of a low dose DA on PRL secretion (Youngren *et al.*, 1996). A stimulatory effect of DA at the hypothalamic level is also suggested by the observation that perfusion of turkey hypothalami *in vitro* with DA stimulates VIP release (Chaiseha *et al.*, 1995).

In summary, studies *in vitro* and *in vivo* suggest that DA may act at the level of the anterior pituitary to inhibit the action of hypothalamic releasing factors and at the level of the hypothalamus to stimulate PRL release acting on D₁ DA receptors to modulate the release of VIP.

1.4.3 Gonadal steroids.

Gonadal steroids have direct effect on the secretion of PRL from the anterior pituitary gland.

1.4.3.1 Oestrogen.

Oestrogen plays an important role in the regulation of PRL synthesis and secretion depending upon the dose administered and the duration of exposure. In ovariectomised turkeys, systemic administration of oestradiol benzoate for several days stimulates PRL secretion (El Halawani *et al.*, 1983; Saeed and El Halawani, 1986). Oestrogens may stimulate PRL secretion by acting directly on lactotropes or on the hypothalamus.

1.4.3.1.1 Direct effects on lactotropes. In the rat, oestrogen regulates PRL synthesis, storage and secretion acting through specific binding sites in the anterior pituitary gland (Nicoll and Meites, 1962, Vician *et al.*, 1979, Lieberman *et al.*, 1982) and exerts a direct mitotic effect on lactotropes (Amara *et al.*, 1987). The addition of oestradiol to cultures of anterior pituitary cells increases the amount of PRL mRNA (Lieberman *et al.*, 1978), increases the transcription of the PRL gene (Maurer, 1982), and stimulates *de novo* synthesis of PRL (Wiklund *et al.*, 1981; Lamberts and MacLeod, 1990). In chickens, incubation of anterior pituitary from laying hens with oestradiol stimulates PRL secretion (Hall and Chadwick, 1978, Hall *et al.*, 1984a) and depletes pituitary stores of PRL (Kono *et al.*, 1980).

In mammals and birds, oestrogen modifies the anterior pituitary responsiveness to PRL regulating factors. In rats, oestradiol administration *in vivo* or *in vitro* impairs the responsiveness of the lactotropes to dopamine by decreasing the number of dopamine receptors, while it increases in the response of PRL to TRH by increasing the number of

TRH receptors (Lamberts and MacLeod, 1990). In birds too, there is evidence that oestrogen acts on the anterior pituitary gland to modulate responsiveness to hypothalamic factors regulating PRL secretion. Incubation of chicken anterior pituitary glands with 17- β oestradiol increases responsiveness to the PRL releasing activity of VIP (Macnamee *et al.*, 1986) and increases sensitivity to inhibitory effects of DA on PRL release (Hall *et al.*, 1984a). Studies on oestrogen treated ovariectomised turkeys show a potentiating role for oestrogen on thyrotropin releasing hormone (TRH) induced PRL release (Saeed and El Halawani *et al.*, 1986). Although TRH stimulates PRL release from cultured rat anterior pituitary cells (Keith *et al.*, 1986), it does not stimulate PRL release from cultured turkey anterior pituitary cells (Fehrer *et al.*, 1985). It therefore appears that the stimulatory effects of TRH on PRL release in the turkey, and probably in other birds, may be mediated through the hypothalamus.

1.4.3.1.2 Direct effects on the hypothalamus. Oestrogens modulate the release of hypothalamic PRL inhibiting and releasing factors in the rat (Demarest *et al.*, 1984, Toney and Katzenellenbogen, 1986). Short-term exposure (3-5 days) to oestrogen increases the turn over and synthesis of dopamine in the tuberoinfundibular system as well as the dopamine concentration in the hypophysial stalk plasma. After long-term oestrogen exposure, dopamine release into the hypothalamic portal circulation is reduced (Cramer *et al.*, 1979, Arita and Kimura, 1987). It is not known in birds, whether oestrogen exerts a direct effect on the hypothalamus to modulate PRL release.

1.4.3.2 Progesterone and testosterone.

PRL synthesis and release from primary cultures of rat, ovine, and monkey anterior pituitary cells is not directly influenced by progesterone (Lamberts and MacLeod, 1990). However, progesterone inhibits oestrogen-induced PRL synthesis in

cultured rat pituitary cells (Chen and Meites, 1970). In contrast, in turkey, high concentrations of progesterone enhance the basal release of PRL from cultured anterior pituitary cells (Knapp *et al.*, 1988). Acute exposure (24h) of cultured turkey pituitary cells to progesterone reduces VIP- induced PRL release, while chronic exposure (96h) to progesterone enhances VIP induced PRL release (Knapp *et al.*, 1988). In broad agreement with the these findings *in vitro*, injections of high doses of progesterone, in ovariectomised mature female turkeys increases plasma PRL (El Halawani *et al.*, 1983) and enhances the magnitude of the PRL response to TRH (Saeed and El Halawani, 1986).

Testosterone, in pharmacological doses, is also reported to modulate PRL synthesis at the level of the anterior pituitary gland in both mammals (MacLeod *et al.*, 1969) and birds (Hall and Chadwick, 1978; Kono *et al.*, 1980). Administration of testosterone *in vivo* depresses pituitary PRL content in castrated immature cockerels, and decreases circulating PRL concentrations in intact immature cockerels (Kono *et al.*, 1980). However, incubation of chicken and or turkey pituitaries with testosterone may inhibit or stimulate the basal PRL secretion (Hall and Chadwick, 1978; Hall *et al.*, 1984b; Knapp *et al.*, 1988). Testosterone may also modulate the action of hypothalamic factors controlling PRL release. Thus, preincubation of chicken pituitaries with testosterone reduces the ability of hypothlamic extract to stimulate PRL release (Hall *et al.*, 1984b). Similarly, in the turkey, VIP-induced PRL release from the anterior pituitary cells *in vitro* is reduced after preincubation with testosterone (Knapp *et al.*, 1988). It therefore seems that in birds, progesterone and testosterone may modulate PRL release at the level of anterior pituitary gland in either as an inhibitory or stimulatory manner. There is no information on possible effects of these steroids acting at the hypothalamic level to control PRL secretion.

1.5 Photoperiodic mechanisms transducing PRL release.

Information on mechanisms controlling photoinduced PRL release in birds is limited but it is possible that they share much in common with mechanisms controlling photoinduced gonadotrophin release (Follett *et al.*, 1985; Follett and Pearce-Kelly, 1991; reviews, Follett, 1984; Sharp, 1983, 1984, 1992, 1993, 1996). The major difference is that the final targets for neural pathways transducing photoperiodic information are the GnRH-I neurons for gonadotrophin release (Perera and Follett, 1992) and neurons producing PRL releasing/ inhibiting factors for PRL release. A brief account is given of the mechanism controlling photoinduced gonadotrophin secretion on the assumption that it is likely to be similar for photoinduced PRL secretion.

1.5.1 Photoreception.

In birds, unlike mammals, the presence of light used for photoperiodic signalling is detected by extra-retinal photoreceptors rather than by the eyes (Wilson, 1991; review, Kuenzel, 1993). For example, in the house sparrow photoinduced gonadal growth is about 40 times less in experimental birds whose skulls were covered with Indian ink, to reduce the penetration of light into the brain, than in control birds not injected with Indian ink (Menaker *et al.*, 1970). Since the eyes were present in both control and experimental groups the differences in gonadal growth was due to the amount of light passing directly through the skull to reach non-retinal photoreceptors. Extra-retinal photoreceptors has also been demonstrated in the turkey in which, bilateral ocular enucleation and/ or pinealectomy has no effect on the increase in plasma LH concentration observed after photostimulation (Siopes and El Halawani, 1986). Further evidence for extra-retinal photoreception has been reported in the American tree sparrow by Wilson (1991), who found that bilateral ocular enucleation together with pinealectomy does not affect the ability of the reproductive system to respond to

photoperiodic stimuli. In the same study it was shown that an epicranially-implanted dim green light source stimulated the reproductive activity, suggesting the presence of deep- brain photoreceptors.

1.5.2 The biological clock and the pineal gland.

The duration of the photoperiod is measured by a biological clock which, in mammals, is located in the suprachiasmatic nuclei (SCN) in the hypothalamus (Moore, 1983; Turek, 1985; Rusak, 1989). Information about the presence or absence of light passes to the SCN via the retinohypothalamic tract (Moore, 1973). In birds, there is also evidence for a retinohypothalamic projection to areas in the hypothalamus which may be homologous with the mammalian SCN (Cassone and Moore, 1987; Norgren and Silver, 1989). However, there is no structure in the avian hypothalamus that has the well- differentiated features of the mammalian SCN (Kuenzel and van Tienhoven, 1982). Furthermore, there is no evidence that an avian homologue of the mammalian SCN plays a role in the transduction of photoperiodic information (King, 1995). In mammals, photoperiodic information is conveyed from the SCN through the superior cervical ganglion to the pineal gland (Evered and Clark, 1985). During the dark period (night), the SCN sends a neuronal signal to the pineal gland to increase the secretion of melatonin into the blood. During the light period this SCN signal is suppressed resulting in a decrease in pineal melatonin synthesis or release. The duration of the increase in the concentration of the plasma melatonin at night provides mammals with the photoperiodic signal which is used to control seasonal breeding (Bitmann and Karsch, 1984). The importance of the pineal gland in the photoperiodic control of reproduction in mammals is illustrated by studies in hamsters (Matt and Stetson, 1980) and sheep (Bittman *et al.*, 1985) showing that pinealectomy abolishes photoinduced responses in reproductive function.

The pineal gland in birds also generates a nocturnal rhythm in the concentration of plasma melatonin (Liou *et al.*, 1987; Kumar and Follett, 1993) although the circadian pacemaker involved is located within the pineal gland rather than in the hypothalamus (Kumar and Follett, 1993). Although diurnal changes in the concentration of blood melatonin in birds, provide a calendar of photoperiodic information, they are not used to control the timing of seasonal reproduction. For example, in the American tree sparrow, pinealectomy and/ or removal of the eyes (an additional source of melatonin) has no effect on photoinduced gonadal growth or the subsequent development of photorefractoriness (Wilson, 1991).

Observations on birds showing that the pineal gland does not play a role in photoinduced reproductive function do not exclude the possibility that the avian pineal gland might be involved in the photoperiodic control of PRL secretion. One study has investigated this possibility and showed that in the turkeys, pinealectomy does not prevent, but reduces a photoinduced increase in plasma PRL (Siopes and El Halawani, 1989).

1.5.3 Neuroendocrine output.

The final photoinduced output controlling LH release, as demonstrated in the Japanese quail is GnRH (Perera and Follett, 1992). In the quail, the photoinduced increase in GnRH secretion begins 22-23 h after dawn, resulting in an associated increase in plasma LH. This increase in plasma LH continues for about 10 days as a “carry over effect” if the quail are returned to short days after one long day (Follett *et al.*, 1977; Nicholls *et al.*, 1983; Follett and Pearce-Kelly, 1991; Perera and Follett, 1992; Meddle and Follett, 1995). The continued secretion of LH on short days is due to the maintenance of increased GnRH release from the hypothalamus (Perera and Follett, 1992). The final photoinduced output from the hypothalamus controlling PRL release has not been identified, but the most likely candidate is VIP (Section 1.4.1). There is no

information on the possibility of a 'first day' release or 'carry over effect' for photoinduced PRL secretion in birds.

1.5.4 Photoperiodic time measurement.

The measurement of photoperiodic time might be achieved using a 'hour glass' or a circadian rhythm. The hour glass hypothesis proposes that transfer from darkness to light initiates the synthesis of a biological product, the concentration of which gradually increases until it triggers the photoperiodic response. Evidence in support of this hypothesis is limited (Saunders, 1977) and most organisms use a circadian rhythm or rhythms to measure photoperiodic time (Saunders, 1977; Follett and Follett, 1981; Sharp, 1983). The circadian clock hypothesis was first proposed by Bunning (1936) who envisaged a circadian rhythm of photoinducibility in organisms exposed to a 12L:12D day with a 'photo-insensitive' period during the daytime, and a 'photo-sensitive' period during the night. The photoperiodic response is induced when daylength increases so that light is present during the photosensitive phase of the rhythm of photoinducibility. This 'external coincidence' model of photoperiodic time measurement has been modified to develop a 'internal coincidence' model of photoperiodic time measurements, in which the presence of light during a photosensitive phase is not required for photoinduction (Fig. 1.2). The 'external coincidence' model for photoinduced reproduction is widely accepted by scientists working on avian reproduction. In part, this is because of the outcome of a classical experiment carried out by Follett and colleagues (1975) in the White-crowned sparrow, in a critical test of the external coincidence model of photoinduced gonadotrophin secretion (Fig. 1.3). The birds were transferred from short days into darkness and at various times thereafter given a single eight-hour photoperiod. Plasma LH was measured before and after the treatment, and inductiveness assessed by determining whether the secretion of the hormone had increased. The results clearly indicated that the photoinduction of LH release only

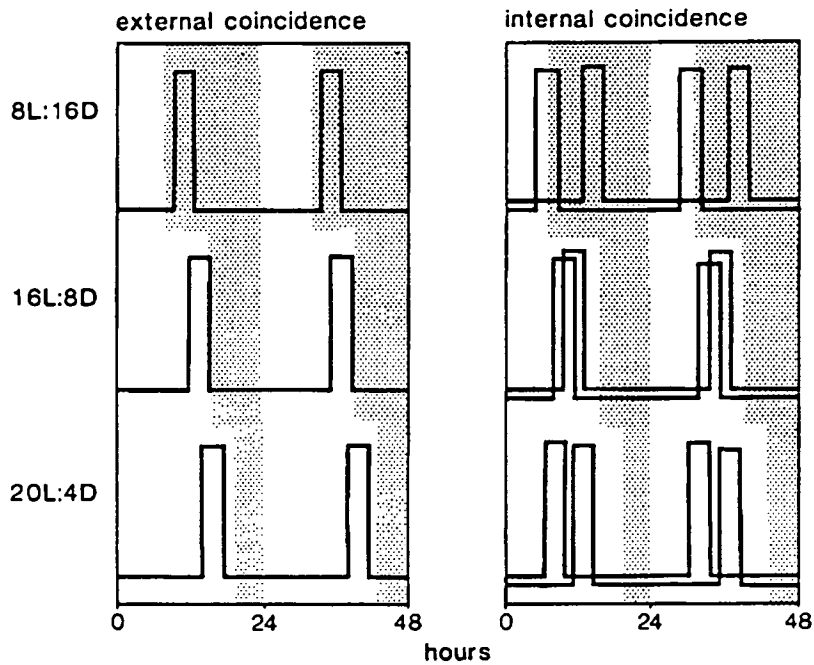


Fig. 1.2 Diagram to illustrate the way circadian rhythms measure the daylength. In the external coincidence model a single circadian rhythm of photoinducibility is shown with a square wave form. The crest of the wave is the photoinducible phase: when this phase is coincident with light, the daylength is judged as long. In the internal coincidence model two circadian rhythms, again shown in a square wave form, are differentially entrained by a given light-dark cycle. As the light:dark ratio changes, the relationship between the two rhythms also changes. When the daylengths are stimulatory, the two rhythms are entrained into an inductive relationship which in this model is assumed to occur when the crests of the two rhythms coincide. (From Sharp, 1984).

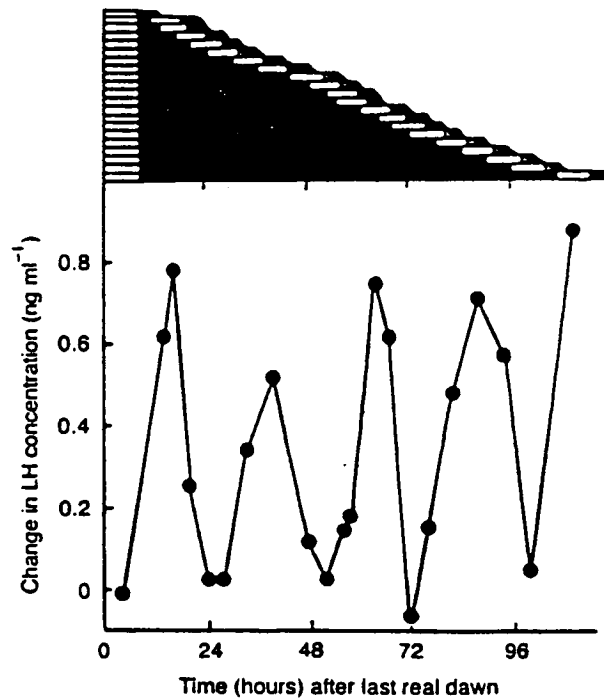


Fig. 1.3 A 'resonance' experiment to show that birds use their circadian system to measure daylength. White-crowned sparrows were kept on an 8 hr day and a pre-experimental blood sample was taken in the last light period before transfer to continuous darkness. At various times thereafter each bird was exposed to a single 8 hr period of light. These lighting treatments are shown in the upper part of the figure. A second blood sample was taken a few hours after the experimental light pulse. The graph shows the change in plasma LH concentration between the two samples in relation to each treatment. The data shows a daily periodicity in photosensitivity to the 8 hr pulse light. It is not the amount of light which is important in triggering gonadotrophin release but when it falls relative to the underlying circadian rhythmicity within the the bird. (From Follett et al., 1975)

occurred if the light period coincided with the phase of rhythm with a period close to, but not equal to 24 h , indicating the presence of an underlying rhythm of photoinducibility.

1.5.5 The avian photoperiodic gonadotrophin and PRL responses.

The seasonal changes in gonadotrophin and prolactin secretion and the associated development of photorefractoriness (Section 1.3.4) implies that they are dependent on photoperiodic history. A model describing how photoperiodic history determines the photoinduced secretion of gonadotrophins in birds is shown in Fig. 1.4. When photoperiodic birds are exposed to winter-like (short) daylengths for a long period, these daylengths are photoperiodically neutral and do not inhibit gonadotrophin releasing hormone (GnRH-I) neurons. The activity of GnRH-I neurons in these circumstances depend on the genotype allowing some avian species, including the domestic chicken (Sharp, 1993, 1996), to come into breeding condition very early in the year, when daylengths are short and non-stimulatory. There is no information on PRL secretion in photoperiodic birds kept for prolonged periods on short days.

As the photoperiod increases in the Spring a 'critical' photoperiod is reached, resulting in the stimulation of gonadotrophin or PRL secretion. The critical daylength for gonadotrophin release ranges between 10-11 h in chickens (Dunn and Sharp, 1990), 11-12 h in quail (Urbanski and Follett, 1982) and 11-13 in starlings (Dawson, 1987). Information on the critical daylength for PRL release is limited to the starlings, where it is reported to be 13-14.5 h (Ebling *et al.*, 1982; Dawson and Goldsmith, 1983).

As the photoperiod increases further there is a direct relationship between gonadotrophin release and photoperiod until a saturation daylength is reached which stimulates the maximum release of gonadotrophins. Photoperiods between the critical and saturation photoperiods are described as being 'marginally photostimulatory' (Sharp, 1984). In the quail, the saturation photoperiod for LH release is about 14 h

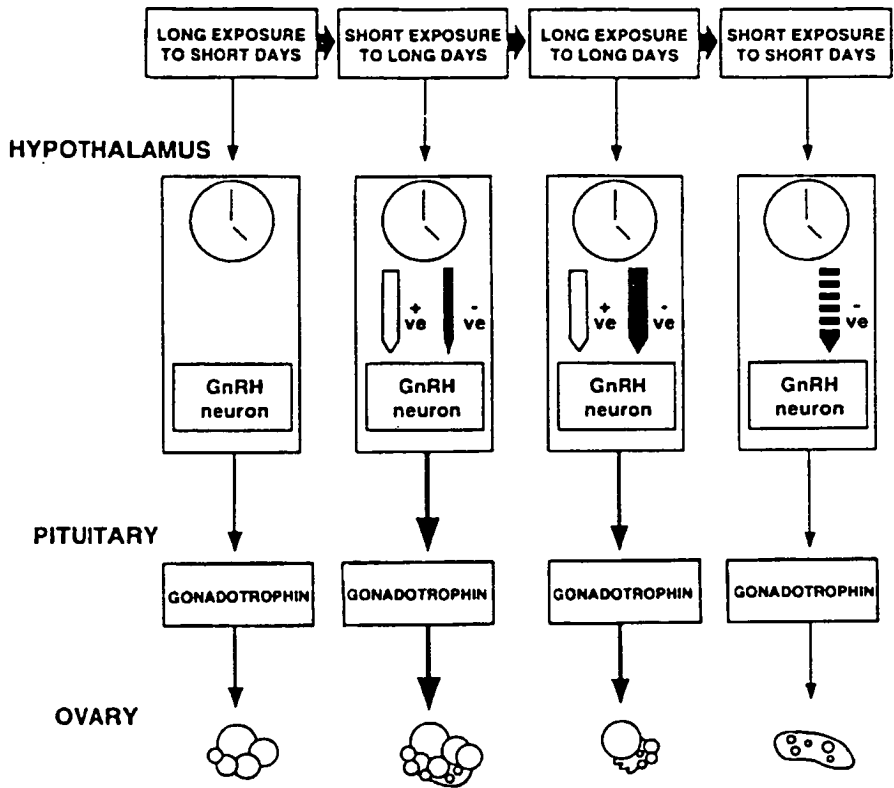


Fig. 1.4 A schematic representation of the photoperiodic response of birds. The hypothalamus contains a biological clock, depicted as the face of an analogue clock, which measures the passage of the photoperiodic time. If the photoperiod is increased, two types of input to the gonadotrophin hormone (GnRH) neurons are activated, one stimulatory (+ ve, white arrow) and the other inhibitory (-ve, black arrow). The stimulatory input is fully activated immediately after photostimulation but the inhibitory input develops more slowly. If the photoperiod is decreased, the stimulatory input disappears immediately leaving the inhibitory input, which dissipates gradually after several weeks or months of exposure to short days (indicated by broken arrows). Photoperiodically induced changes in the inputs to GnRH neurons result in the secretion of GnRH and the gonadotrophins, indicated by the thickness of the solid lines with arrows. Changes in gonadotrophin secretion control the development of the ovary (shown here) or the testes. (From Sharp, 1993)

(Urbanski and Follett, 1982), while in the chicken it is about 12 h (Dunn and Sharp, 1990).

The increase in photoperiod above the critical photoperiod in Spring induces a stimulatory input to GnRH-I neurons, and presumably, to neurons containing the neuropeptides/ neurotransmitters which stimulate PRL release. A further increase in photoperiod results in the development of an additional, inhibitory, input to these two neuronal systems resulting in the development of photorefractoriness. In birds, such as quail and chicken which become relatively photorefractory for photoinduced LH release (Robinson and Follett, 1982; Sharp, 1993), this inhibitory input is revealed by reducing the photoperiod to a duration which would otherwise be photostimulatory in Spring. Thus, in relative photorefractory birds, gonadotrophin secretion decreases and the gonads regress when exposed to decreasing but long daylengths in late Summer or early Autumn (Robinson and Follett, 1982).

In the only species in which relative refractoriness has been studied, the quail, there is no evidence for the development of relative photorefractoriness for photoinduced PRL. Thus transfer of relatively photorefractory quail from 20 h to marginally stimulatory daylength of 13 h , does not result in a decrease in the concentration of plasma PRL (Juss, 1993).

A second form of photorefractoriness, absolute photorefractoriness, is induced in many temperate zone birds as a result of the development of long day-induced inhibitory inputs to the GnRH-I/ PRL stimulatory factor neurons, resulting in the depression of plasma gonadotrophin and PRL secretion (Section 1.3.4). This is the mechanism which allows birds to stop breeding in late Summer when daylengths are still long (Section 1.3.4). In birds which become absolutely refractory, the long-day induced inhibitory inputs to GnRH-I/ PRL stimulatory factor neurons completely override the long-day stimulatory inputs. As a result, in contrast with relatively photorefractory birds, a further increase in photoperiod will not stimulate gonadotrophin or PRL secretion in

birds showing absolute photorefractoriness for these hormones. The daylengths required to induce the development of photorefractoriness for both LH and PRL release are longer than those required to induce LH or PRL secretion. Thus, transfer of birds to marginally stimulatory daylengths does not permit the development of reproductive photorefractoriness. This has been observed in quail, chickens, starlings and Willow ptarmigans exposed to marginally stimulatory daylengths of 13, 11, 11 and 14 respectively (Robinson and Follett, 1982; Sharp *et al.*, 1992; Dawson and Goldsmith, 1983; Stokkan *et al.*, 1992). It is unknown whether the development of absolute photorefractoriness for PRL secretion is inhibited in birds exposed to marginally stimulatory daylengths.

When daylengths decrease below the critical photoperiod in Autumn, the stimulatory input to GnRH-I neurons is withdrawn immediately (Fig. 1.4). In contrast, the long-day induced inhibitory input dissipates slowly. The dissipation of absolute or relative photorefractoriness for gonadotrophin release requires 21 days in quail (Follett and Pearce-Kelly, 1990) and 28 days in starlings (Dawson, 1991). There is no information available for the time required to dissipate refractoriness for PRL release.

Most birds do not breed in the year in which they are hatched because the chances of their offspring surviving is low. Birds that hatch in the early Summer therefore require a mechanism to prevent them from coming into breeding condition. In order to prevent premature breeding, the neuroendocrine system matures to become refractory to the stimulatory effects of long days. Photosensitivity is thus first acquired after exposure to the short days of winter. The age at which the neuroendocrine system first becomes responsive to changes in daylength differs between species but generally occurs during the early stages of post-hatch somatic growth (Harvey *et al.*, 1979a; Dunn *et al.*, 1990; McNaughton *et al.*, 1992). There is no information on the ontogeny of development of the photorefractoriness for PRL release in juvenile birds.

1.6 Research Objectives.

- To determine the effect of castration, fasting and changes in ambient temperature on PRL secretion.
- To determine the influence of sex and age in the ontogeny of the PRL and LH photoperiodic responses.
- To establish the critical, marginal and saturation photoperiods for PRL secretion and to compare them with the critical, marginal and saturation photoperiods for LH secretion.
- To determine the influence of photoperiodic history on the critical photoperiod for plasma PRL secretion.
- To establish whether there is a photorefractory component for photoinduced PRL secretion.
- To establish whether exposure to a single long day will increase PRL secretion and whether this is associated with a “carry over effect”.
- To establish the patterns of PRL and LH secretion in intact male and female chickens during a photoinduced breeding cycle and compare them with the turkey, a species known to demonstrate absolute photorefractoriness for reproduction and PRL secretion.
- To establish whether photoinduced PRL release is mediated by the avian PRL releasing hormone, vasoactive intestinal polypeptide (VIP).
- To assess the function of photoinduced PRL secretion and make a comparison with the turkey.

MATERIALS AND METHODS

The full addresses of the suppliers of reagents and equipment are given in Annex 1.

2.1 Animals and husbandry conditions.

The birds used in these experiments were bantams and Spotted Nebraska and Slate turkeys, hatched from eggs from the Roslin Institute flocks or ISA Brown chicks purchased at day old (ISA Poultry Service). They were all reared in floor pens or in individual cages. The birds had free access to feed unless otherwise mentioned. Water was freely available through drinking nipples. All day-old bantam chicks were vaccinated against Marek's disease and received a booster after 3 weeks. At 4 weeks, all birds were vaccinated for Newcastle and Gumboro disease and boosted at 16 weeks of age. All the birds used in these experiments were kept in an ambient temperature of 20 ± 3 °C. The light intensity in the middle of the rooms was 250-350 lux, measured using a Megatron DL 3 light meter.

2.2 Surgical castration.

The male bantam and ISA Brown chicks (2-4 weeks old) were anaesthetised using "Equithesin" (Gandal, 1969), containing (per 100 ml) 4.2 g magnesium sulphate (7 H₂O), 0.96 g pentobarbitone sodium, 4.3 g chloral hydrate, 13.6 ml ethanol (70 %), 35 ml propylene glycol and 51.4 ml de-ionised H₂O. All chemicals were obtained from SIGMA. The anaesthetic was injected intramuscularly in a breast muscle at a dose of 2.5 ml/ kg body weight. Once unconscious, each bird was laid on its right side on an acrylic board, with its legs and wings secured to the board. The exposed left flank was then

swabbed with 70% alcohol, making the rib cage visible through the skin. An incision approximately one cm long was made through the skin with a scalpel blade, between the last two caudal ribs. A pair of dissection scissors (blunt ended) were then gently pushed through the underlying muscle and the connective tissues were parted to expose the body cavity beneath the ribs. The incision was held apart with a pair of toothed-screw lock forceps, and the intestines were held back with a small spatula. Air sacs present in the body cavity were burst in order to expose the testis. Using a fine pair of curved forceps the left testis was carefully removed without causing damage to the major blood vessels nearby. After removal of the testis, the toothed-screw lock forceps were removed and the last two caudal ribs were sutured together using sterile plain catgut (3/0, Ethicon Ltd). The muscles and the skin were then sutured using sterile plain catgut (3/0) and the wound was dusted with Aureomycin antibiotic powder (Cynamid). The bird was then laid on its left side and the same surgical procedures were repeated to remove the right testis. Once the bird recovered from the anaesthesia it was returned to its home cage. Less than 1 % of the birds died as a consequence of the surgical procedure. Castration took approximately 10 min, and the success of the surgical procedure was 90%, as assessed by the lack of growth of the comb and wattles. The growth of these structures is dependent on the presence of testicular tissue.

2.3 Collection of plasma samples.

Blood samples (1 ml) were withdrawn into heparinised syringes from the brachial wing vein and the plasma fractions were obtained by centrifugation at 250 x g, 4⁰C for 15 min. They were stored at -20 ⁰C until required for assay.

2.4 Hormone measurements.

All hormone radioimmunoassays were carried out using a radioimmunoassay diluent containing (per 2000 ml)

160 ml 0.5 M phosphate buffer, pH 7.5

17.5 g NaCl

5.84 g EDTA (disodium salt)

2 g sodium azide (BDH)

40 ml horse serum (GIBCO)

The final volume was made to 2 litres with deionized water and the pH was adjusted with 1 M NaOH to pH 7.5.

2.4.1 Radioimmunoassay of chicken LH.

The chicken radioimmunoassay procedure was a modification of the double antibody method described by Sharp *et al.* (1987). The preparation of the native chicken LH (code RI-LH-1) used for standards and radioiodination was described by Sharp *et al.* (1987). The sensitivity of the assay as measured by the ED₈₀ was 0.10 ng/ ml. The inter- and intra-assay coefficients of variation were 6 and 8% respectively.

2.4.1.1 Preparation of radiolabelled chicken LH.

Chicken LH was radiolabelled using chloramine T (Sharp *et al.*, 1987). All reagents were dissolved in 50 mM sodium phosphate buffer, pH 7.5, unless otherwise specified. A small stirring bar, made from a short piece of paper clip, was added to the reaction vessel (1.5 ml microcentrifuge tube) containing 4.8 µg of native chicken LH in 25 µl of 50 mM phosphate buffer. The iodination procedure was carried out with constant mixing using a magnetic stirrer. Thirty seven Mbq ¹²⁵I[NaI] (IMS-30, Amersham International plc) in 10 µl was added to the reaction tube and the iodination reaction was

started with the addition of 10 µl of freshly prepared 3.55 mM chloramine T sodium salt (Fisons). The reaction proceeded at room temperature (17-19°C) for 45 sec and was terminated with 5.26 mM sodium metabisulphite (100 µl, Fisons) and 0.6 M potassium iodide (100 µl, Fisons). The reaction mixture was transferred to a PD-10 Sephadex G-25 column (LKB-Pharmacia) pre-equilibrated with 50 mM phosphate buffer (pH 7.5) containing 0.2% (w/v) gelatine (column buffer). The reaction tube was rinsed with 200 µl column buffer and added to the column. Column buffer was continuously applied until 25 fractions of 10 drops each had been eluted into LP4 plastic tubes (Denley-Luckham Ltd). Radiolabelled RI-LH-1 was located, using a gamma counter, in fractions 4 -7 with the free iodine being eluted in fractions 13- 18. The radiolabelled fractions were pooled and diluted in radioimmunoassay diluent at approximately 500,000 counts/minute/ 10 µl and stored for up to 4 weeks at 4°C.

2.4.1.2 Preparation of standards.

Lyophilised native chicken LH stored at -20°C in aliquots of 192 µg per tube, was reconstituted with 50 mM phosphate buffer (1 ml) to produce the stock standard (192 µg/ml). This was further diluted with 50 mM phosphate buffer (pH 7.5) to produce the working standard of 5 µg/ml which was used to prepare sets of 14 standards for the assay. These were designated as Std. 1 (5.0 ng/ml), Std. 2 (4.0 ng/ml), Std. 3 (2.5 ng/ml), Std. 4 (2.0 ng/ml), Std. 5 (1.0 ng/ml), Std. 6 (0.5 ng/ml), Std.7 (0.25 ng/ml), Std. 8 (0.125 ng/ml), Std. 9 (0.0625 ng/ml), Std. 10 (0.0313 ng/ml), Std. 11 (0.0156 ng/ml), Std. 12 (0.0078 ng/ml), Std. 13 (0.0039 ng/ml), Std. 14 (0.0019 ng/ml).

2.4.1.3 Radioimmunoassay procedure.

The radioimmunoassay procedure is summarised in Table 2.1. Plasma samples and standards (200 µl each) were dispensed into plastic LP2 tubes (Denley-Luckham)

with an Hamilton MicroLab-M automated dispenser (Howe and Co Ltd). They were mixed with anti-chicken LH primary antibody (50µl, code: LH 3/3, diluted to 1:19000 in radioimmunoassay diluent) and incubated overnight at 4⁰C. The radiolabelled [¹²⁵I] LH was added (50 µl) to all the tubes to give an approximate count of 12000 cpm and all the tubes were incubated overnight at 4⁰C. On the third day, donkey anti-rabbit serum (50 µl, DARS, 1: 20 dilution, Scottish Antibody Production Unit) and normal rabbit serum (50 µl, NRS, 1: 200 dilution, Scottish Antibody Production Unit) were added, mixed and further incubated overnight at 4⁰C. On the fourth day of the assay all the tubes excepting the total count tubes were centrifuged (Sorvall RC-3B, Dupont (UK) Ltd) at 2000 x g for 30 min at 4⁰C. Starch solution (6%, 50 µl) was added to each tube, to prevent disturbance of the precipitate during aspiration and was further centrifuged for 20 min. The supernatant fraction was then aspirated and the pellets and total count tubes were counted for 60 sec on a gamma counter (1277 Gamma Master, LKB- Pharmacia) and the data analysed using the AzzayZapTM programme (AzzayZap Universal Assay calculator; BIOSOFT).

Table 2.1 The protocol for the radioimmunoassay of chicken LH

Solution	TC	NSB	TB	STD	SAM
(Day 1)					
DIL	NIL	250 µl	200 µl	NIL	NIL
SAMPLE	NIL	NIL	NIL	NIL	200 µl
STANDARD	NIL	NIL	NIL	200 µl	NIL
LH AB	NIL	NIL	50 µl	50 µl	50 µl

(Day 2)

PRL LABEL	50 µl	50 µl	50 µl	50 µl	50 µl
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(Day 3)

DARS	NIL	50 µl	50 µl	50 µl	50 µl
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NRS	NIL	50 µl	50 µl	50 µl	50 µl
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(Day 4)

CENTRIFUGATION AT 2000 x g at 4 °C for 30 min.

STARCH	NIL	50 µl	50 µl	50 µl	50 µl
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CENTRIFUGATION AT 2000 x g at 4 °C for 20 min.

Abbreviations:TC (total counts); NSB (non specific binding); TB (total binding);
STD (PRL standards); SAM (samples); NRS (normal rabbit serum);
DARS (donkey anti rabbit serum); NIL (no addition made); DIL
(radioimmunoassay diluent); LH AB (native chicken LH antibody, code 3/3)

2.4.2 Radioimmunoassay of chicken PRL.

The radioimmunoassay procedure used for measuring PRL in avian plasma was modified from Talbot and Sharp (1994). The recombinant-derived chicken prolactin used for the standards and radioiodination was as described by Talbot and Sharp (1994). The sensitivity of the assay as measured by the ED₈₀ was 0.10 ng/ ml. The inter- and intra-assay coefficients of variation were 9.8 and 16.5% respectively.

2.4.2.1 Preparation of radiolabelled chicken PRL.

The method described by Talbot and Sharp (1994) was modified by using iodo-beads iodination reagent (Pierce and Warriner Ltd) instead of chloramine T in the iodination reaction. Recombinant-derived chicken PRL (5 µg in 5 µl buffered with 0.3 M

phosphate buffer) was mixed with 37 Mbq [¹²⁵I] in 10 µl with a magnetic stirrer. One bead of Iodo-bead iodination reagent, previously washed in 500 µl 0.3 M phosphate buffer, was then added to initiate the iodination reaction. The reaction time was terminated after 5 min by the addition of 800 µl column buffer made up of 0.1 M Tris buffer (pH 7.5) containing 0.1% (v/v) Tween 20 (SIGMA) and 0.1% (w/v) sodium azide. The reaction mixture was separated on a PD-10 Sephadex G-25 column (LKB-Pharmacia), pre-equilibrated with column buffer and 20 fractions of 12 drops each were collected. Radiolabelled PRL located in fractions 7-10 were further chromatographed, to remove aggregates, on a Sephacryl HR 100 column (SIGMA) equilibrated with the same column buffer. The column was run at a flow rate of 1 ml/min for 1 h and 1 ml fractions were collected at 1min intervals. The four fractions with the highest amount of radioactivity usually located between fractions 35-39 were pooled and diluted 1: 0.5 with radioimmunoassay diluent and stored at -70°C.

2.4.2.2 Preparation of standards.

Recombinant-derived chicken PRL (5µg in 48 µl sodium bicarbonate buffer) was diluted with radioimmunoassay diluent to give a working stock standard of 500 ng/ml. The top standard (125 ng/ml) was prepared by diluting the stock standard (1:3) with radioimmunoassay diluent. A further series of 1:1 dilutions produced a final series of standards, designated Stds 14 -1, containing (125 ng/ml, 62.5 ng/ml, 31.25 ng/ml, 15.63 ng/ml, 7.81ng/ml, 3.91ng/ml, 1.95 ng/ml, 0.98 ng/ml, 0.49 ng/ml, 0.24 ng/ml, 0.12 ng/ml, 0.06 ng/ml, 0.03 ng/ml and 0.15 ng/ml) .

2.4.2.3 Radioimmunoassay procedure.

The radioimmunoassay procedure for PRL (Table 2.2) was similar to that described for LH (2.3.1.3) except that standards and samples were in smaller volumes

(100 µl). The rabbit anti-recombinant-derived chicken prolactin (code 31/1) was used at a final dilution of 1:48000.

Table 2.2 The protocol for the radioimmunoassay of chicken PRL.

Solution	TC	NSB	TB	STD	SAM
(Day 1)					
DIL	NIL	150 µl	100 µl	NIL	NIL
SAMPLE	NIL	NIL	NIL	NIL	100 µl
STANDARD	NIL	NIL	NIL	100 µl	NIL
PRL AB	NIL	NIL	50 µl	50 µl	50 µl
(Day 2)					
PRL LABEL	50 µl	50 µl	50 µl	50 µl	50 µl
(Day 3)					
DARS	NIL	50 µl	50 µl	50 µl	50 µl
NRS	NIL	50 µl	50 µl	50 µl	50 µl
(Day 4)					
CENTRIFUGATION AT 2000 x g at 4 °C for 30 min.					
STARCH	NIL	50 µl	50 µl	50 µl	50 µl
CENTRIFUGATION AT 2000 x g at 4 °C for 20 min.					

Abbreviations:TC (total counts); NSB (non specific binding); TB (total binding); STD (PRL standards); SAM (samples); NRS (normal rabbit serum);

DARS (donkey anti rabbit serum); NIL (no addition made); DIL (radioimmunoassay diluent); PRLAB (recombinant derived chicken PRL antibody, code 31/1)

2.5 Measurement of VIP antibody titres in chicken and turkey blood.

2.5.1 Preparation of radiolabelled chicken VIP.

The iodination procedure for VIP was as described by Sharp *et al.* (1989). Chicken (c)VIP (5 µg, Peninsula Laboratories plc) was iodinated using chloramine T as previously described for LH (Section 2.4.1.1), excepting that the reaction was terminated after 60 sec, by the addition of 50 mM sodium metabisulphite (10 µl) and Buffer A (0.1% Trifluoroacetic acid and 10% Acetonitrile, 50 µl).

The radioiodinated cVIP was purified on a gradient reverse phase high-performance liquid chromatography (RP-HPLC) system (Water 600E, Millipore Corporation) with a Vydac C18 TP514 column (Hichrom). The ¹²⁵I-labelled VIP was eluted with buffers A and B (0.1% Trifluoroacetic acid and 90% Acetonitrile) using a linear gradient starting with 0% Buffer B (v/v) to 60% Buffer B (v/v) over 30 min , at a flow rate of 1.5 ml /min. The major peak appeared after 18 min and was collected and stored at 4⁰C until required for assay.

2.5.2 Assay procedure for measurement of VIP antibody in chicken and turkey blood.

The assay procedure for measuring the VIP antibody is summarised in Table 2.3. Plasma samples (100µl) were dispensed into plastic LP2 tubes (Denley-Luckham) using an automated dispenser (Hamilton MicroLab-M). They were then mixed with ¹²⁵I-labelled VIP (100 µl, 12000 cpm/ tube), and incubated at 4⁰C overnight. On the second

day, anti-chicken serum (50 µl, ACS, 1: 6 dilution, Scottish Antibody Production) and non-immune chicken serum (50 µl, NCS, 1: 200 dilution, Scottish Antibody Production) were added, mixed and further incubated overnight at 4⁰C. On the third day of the assay all the tubes, excepting total counts tubes were centrifuged (Sorvall RC-3B) at 2000 x g for 30 min at 4⁰C. Starch solution (50 µl, 6%) was added to each tube and further centrifuged for 20 min. The supernatant fraction was then aspirated and the pellets and total counts were counted for 60 sec. on a gamma counter (1277 Gamma Master, LKB-Pharmacia). The data were analysed with AzzayZapTM (AzzayZap Universal Assay calculator, BIOSOFT) to determine the % of bound.

Table 2.3 The protocol for measurement of the chicken VIP antibody titre.

Solution	TC	NSB	TB
(Day 1)			
DIL	NIL	100 µl	NIL
SAMPLE	NIL	NIL	100 µl
VIP LABEL	100 µl	100 µl	100 µl
(Day 2)			
ACS	NIL	50 µl	50 µl
NCS	NIL	50 µl	50 µl
(Day3)			
CENTRIFUGATION AT 2000 x g at 4 ⁰ C for 30 min.			
STARCH	NIL	50 µl	50 µl
CENTRIFUGATION AT 2000 x g at 4 ⁰ C for 20 min.			

Abbreviations: TC (total counts); NSB (non specific binding); TB (total binding); SAM (samples); NCS (normal chicken serum); ACS (donkey anti chicken serum); NIL (no addition made); DIL (radioimmunoassay diluent)

2.6 Data presentation and analysis.

The data were subjected to split-plot analysis of variance (Cochran and Cox, 1966), analysis of variance for repeated measures, analysis of variance (one-way) followed by Fisher's protected least squares differences (PSLD) test (Steel and Torrie, 1982) where appropriate or a paired and unpaired Student's t-tests (two tailed). The results are presented as mean \pm standard error of the mean (sem). The results were analysed with a statistical program (Super AnovaTM, Abacus Concepts Inc) using an Apple Macintosh computer.

EFFECT OF CASTRATION, CHANGES IN AMBIENT TEMPERATURE, AND FASTING AND REFEEDING ON PRL SECRETION.

3.1 Introduction.

The secretion of PRL is believed to be influenced by several non photoperiodic factors which must be taken into account when interpreting the results of the photoperiodic experiments described in subsequent chapters. A major factor influencing PRL secretion is the presence of gonadal steroids, especially oestrogen. For example, in the turkey, ovariectomy prevents the increase in plasma PRL normally seen at the onset of sexual maturation in birds reared on a constant daylength, while administration of oestradiol to ovariectomised turkeys increases plasma PRL (El Halawani *et al.*, 1986). In the domestic chicken, studies *in vitro* show that incubation of pituitary glands from juvenile birds with oestrogen for 20 h results in an increase in base-line release of plasma PRL (Hall *et al.*, 1984a) and an enhanced PRL response to vasoactive intestinal polypeptide (Hall and Chadwick, 1985).

An environmental factor which might affect PRL secretion is ambient temperature. For example, in rats a decrease in ambient temperature sufficient to induce mild hypothermia (35⁰C) causes a fall in plasma PRL which is reversed when the temperature is increased to its initial value (Okuda *et al.*, 1986). This cold-induced decrease in plasma PRL is mediated by an increased activation of tuberal hypothalamic dopamine neurons (Okuda *et al.*, 1986). There is no information on the effect of changes in ambient temperature on plasma PRL in birds.

A further factor reported to affect PRL secretion is the withdrawal of food. For example, in chronically starved rats, the concentration of plasma PRL is suppressed (Xie, 1991). There is no information on the effect of starvation on PRL secretion in birds, although it is known that the administration of PRL to birds increases food intake (Ensor, 1975; Buntin and Tesch, 1985; Boswell *et al.*, 1995).

The experiments described in this chapter were undertaken to establish in cockerels whether the concentration of plasma PRL is modulated by 1) testicular steroids during sexual maturation, 2) changes in ambient temperature and 3) fasting and refeeding. The data were subjected to Student's t-tests (two tailed) for testing the difference between the means.

3.2 Effect of castration on the concentration of plasma PRL during somatic maturation.

Sixty four ISA Brown cockerels were reared on short days (8 h light/ day) in floor pens with free access to feed and water. Half the birds were castrated at 2 weeks; the remainder were sham-operated and retained as the control group. At 4, 8, 16 and 24 weeks, 8 birds from each group (8 castrates and 8 intact) were bled for the measurement of plasma PRL.

The concentrations of plasma PRL in castrates and intact birds at 4 weeks were not significantly different; at all other ages the concentration of plasma PRL was higher in intact than in castrated birds (Fig. 3.1). PRL concentrations in castrated and intact birds at 4 weeks were significantly higher than at 8 ($P<0.001$) and 16 ($P<0.001$) weeks. The concentrations of plasma PRL in 24 -and 4 -week-old intact birds were not significantly different. However, the concentrations of the plasma PRL in 24 -week -old castrated birds was lower than in 4 -week-old castrated birds ($P<0.001$). Plasma PRL concentration of the 24 -week-old intact birds were similar to that of 4 -week-old birds. Twenty- four- week old castrated birds had a significantly higher concentrations of

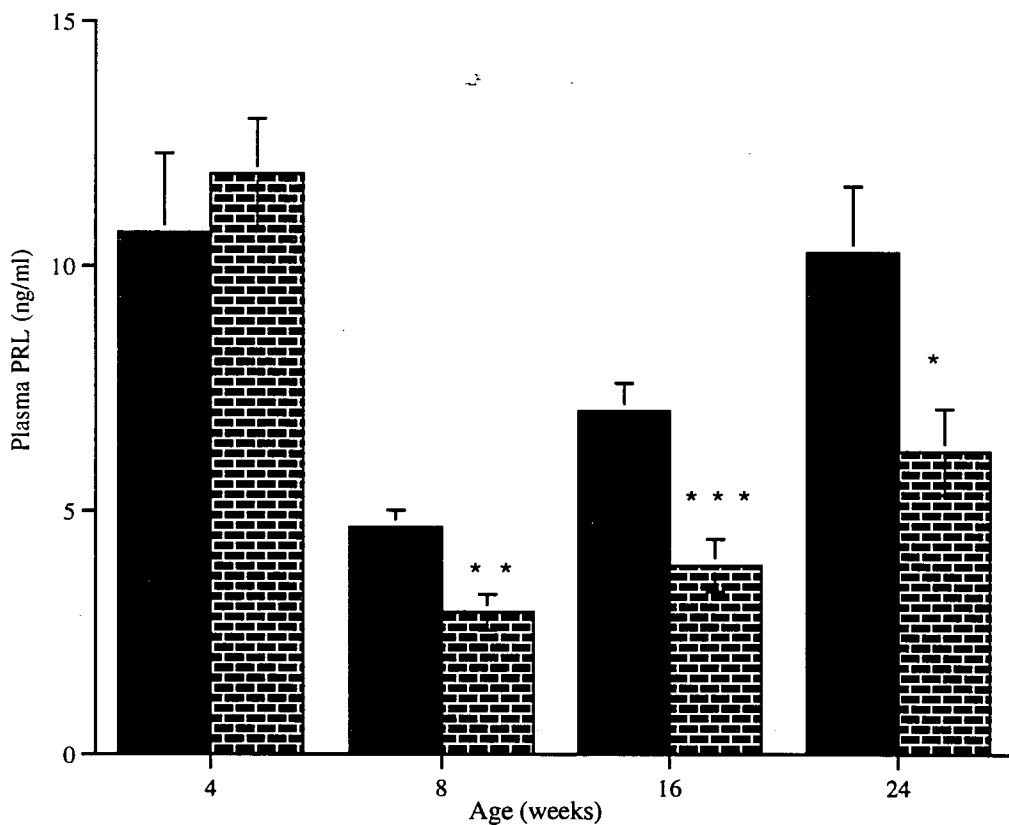


Fig.3.1 A comparison of concentrations of plasma PRL in intact (■) and castrated (▒) cockerels maintained on a 8 h photoperiod during somatic maturation. The cockerels were surgically castrated or sham operated at 2 weeks of age. The onset of sexual maturation began at about 16 weeks and the birds are sexually mature at 24 weeks. Different groups of birds were sampled at each age. Values are means \pm s.e.m (n=8). * P <0.05, ** P <0.005, *** P <0.001 compared with controls. The birds were held on short days (8L:16D) throughout the study.

plasma PRL than 8 ($P<0.005$) or 16 ($P<0.05$) week-old castrated birds. Plasma PRL concentrations in 8 and 16 -week-old castrated birds were not significantly different.

3.3 Effect of change in ambient temperature on the concentration of plasma PRL.

Groups of 12 castrated birds (>80 week old) were kept on a photoperiod of 12 h at 20⁰C for 3 weeks in two climate chambers. The 12 h photoperiod was chosen because, in a previous study (Chapter 6), it was observed that in birds kept on this photoperiod the concentration of plasma PRL is maintained at an unchanged, moderately elevated, value without the development of photorefractoriness (Chapter 6, Section 6.2, 6.3). Hence, at this photoperiod, any stimulatory or inhibitory effect of a change in ambient temperature on the concentration of plasma PRL should be readily measurable. The ambient temperature in one of the chambers was increased to 30⁰C for 2 weeks and returned to 20⁰C, while the temperature in the other chamber was reduced to 5⁰C for 2 weeks and then returned to 20⁰C. Blood sample was collected from each bird immediately before, the ambient temperatures were changed. Birds were bled on the 14 th day of exposure to the increased or decreased ambient temperature. After return to the original ambient temperature (20⁰C), the birds were again bled after 14 days. Blood samples were all taken at the same time of the day, 6 h (14.00 h) after the lights came on (08.00 h), to avoid any confounding effect of diurnal rhythmicity. The exposure period of 2 weeks to each ambient temperature was chosen to allow the birds to acclimatise fully, to avoid measuring PRL responses which could be due to stress associated with the change in temperature.

The concentrations of plasma PRL in birds exposed to increased or decreased ambient temperature are shown in Fig. 3.2. There was no significant ($P<0.05$) change in

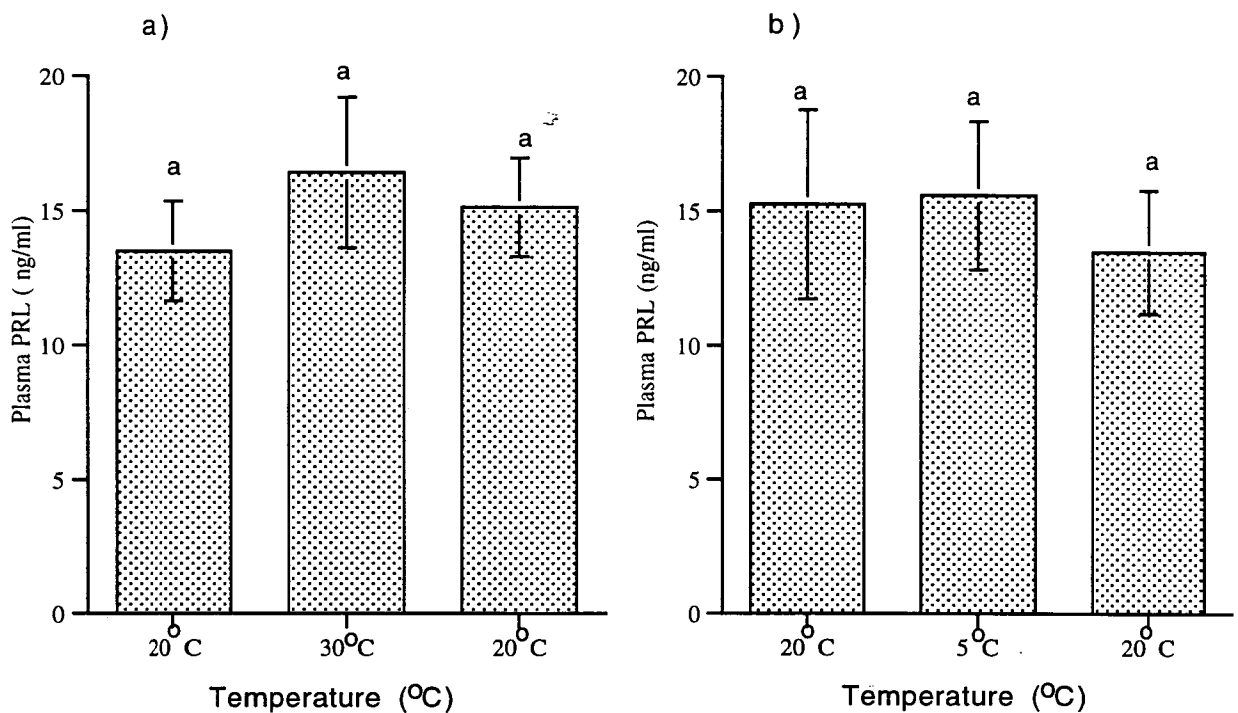


Fig. 3.2 Effect of an a) increase or b) decrease in ambient temperature on plasma PRL levels in castrated bantams. The birds were transferred from 20°C to 30°C or 5°C for 2 weeks and back to 20°C. The birds were held at each temperature for 2 weeks and sampled during the second week in the middle of the light period. The birds were held on 12L:12D, throughout the study. Values with the same superscript are not significantly different. Values are means \pm s.e.m (n=12).

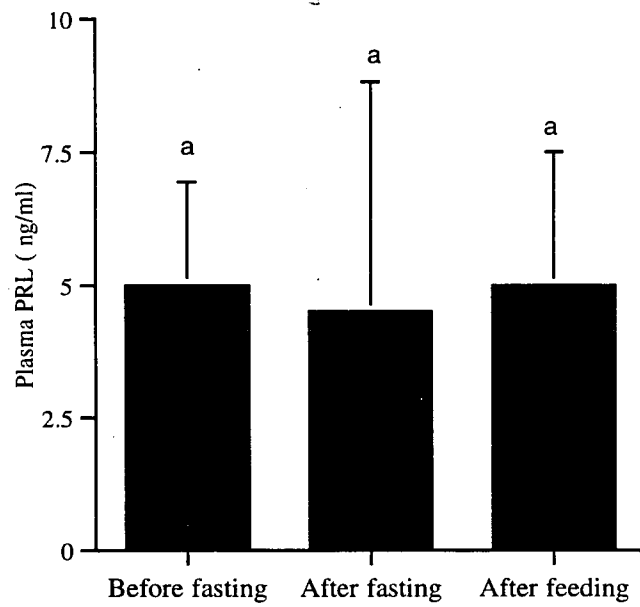


Fig. 3.3 Concentration of plasma PRL in castrated cockerels held on 8 h light/ day before fasting, after 24 h fasting and after 1 hour refeeding. Values with the same superscript are not significantly different. Values are means \pm s.e.m (n=8).

the plasma PRL concentrations after an increase or decrease of ambient temperature and after return to 20°C.

3.4 Effect of a 24 - h fasting and refeeding on the concentration of plasma PRL.

Eight castrated bantam cockerels (36 weeks old) kept on a short photoperiod (8 h light/ day) for more than 3 months were housed in individual cages with free access to feed and water. On the day of the experiment the birds were deprived of feed for 24 h but had free access to water. After a 24 h fast the birds were given free access to feed. Blood samples were collected just before feed deprivation, after fasting and one hour after refeeding.

Plasma PRL concentrations before and after fasting and one hour after refeeding are shown in Fig. 3.3. There was no significant ($P<0.05$) effect of fasting or refeeding on the concentration of plasma PRL.

3.5 Discussion.

The observation that, except at 4 week of age, the concentration of plasma PRL was higher in intact than in castrated bantams demonstrates that testicular hormones play a role in maintaining increased plasma PRL. A consistent effect of testosterone on PRL secretion has not been established; both inhibitory and stimulatory effects have been reported (Section 1.4.3.2). It is suggested that the increased concentration of plasma PRL in intact birds is due to aromatisation of testicular androgen to oestrogen since it is well established that oestrogen facilitates increased PRL secretion by a direct action on the anterior pituitary gland (Section 1.4.3.1.1). The blood of juvenile and adult cockerels has been reported to contain about 68 and 91 pg/ ml oestradiol respectively (Liu, 1993). These concentrations of oestrogen may be sufficient to exert a stimulatory effect on PRL secretion at the level of the anterior pituitary. The absence of a difference

in concentration of plasma PRL in 4 week-old intact and castrated birds suggests that at this age, the testes secrete insufficient amount of oestrogen to enhance PRL secretion.

The observation that the plasma PRL was higher at 4 weeks old, was low at 8 and 16 weeks, and increased at 24 weeks irrespective of whether the birds were intact or castrated is consistent with earlier studies in male broilers (Sterling *et al.*, 1984). In broilers plasma PRL is high at 3 weeks of age, falls by 9 weeks and show a progressive increase between 17 and 26 weeks of age (Sterling *et al.*, 1984). The present study shows that castration changes the amplitude of PRL secretion and not the age-related changes in plasma PRL concentrations. It thus appears that the age-related changes in plasma PRL concentration are independent of gonadal steroids.

The present study demonstrated that an increase or decrease in ambient temperature in the thermo-neutral range (Whittow, 1986) has no effect on plasma PRL secretion in castrated bantams. This observation is consistent with findings in female turkeys held for 4 weeks at 10⁰C, 24⁰C or 30⁰C on short days. Differences in ambient temperature had no effect on plasma PRL either before or after photostimulation (El Halawani *et al.*, 1984b). In sexually immature cockerels (3-5 week-old), acute exposure to increased environmental temperature (45⁰C) has not been reported to affect the plasma PRL. However, an acute decrease in ambient temperature (4⁰C) has been reported to increase plasma PRL (Harvey *et al.*, 1977). It is possible that in the latter study, the cold-induced change in plasma PRL was a stress-related response which was avoided in the present study by allowing the birds to acclimatise to the changed temperature before the blood samples were taken.

The current observation on plasma PRL in fasting and refeeding bantams suggests that the PRL secretion is not readily affected by short-term fasting. These observations are consistent with findings in non-laying turkeys reared on long days, where feed and/ or water deprivation for 3 days had no effect on the plasma PRL levels (Zadworny *et al.*, 1985). In contrast, in a study on White Leghorn layers, plasma PRL decreased after 11

days fasting in both short and long photoperiods. The concentration of plasma PRL rose after refeeding in both groups (Millam and El Halawani, 1986). Similarly, a decrease in plasma PRL was also observed in 2 -and 6 -week old intact cockerels held on long days and fasted for 24 and 12 - 48 respectively (Harvey *et al.*, 1978). It is possible that a similar depression on plasma PRL was not observed in the current study because castration and exposure to short days resulted in plasma PRL being at basal values, which could not be depressed further.

In conclusion it is inferred that testicular steroids increase the plasma PRL concentration during somatic maturation. Chronic exposure to changes in ambient temperature or 24 h fasting and refeeding has no effect on plasma PRL concentrations in somatically mature castrated cockerels.

ONTOGENY OF THE PHOTOPERIODIC CONTROL OF PRL AND LH SECRETION.

4.1 Introduction.

Prolactin (PRL) secretion is stimulated by an increase in photoperiod in several species of birds (Section 1.3.4) but information in the developmental stage at which PRL secretion can first be stimulated by an increase in photoperiod is unknown. The aim of this study was to determine, in the bantam chicken, the posthatch age at which the hypothalamo-hypophysial axis first responds to an increase in daylength by an increase in plasma PRL and to determine whether this is correlated with the development of the photoinduced luteinising hormone (LH) response (Dunn *et al.*, 1990).

4.2 Experimental design.

Bantam chicks reared on a short day lighting pattern of 8 h light and 16 h darkness (8L:16D) were transferred in groups of 12 (6 males and 6 females) to a long day lighting pattern of 20L:4D at 4, 8, 12 and 16 weeks. Control groups (6 males and 6 females) were retained on short days. Plasma PRL and LH were measured at 3- 4 day intervals for 36 days after photostimulation. The birds were then sacrificed to record the weights of the testes, ovaries and oviducts.

4.3 Photoinduced PRL secretion during somatic maturation.

Males and females showed a significant increase in plasma PRL after photostimulation at 4, 8, 12 and 16 weeks (Fig. 4.1a). In all the groups, a significant increase in plasma PRL was seen after 3 days of photostimulation (males, 4 weeks

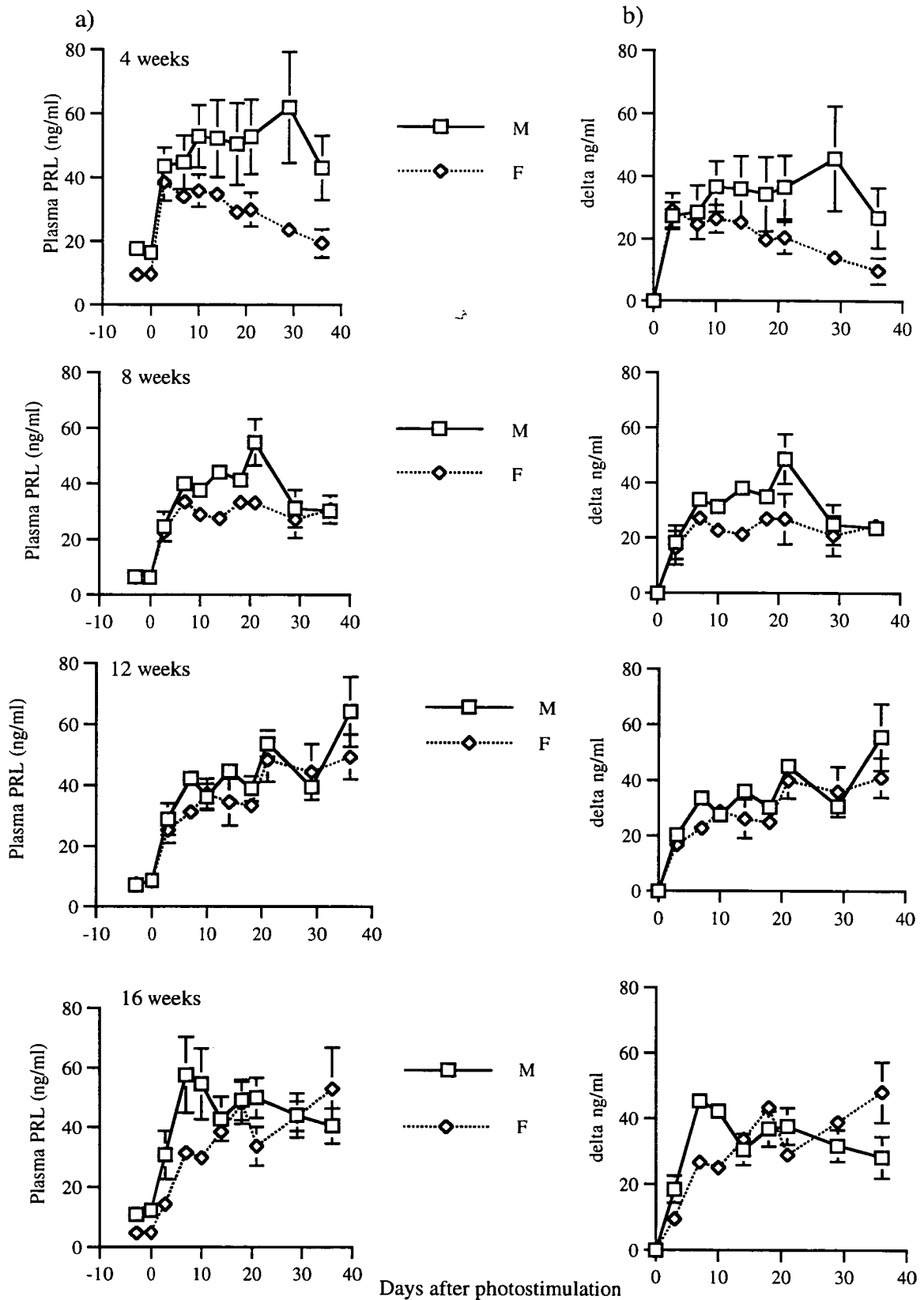


Fig. 4.1 Plasma PRL concentrations after transfer from 8 to 20 h light/day at 4, 8, 12 and 16 weeks in male (M) and female (F) bantams expressed as a) ng/ml and b) difference in plasma PRL concentration from that before photostimulation (delta ng/ml). Values are means \pm s.e.m (n=6).

$P < 0.001$, 8 weeks $P < 0.05$, 12 weeks $P < 0.005$, 16 weeks $P < 0.01$; females, 4 weeks $P < 0.05$, 8 weeks $P < 0.001$, 12 weeks $P < 0.005$, 16 weeks $P < 0.001$, ANOVA repeated measures followed by Fishers's protected least squares difference). There was no significant ($P > 0.05$) difference between the sexes in the increase in plasma PRL concentration after photostimulation in any of the age groups. Increased concentrations of plasma PRL were maintained for a further period of 33 days, until the birds were killed (Fig. 4.1a). The exception to this was females photostimulated at 4 weeks. In this group, plasma PRL decreased between 29 and 36 days to concentrations which were not significantly different from pre-photostimulation values (Fig. 4.1a,b). Concentrations of plasma PRL after 36 days of photostimulation in all groups, irrespective of age, were significantly higher than in non photostimulated control groups (Table 4.1). The concentration of plasma PRL increased in both short day control males and females of 12 and 16 weeks of age, but the increase was less compared to photostimulated birds of the same age group (Table 4.1). The mean concentration of plasma PRL after photostimulation was not significantly ($P > 0.05$) different between the males in different age groups. However, the mean concentrations of plasma PRL in females after photostimulation at 4 and 8 weeks were lower than those after photostimulation at 16 weeks (Fig. 4.1a). The photoinduced changes in plasma PRL (delta PRL) concentrations were not significantly ($P > 0.05$) different between males and females in any of the age groups (Fig. 4.1b).

Table 4. 1 The effect of transfer from 8 h to 20h light/day for 36 days at 4, 8, 12 and 16 weeks of age on plasma prolactin in intact male and female bantams.

Plasma prolactin (ng/ml)				
	Age at which photostimulated (weeks)			
	4	8	12	16
Males				
Short day controls				
Day 0	11.81± 0.89	5.92 ±1.38	5.83 ± 0.55	9.17 ±1.32
Day 36	5.92 ± 1.38a	9.46 ± 1.36	12.00 ± 1.61a	12.47 ± 1.79a
Photostimulated				
Day 0	16.33 ± 2.76	6.17 ± 1.01	8.67 ± 1.26	12.24 ± 3.04
Day 36	43.08 ± 10.14a	30.17 ± 2.48d	64.17 ± 11.41c	40.41 ± 5.89b
Females				
Short day controls				
Day 0	10.67 ± 2.97	5.08 ± 0.27	5.17 ± 0.47	5.04 ± 0.48
Day 36	5.08 ± 0.27	5.79 ± 0.56	8.67 ± 0.79a	12.10 ± 1.16a
Photostimulated				
Day 0	9.50 ± 0.99	6.17 ± 0.87	8.56 ± 1.12	4.76 ± 0.62
Day 36	19.33 ± 4.40	30.67 ± 4.97c	49.33 ± 7.43c	52.76 ± 13.93a

Values are mean ± s.e.m (n=6), a= $P<0.05$, b= $P<0.01$, c= $P<0.005$ and d= $P<0.001$ compared to the values within the same group before photostimulation

4.4 Photoinduced LH secretion during somatic maturation.

Changes in LH secretion after photostimulation during somatic development are shown in Fig. 4.2 and Table 4.2. An increase in plasma LH concentration was observed after 3 days of photostimulation in males at 4 ($P<0.05$), 8 ($P<0.01$) and 12 ($P<0.05$) weeks and in females at 8 ($P<0.05$), 12 ($P<0.05$) and 16 ($P<0.005$) weeks. Significant increases in LH secretion were not observed after photostimulation in females at 4 weeks and males at 16 weeks. After photostimulation at 4 weeks, the concentration of LH was higher ($P<0.05$) in males than in females but after photostimulation at 16 weeks, plasma LH increased more ($P<0.01$) in females than in males. A significant increase in plasma LH was seen in both sexes for 36 days after photostimulation at 4 and 8 weeks (Table 4.2). Similarly, an increase in plasma LH was observed for 36 days in females, but not in males after photostimulation at 16 weeks (Table 4.2). After photostimulation at 12 weeks the initial significant increases in plasma LH in the males and females were not maintained after 3 and 10 days respectively. The concentrations of plasma LH in short day control male and female groups did not change significantly during the 36 -day experiment (Table 4.2).

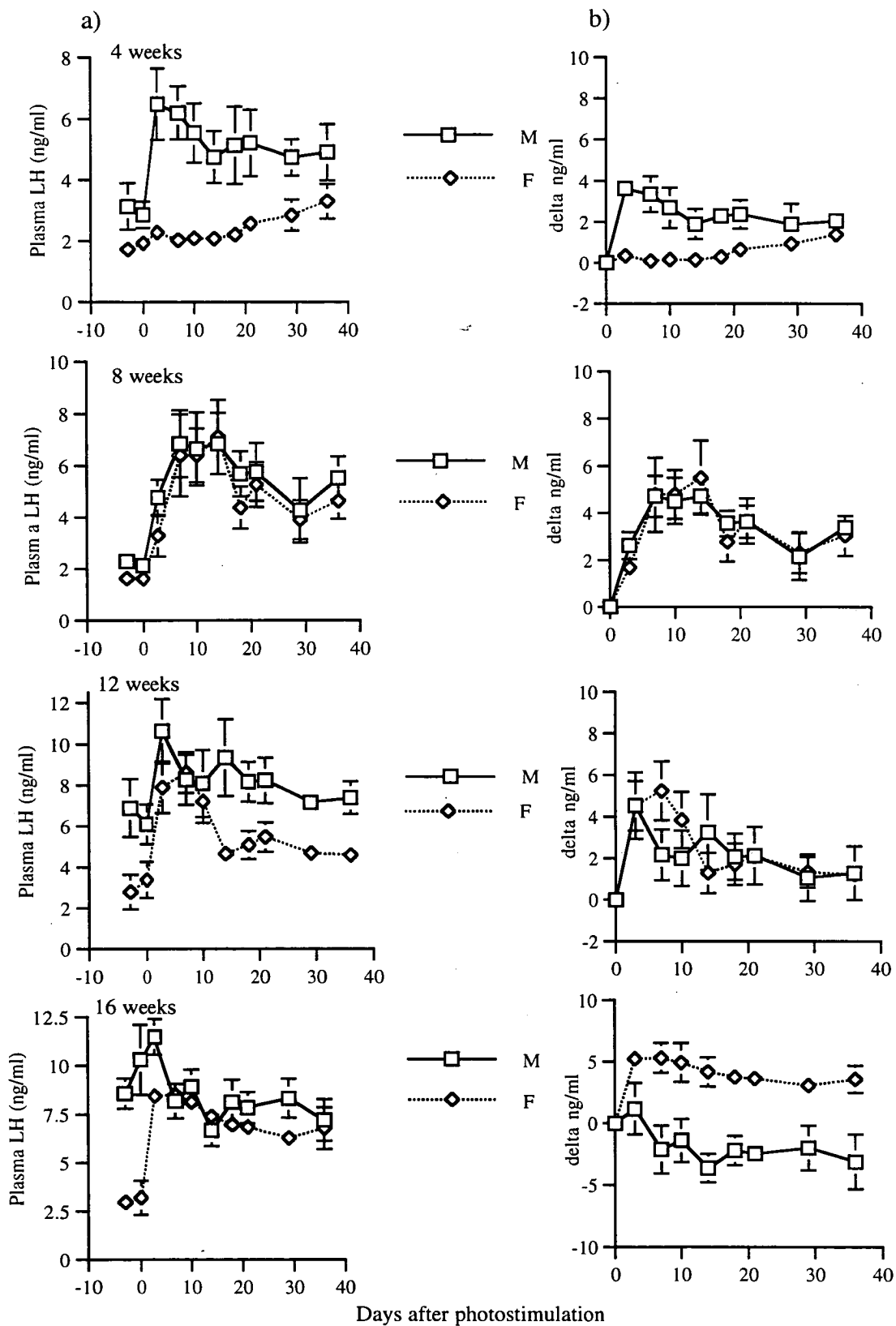


Fig. 4.2 Plasma LH concentrations after transfer from 8 to 20 h light/day at 4, 8, 12 and 16 weeks in male (M) and female (F) bantams expressed as a) ng/ml and b) difference in plasma LH concentration from that before photostimulation (delta ng/ml). Values are means \pm s.e.m (n=6).

Table 4. 2 The effect of transfer from 8 h to 20h light/day for 36 days at 4, 8, 12 and 16 weeks of age on plasma luteinising hormone in intact male and female bantams.

Plasma luteinising hormone (ng/ml)				
	Age at which photostimulated (weeks)			
	4	8	12	16
Males				
Short day controls				
Day 0	5.97 ± 1.84	5.44 ± 2.01	9.52 ± 1.41	5.34 ± 0.87
Day 36	5.44 ± 2.01	11.70 ± 3.11	5.89 ± 0.43	4.24 ± 1.07
Photostimulated				
Day 0	2.86 ± 0.43	2.14 ± 0.4	6.10 ± 0.97	10.31 ± 1.81
Day 36	4.91 ± 0.92a	5.52 ± 0.84c	7.38 ± 0.8	7.18 ± 1.08
Females				
Short day controls				
Day 0	2.15 ± 0.49	2.98 ± 0.65	4.27 ± 2.21	3.01 ± 0.62
Day 36	2.98 ± 0.65	2.12 ± 0.36	2.51 ± 0.29	3.27 ± 0.74
Photostimulated				
Day 0	1.93 ± 0.27	1.62 ± 0.37	3.38 ± 0.88	3.19 ± 0.74
Day 36	3.31 ± 0.57a	4.64 ± 0.7a	4.59 ± 0.47	6.67 ± 1.64a

Values are mean ± s.e.m (n=6), a= $P < 0.05$ and c= $P < 0.005$ compared to the values within the same group before photostimulation.

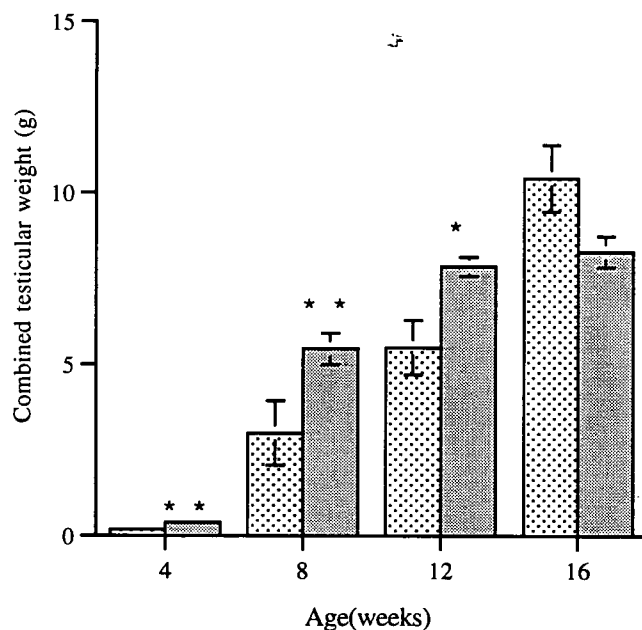


Fig. 4.3. The testis weights of bantam cockerels after photostimulation (■ 20L:4D) for 36 days at 4, 8, 12 and 16 weeks of age. Control birds at the same ages were retained on short days (▨ 8L:16D) and sacrificed at the same time as the photostimulated birds. Values are mean \pm sem (n=6). * P <0.05, ** P <0.005 compared to non photostimulated control birds.

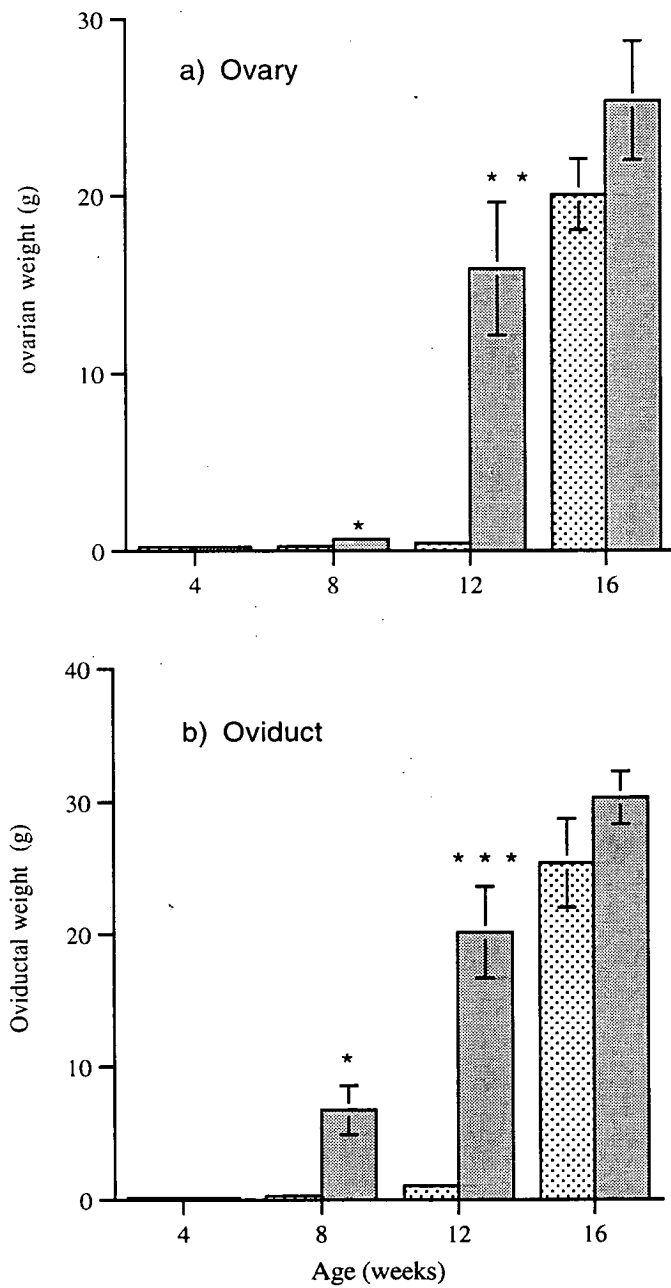


Fig. 4.4. The a) ovarian and b) oviductal weights of bantam hens after photostimulation (■ 8L:16D transferred to 20L:4D) for 36 days at 4, 8, 12 and 16 weeks of age. Control birds at the same ages were retained on short days (▨ 8L:16D) and sacrificed at the same times as the photostimulated birds. Values are mean \pm sem (n=6). * P <0.05, ** P <0.005, *** P <0.001 compared to non photostimulated control birds.

4.5 Photoinduced changes in gonadal and oviductal weights during somatic maturation.

The effects of days of photostimulation on the testicular, ovarian and oviductal weights in males and females at 4, 8, 12 and 16 weeks are presented in Figs. 4.3 and 4.4 respectively. The testicular weights were significantly higher after photostimulation for 36 days at 4 ($P<0.005$), 8 ($P<0.005$) and 12 ($P<0.05$) weeks than in control birds kept on short days. There was no significant difference in testicular weights between experimental males that were photostimulated at 16 weeks and their short day controls (Fig. 4.3). The increases in testicular weight expressed as a % after photostimulation at 4 ($210.32\pm 28.1\%$), at 8 ($620.4\pm 335.5\%$) and 12 ($166.3\pm 36.4\%$) weeks were not significantly different. Ovarian and oviductal weights were significantly higher after photostimulation at 8 weeks ($P<0.05$ and $P<0.05$) and 12 weeks ($P<0.001$ and $P<0.005$) than in short day controls (Figs. 4.4 a, b). Although some of the birds photostimulated at 16 weeks of age came into lay during the last week of photostimulation, the mean weights of their ovaries and oviducts were not significantly ($P>0.05$) different from those in the short day controls which did not come into lay. Similarly, there were no differences between the ovarian and oviductal weights in the females photostimulated at 4 weeks and their short day controls (Figs. 4.4 a, b).

4.6 Discussion.

The increase in plasma PRL levels observed in chickens of both sexes after photostimulation at 4 weeks of age indicates that the neuroendocrine pathways involved in photoperiodic control of PRL secretion are functional at this early age. An increased concentration of plasma PRL was maintained after 36 days of photostimulation with the exception of photostimulated hens at 4 weeks. Harvey *et al.* (1979a) found that in layer and broiler strains of chicken maintained on long days from hatch, males had higher

concentrations of plasma PRL at 6, 7, 11, 12 and 15 weeks of age than females, but from 16 to 24 weeks of age the females had a higher concentration of plasma PRL than males. In the present study, no sex differences were observed in the concentration of plasma PRL in juvenile chickens at any age before photostimulation. Similarly, except at 4 weeks, there were no sex differences in the photoinduced increase in plasma PRL. The differences observed between the present study and that of Harvey *et al.* (1979a) might be due to the differences in the strains used and /or to the differences in photoperiodic history. Although there were no sex differences in the response to photostimulation, there was a sex difference in the ability to maintain the photoinduced increase in plasma PRL concentration in 4-week-old birds. The inability of the 4-week-old females to maintain increased plasma PRL concentrations for more than 29 days after photostimulation (Fig. 4.1a) could be due to the absence of a stimulatory effect of plasma oestrogen at the level of the anterior pituitary gland (Section 1.4.3.1.1). This view is supported by the observation that in 4-week-old females, photostimulation failed to induce a steep increase in LH secretion and was not associated with a stimulation of ovarian or oviductal growth (Figs 4.2, 4.4). This suggests that in contrast with older females, photostimulation at 4 weeks did not induce a sufficient increase in ovarian oestrogen production to facilitate PRL secretion (Section 1.4.3.1). In contrast, in 4-week-old males, photostimulation induced both an increase in plasma LH and testicular growth (Fig. 4.3) and this increase in testicular activity and associated increase in plasma steroids are suggested to be sufficient to maintain plasma PRL at a higher values than in the females at the same age.

The reduced photoinduced LH response observed in females at 4 weeks is inconsistent with earlier study in female broilers (Dunn *et al.*, 1990). In that study, it was shown that in 3-week-old female broiler chickens, a significant increase in plasma LH level occurs after 4 days of photostimulation (8L:16D to 20L:4D). The experiments of Dunn *et al.* (1990) suggest that the maturation of the neuroendocrine pathways

controlling photoinduced LH release may partly depend on the fat content of the diet. It could be that the nutritional status of the 4-week-old bantam females was not adequate to allow the full development of the photoinduced LH response. However, no explanation can be offered to account for the sex difference in the photoinduced release of LH at 4 weeks of age. The robust response observed in the males was clearly not affected by nutritional status.

The concentration of plasma LH increases progressively in cockerels reared on 14 h light/day between 9 and 19 weeks of age (Sharp, 1975; Sharp *et al.*, 1977; Wilson, 1978). The non-significant increase in the concentration of plasma LH observed in 16-week-old cockerels after 3 days of photostimulation was probably due, in part, to an increase in plasma LH to near-maximal values which was independent of a change in photoperiod. The capacity for further increase after photostimulation may therefore have been reduced. The view that the 16-week-old cockerels were approaching sexual maturity, and therefore had maximum plasma LH values, was confirmed by the finding that the testes of short day control birds killed at 16 weeks were almost fully developed (Fig. 4.3). The capacity of the 16 week females to respond to photostimulation by an increase in LH secretion might be explained by the fact the LH levels do not increase in female chickens reared and maintained on 14 h light/day at the onset of puberty as much as they do in males (Sharp, 1975). This sex difference can be accounted for by differences in the circulating concentrations of plasma steroids (Tanabe *et al.*, 1981; Liu, 1993). It is therefore suggested that, in contrast with the males at 16 weeks, plasma LH in females is sufficiently depressed by oestrogen to allow a photoinduced release of LH to be readily detected.

An increase in testicular growth after photostimulation has been observed as early as 1 week of age in quail (Tanaka *et al.*, 1965) and in the present study a significant ($P < 0.005$) increase in testicular weight was seen after photostimulation at 4 weeks of age. It therefore appears that the acquisition of photosensitivity occurs early in post-



hatch juvenile development in Galliforms. It is possible that when these birds hatch, the neural mechanisms controlling LH and PRL release are sufficiently mature to allow a full photoperiodic response. The present study, showing increased ovarian and oviductal weights after photostimulation at 4, 8 and 12 weeks is consistent with an earlier study in dwarf broiler chickens (Dunn *et al.*, 1990). Photoinduced ovarian growth was correlated with increased oviductal growth in 8 and 12 week-old birds. This can be explained by an increase in oestrogen and progesterone production by the ovaries in response to the photoinduced increase in LH secretion. The growth of the oviduct depends on increased secretion of oestrogen and progesterone from the ovary (Phillips *et al.*, 1985).

In conclusion, the hypothalamo-hypophysial axis of the bantam hens is sufficiently mature at 4 weeks of age to respond to increased daylength by an increase in both PRL and LH secretion. However, the LH response at 4 weeks, may not be as robust in females as it is males.

PHOTOPERIODIC RESPONSE CURVES FOR PRL AND LH IN CASTRATED BANTAMS.

5.1 Introduction.

The shortest daylength required to induce a photoperiodic response is termed the critical daylength, while the shortest daylength required to induce the maximum photoperiodic response is termed the saturation daylength (Section 1.5.5). Between the critical and saturation daylength there is proportionality between the daylength and the photoperiodic response. A plot of daylength versus the photoperiodic response between the critical and saturation daylengths is termed a photoperiodic response curve (PRC). In birds, PRCs have been more commonly presented for rates of testicular or ovarian growth (Farner, 1964; Follett and Maung, 1978). The relationship between gonadal growth and daylength has been shown in quail (Follett and Maung, 1978) and chickens (Sharp, 1993) to reflect the relationship between an increase in daylength and increased luteinising hormone (LH) secretion. The critical daylength for LH release is known to be longer for birds reared on long days than in birds reared on short days (Robinson and Follett, 1982). Although it is well established in birds that prolactin (PRL) secretion is stimulated when daylength increases (Section 1.3.4), there is no information on the PRC for photoinduced PRL release in the domestic hen.

The objective of this study is to establish in bantam cockerels, a) the critical daylength for birds reared on short days, b) the saturation daylength for birds reared on short days and c) the critical daylength for birds reared on long days, for photoinduced PRL and LH release. The experiments were carried out on castrated birds to avoid the

direct stimulatory and inhibitory effects of gonadal steroids on PRL (Section 1.4.3) and LH release respectively. The data in these experiments were subjected to logarithmic transformation to normalise the variations before subjecting to split -plot ANOVA. The differences between means were tested using Fisher's protected least significant difference test.

5.2 Estimate of the critical and saturation daylengths for photoinduced PRL and LH release in birds reared on short days.

Bantam chicks reared in floor pens on short days (8 h light/ day) from hatch were surgically castrated (Section 2.2) at 4 weeks, transferred to individual cages and maintained on short days. At 20 weeks, groups of 8 birds were transferred to one of the following light regimes: 9L:15D, 10L:14D, 11L:13D, 12L:12D and 14L:10D. Blood samples were collected 4 days before, on the day of transfer, and at intervals of 3-4 days after transfer to the test lighting pattern for a period of 36 days. Blood samples were taken at the same times from a control group maintained on short days (8L:16D).

The prolactin responses in birds reared on short days to increased photoperiods are shown in Fig. 5.1. Birds transferred to 9L:15D showed no significant ($P>0.05$) increase in PRL secretion. The concentrations of plasma PRL increased significantly ($P<0.05$) after 4 days by 27, 39, 89, 135 % in groups transferred to 10L:14D, 11L:13D, 12L:12D and 14L (Fig. 5.2). The concentration of plasma PRL did not change further in birds held on 10L:14D until after 3 weeks of photostimulation, when it decreased to pre-photostimulation values (Fig. 5.1). Similarly, in birds transferred to 11L:13D, the concentration of plasma PRL returned to baseline values after 36 days of photostimulation. In birds exposed to 12L:12D and 14L:10D plasma PRL continued to increase for 10 and 18 days respectively after photostimulation and thereafter remained high until the end of the experiment. Three PRCs are presented because they give slightly

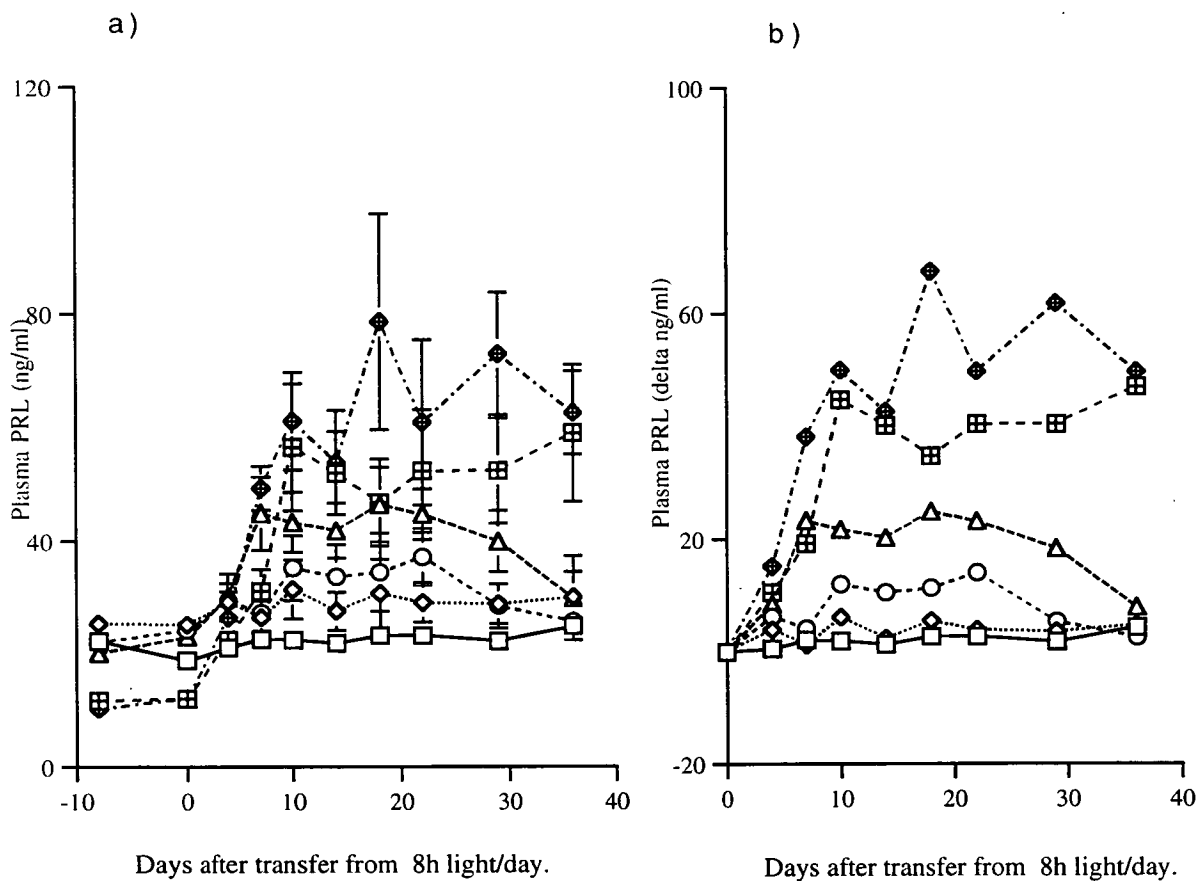
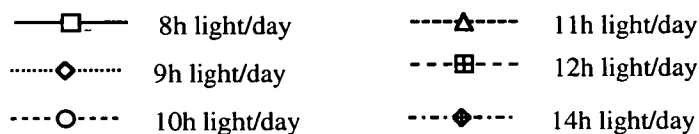


Fig. 5.1 Changes in plasma PRL in 20 week-old castrated bantams reared on 8 h light/ day, after transfer from 8h light/ day to 9, 10, 11, 12 or 14 h light/ day expressed as, a) concentration (ng/ml) and b) as the change (delta) from the prephotostimulation values (ng/ml). Values are mean \pm sem; n=8).



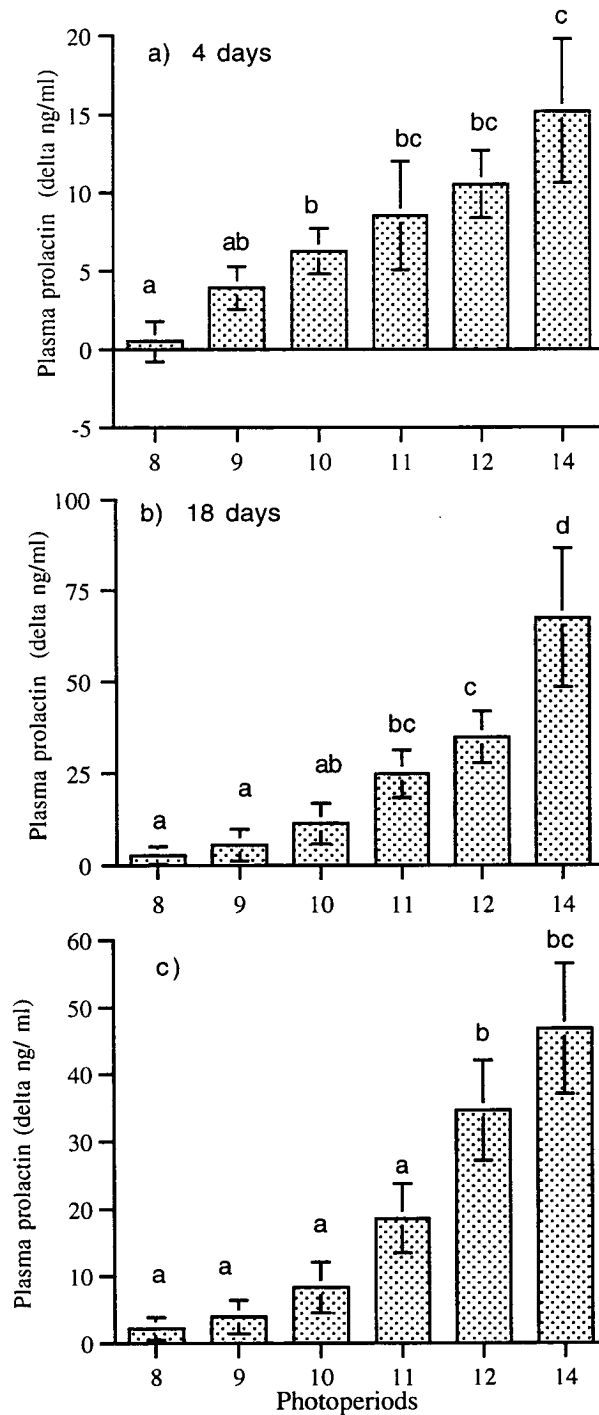


Fig. 5.2. Photoperiodic response curves for plasma PRL in castrated bantams transferred from 8 h light/ day to a range of increased fixed photoperiods, derived from changes in plasma PRL concentration after a) 4 days, b) 18 days, and c) a composite of all days of photostimulation. Values with different superscripts are significantly ($P < 0.05$) different (\pm sem; $n = 8$)

different results depending on how they were calculated. The times chosen were 4 days, 18 days, and a composite of all days after photostimulation (Fig. 5.2). Day 4 was chosen because it was the first sampling point at which a photoinduced increase in PRL were observed. Day 18 was chosen because it corresponds to the period when plasma PRL had reached a plateau in all groups (Fig. 5.1). A composite PRC was constructed using all the data to increase the power of analysis. The PRC after 4 days of photostimulation demonstrated a significant ($P < 0.05$, ANOVA) increase in plasma PRL in birds photostimulated with photoperiods of 10 h or more (Fig. 5.2a). The PRC taken at day 18, when plasma PRL concentrations had reached a plateau, showed a significant ($P < 0.05$, ANOVA) increase in PRL secretion in birds exposed to photoperiods of 11 h or more (Fig. 5.2b). The composite PRC including all data from the times after photostimulation showed a significant ($P < 0.05$, ANOVA) increase in plasma PRL concentrations in birds transferred to photoperiods of 12 h or more (Fig. 5.2c). It therefore appears that the critical daylength for PRL release lies between 10 and 12 h.

The PRCs (Fig. 5.2) suggested that the saturation daylength for plasma PRL was between 12 and 14 h but it was not possible to establish whether photoperiods greater than 14 h might exert a greater stimulatory effect on PRL release.

Estimates of changes in plasma LH after photostimulation were confounded by the progressive increase in plasma LH which occurred in the short day control birds during the 36-day study (Fig. 5.3).

5.3 Additional estimation of the saturation daylength for PRL secretion in birds reared on short days.

The previous study suggested that the saturation daylength for plasma PRL release might be between 12 and 14 h (Fig. 5.2). However, those data did not convincingly demonstrate that daylengths greater than 14 h were equally stimulatory. The purpose of this experiment was to confirm the saturation daylength for

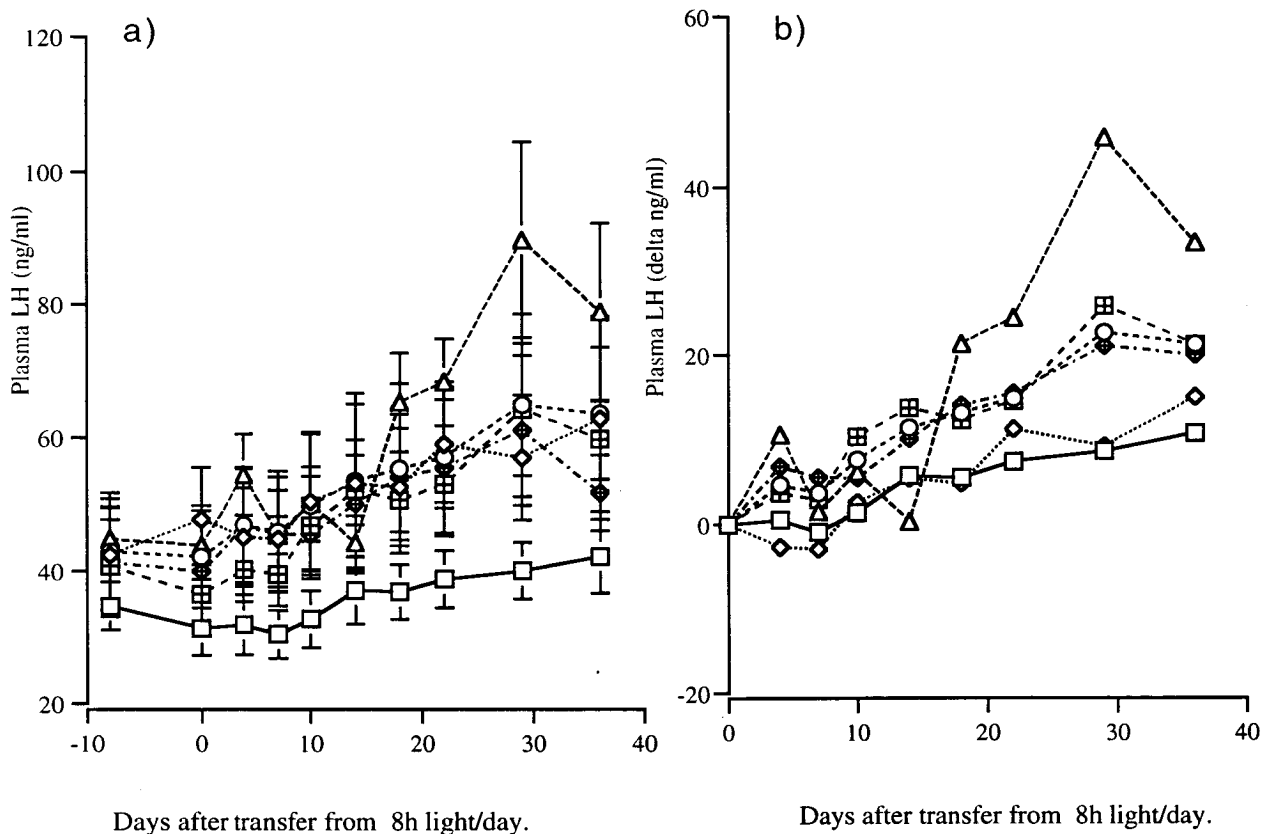


Fig. 5.3 Changes in plasma LH in 20 week-old castrated bantams reared on 8 h light/day, after transfer from 8h light/day to 9, 10, 11, 12 or 14 h light/day expressed as, a) concentration (ng/ml) and b) as the change (delta) from the prephotostimulation values (ng/ml). Values are mean \pm sem; n=8).

—□—	8h light/day	---△---	11h light/day
.....◇.....	9h light/day	---■---	12h light/day
---○---	10h light/day	---◆---	14h light/day

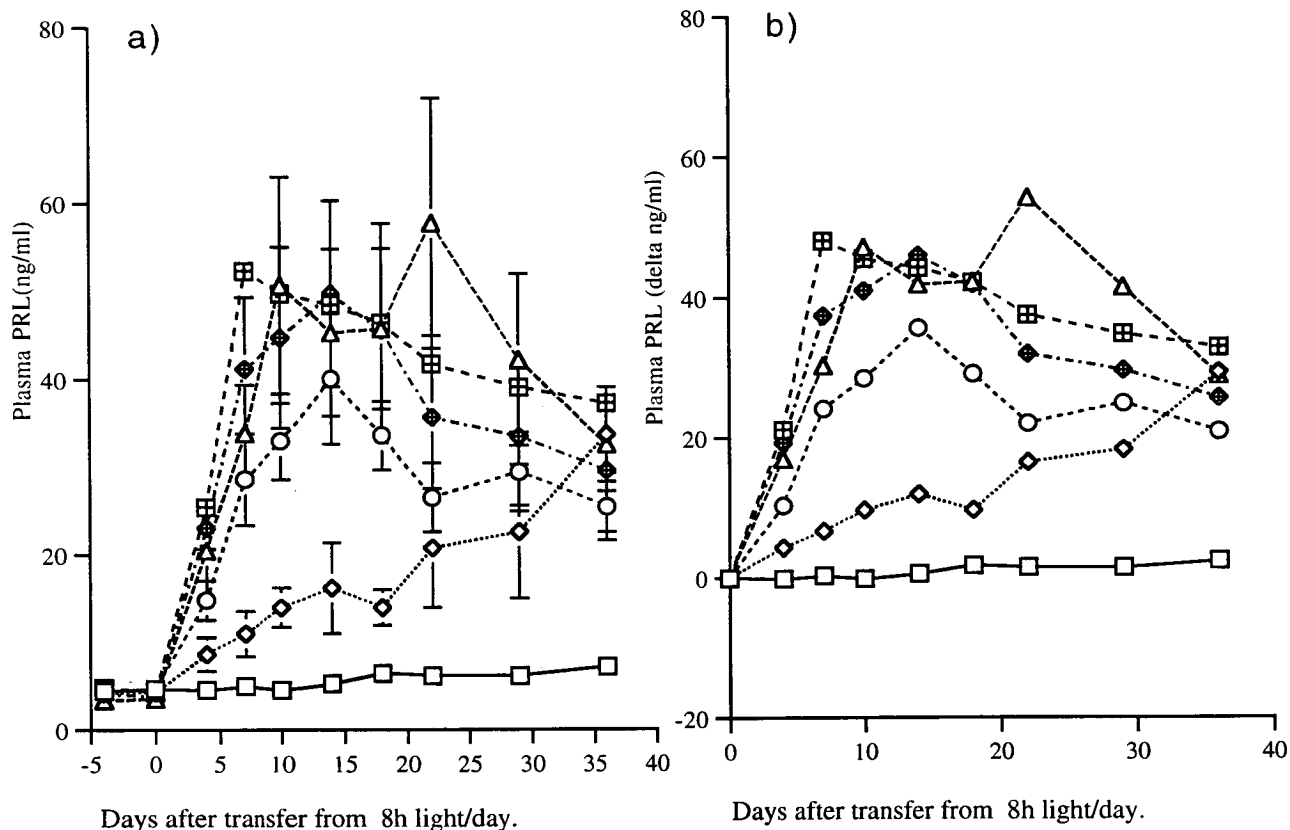
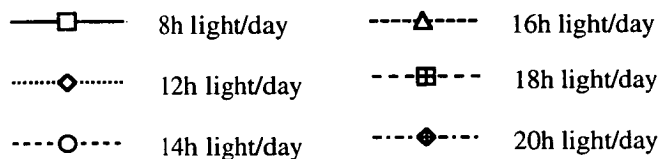


Fig. 5.4 Changes in plasma PRL in 16 week old castrated bantams reared on 8 h light/ day, after transfer from 8 h light/ day to 12, 14, 16 , 18 and 20 h light/ day expressed as a) concentration (ng/ ml) and b) as the change (delta) from the pre-photostimulation value (ng/ ml). Values are mean \pm sem ; n=8.



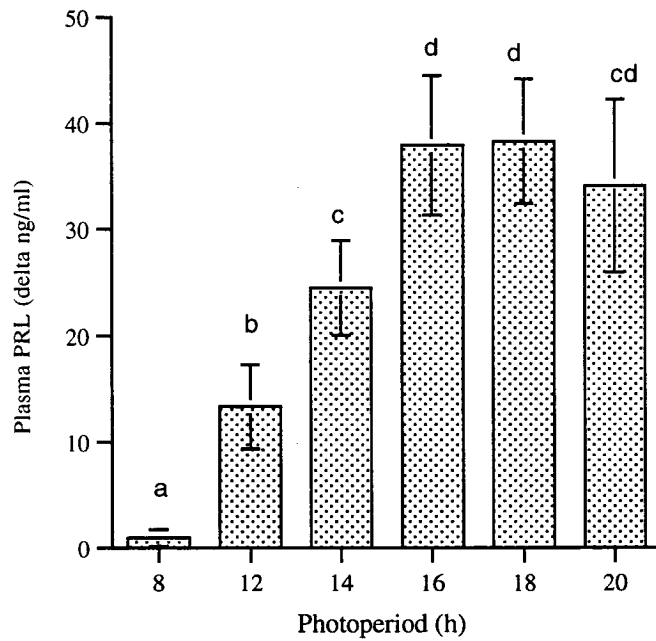


Fig. 5.5 A photoperiodic response curve for plasma PRL for castrated bantams reared on 8 h light/ day derived from composite changes in PRL concentrations after photostimulation. Values with different superscripts are significantly ($P < 0.05$) different (\pm sem; $n=8$)

photoinduced PRL secretion and to compare it with the saturation daylength for LH (Section 5.2).

Six groups (n=8) of birds castrated at 4 weeks were reared on short days (8L:16D). At 16 weeks, the groups were either retained on 8L:16D (control) or transferred to 12L:12D, 14L:10D, 16L:8D, 18L:6D and 20L:4D for 36 days. Blood samples were taken at 3-4 day intervals for LH and PRL measurements.

Changes in plasma PRL after transfer to the various photoperiods are shown in Fig. 5.4. The control birds on 8L:16D did not show any significant ($P>0.05$) change in plasma PRL concentrations throughout the experiment with values ranging between 4.64 ± 0.64 and 7.14 ± 1.67 ng/ml. A significant ($P<0.05$) increase in plasma PRL concentration was seen 4 days after transfer to photoperiods of 12, 14, 16, 18 and 20h. A further significant ($P<0.05$) increase in plasma PRL was observed after 7 days of photostimulation except for the birds transferred to 12L:12D, where a further significant ($P<0.05$) increase was only seen after 29 days of photostimulation. A composite PRC was constructed using incremental changes in PRL secretion after photostimulation (Fig. 5.5). This shows that photoperiods above 16h light/day were equally photostimulatory (Fig. 5.5). A submaximal PRL response was observed in birds transferred to 14 h light/day. Consequently, the saturation photoperiod for PRL release was identified as being between 14 and 16 h.

5.4 Estimation of critical daylength for PRL and LH secretion in birds reared on long days.

The objective of this study was to establish whether the critical daylength for PRL and LH release is increased when the birds are reared on long days as has been observed for LH in quail (Urbanski and Follett, 1982; Robinson and Follett, 1982).

The castrated bantams used for the study were reared on 20h light/day from hatch. They were caged in individual cages with free access to feed and water. At 16

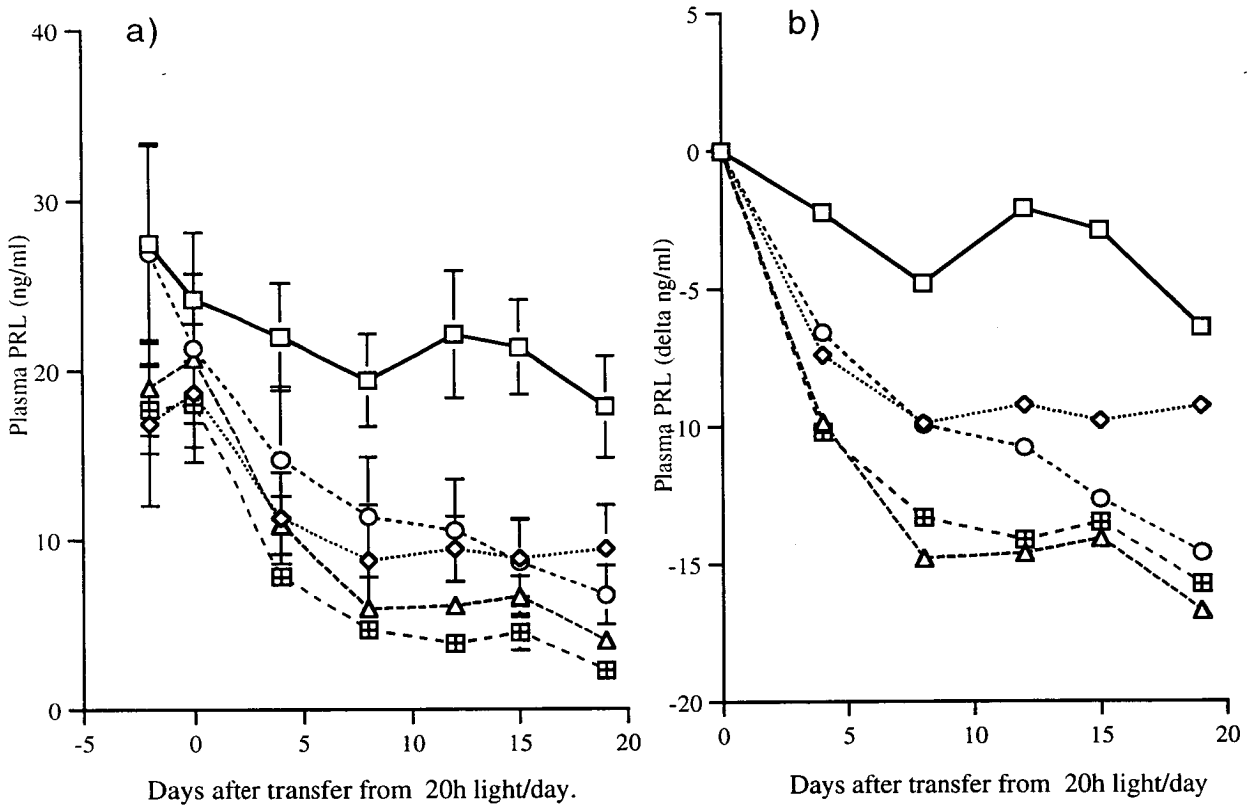


Fig. 5.6 Changes in plasma PRL in 16 week -old castrated bantams reared on 20 h light/ day and after transfer from, 20 h light/ day to 14, 12, 11 and expressed as a) concentration (ng/ ml) and b) as the change (delta) from the values on 20 h light/ day (ng/ ml). Values are mean \pm sem; n=8

—□— 20h light/day - - -△- - - 11h light/day
◇..... 14h light/day - - -■- - - 8h light/day
 - - -○- - - 12h light/day

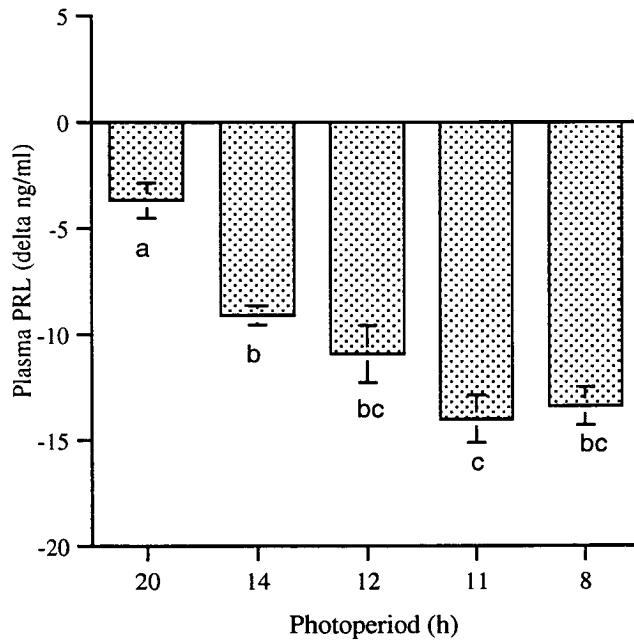


Fig. 5.7 Photoperiodic response curve for plasma PRL in castrated bantams derived from changes in the concentration of plasma PRL after transfer from 20h light/day to 14, 12, 11, and 8 h light/ day. Values with different superscripts are significantly ($p < 0.05$) different (\pm sem; $n=8$)

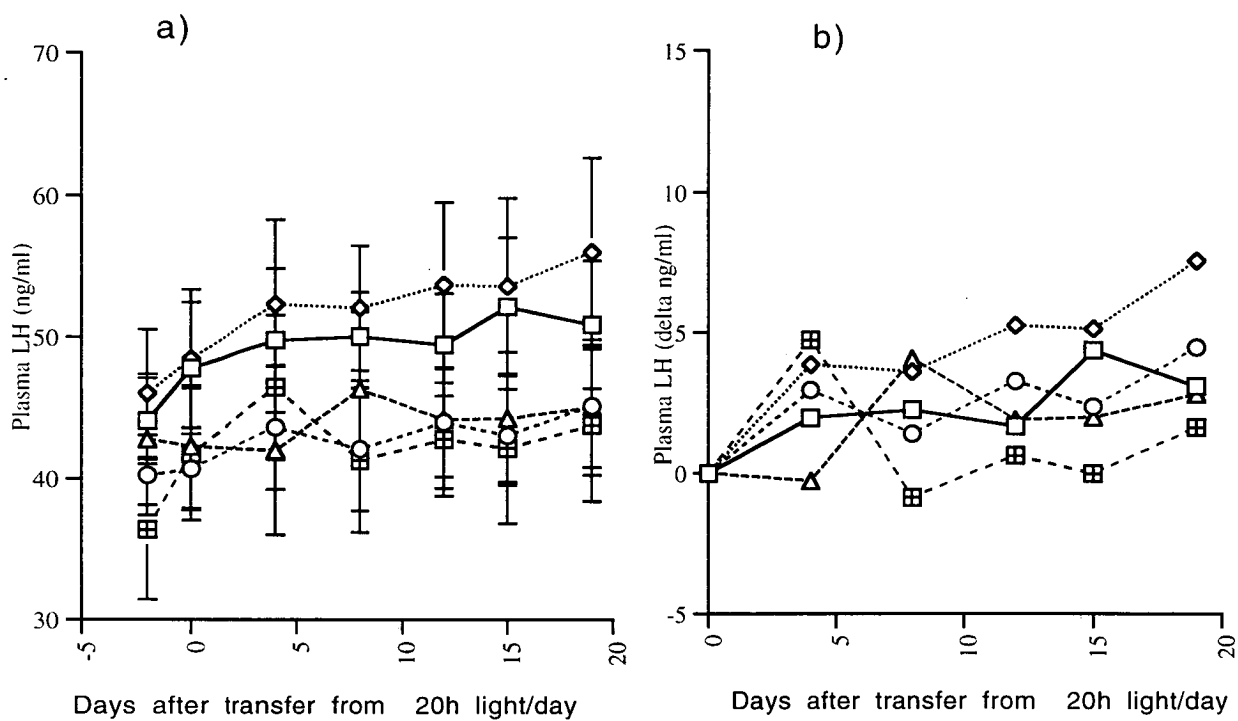
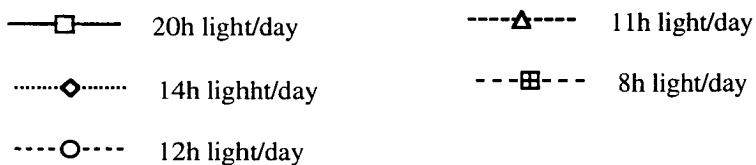


Fig. 5.8 Changes in plasma LH in 16 week -old castrated bantams after transfer from, 20 h light/ day to 14, 12, 11 and expressed as a) concentration (ng/ ml) and b) as the change (delta) from the values on 20 h light/ day (ng/ ml). Values are mean \pm sem; n=8.



weeks of age, groups of 8 birds each were transferred to one of the following light regimes: 14L:10D, 12L:12D, 11L:13D, 8L:16D or retained on 20L:4D. Blood samples were collected 2 days before, on the day of transfer, and at regular intervals of 3-4 days for a period of 19 days.

Changes in plasma PRL concentrations in birds transferred from long days to a range of shorter photoperiods are shown in Fig. 5.6. The control birds exposed to 20L:4D did not show any significant changes ($P>0.05$) during the 19-day experiment. A significant ($P<0.05$) fall in plasma PRL concentration was observed in all groups 4 days after transfer to shorter photoperiods. Thereafter, plasma PRL in the 14h group did not decrease significantly whereas plasma PRL in the 12, 11 and 8 h groups continued to decrease ($P<0.05$). A composite PRC taking into account all the decremental changes in PRL secretion after reducing the photoperiod is presented in Fig. 5.7. This shows that photoperiods of 14 h or less are not as stimulatory as a 20 h photoperiod while photoperiods of 12 h or less are equally non-photostimulatory (Fig. 5.7). It is concluded that the critical daylength for PRL release in birds reared on 20 h light/day is greater than 14 h.

Concentrations of plasma LH in castrated birds reared and maintained on long day lengths showed no significant ($P>0.05$) change after transfer to shorter photoperiods for 19 days (Figs. 5.8).

5.5 Discussion.

In the present study, the critical daylength for plasma PRL secretion could not be precisely defined, being between 10 and 12 h depending on the way the PRC was calculated (Fig. 5.2). This is shorter than in the starling, the only other avian species studied. In the latter species, the critical daylength for plasma PRL was 14-15 h (Goldsmith and Nicholls, 1984). The saturation daylength for photoinduced PRL release in the present study was between 14 and 16 h light/day (Fig. 5.5). Thus for daylengths

between 11 and 14 h there is a direct relationship between the concentration of plasma PRL and the photoperiod.

The critical daylength for plasma LH in the present study could not be defined accurately because LH concentrations were poorly responsive to changes in daylength in castrated birds. It appears that in long-term castrated cockerels the pituitary assumes a degree of autonomy in LH synthesis and release irrespective of the photoperiodic stimulus (Chapter 6). Since a PRC for LH secretion could not be constructed, comparison between PRCs for PRL and LH were done utilising data from previous studies in intact chickens. Observations on intact female dwarf broilers, intact female ISA Brown hens and White Leghorn hens show that the critical daylengths for LH release were 10.5 h, 10.5- 12.75 h and 10 h respectively (Dunn and Sharp, 1990; Sharp, 1988). The critical daylength for photoinduced LH release reported in castrated male quail (11-12 h; Urbanski and Follett, 1982), male starlings (11.5 h; Ebling *et al.*, 1982) and male and female starlings (11-13 h; Dawson, 1987) are similar to that reported for chicken (Dunn and Sharp, 1990; Sharp, 1988).

In intact female ISA Brown hens the saturation daylength for photoinduced plasma LH secretion lies between 12.75 and 15.25h and in intact female dwarf broiler chicken between 10.25 and 12.75 h (Dunn and Sharp, 1990). In castrated quail there is a direct relationship between the concentration of plasma LH and photoperiod between photoperiods of 12 and 14 h. (Urbanski and Follett, 1982). There is a direct relationship between the concentration of plasma LH for photoperiods between 10.25 and 12.75 h in the chicken (Dunn and Sharp, 1990). As a result, the slope of the PRC for photoinduced LH release is much steeper than that for photoinduced PRL release. The significance of this difference is discussed further in chapter 10 (General discussion).

The present study shows that, as for photoinduced LH release, critical, marginal and saturation daylengths for photoinduced PRL release are not fixed and are dependent on the previous photoperiodic history. Japanese quail (castrated males) maintained on

23h light/ day show a decrease in plasma LH when transferred to 13 h light/ day, even though 13 h light/ day is above the critical daylength for LH release in birds maintained on short days. This shift in the critical daylength depends on the photoperiodic history (Urbanski and Follett, 1982; Robinson and Follett, 1982). In the present study, such a shift could not be demonstrated for LH release but was seen for the photoinduced release of plasma PRL. The concentration of plasma PRL in birds reared on long days (20 h light/ day) was significantly ($P<0.05$) depressed after transfer to shorter photoperiods of 14, 12, 11 or 8 h. The critical daylength for birds reared and maintained on long days (20L:4D) therefore appears to be greater than 14 h, while for birds reared on short days (8L:16D) it is between 10-12 h. (Fig. 5.2).

The absence of a decrease in plasma LH in castrated bantams transferred from 20h light/ day to shorter daylength including 8 h light/ day must be a consequence of castration. This is in contrast to the studies in castrated quail where there is a marked decrease in plasma LH after transfer from long to short days (Urbanski and Follett, 1982). In long -term castrated bantams, pituitary LH secretion appears to become less dependent on hypothalamic control and to assume a degree of autonomy. This provides an explanation for the slow LH response (Fig. 5.6) or lack of LH response (Fig. 5.8) to a change in photoperiod. It is possible that if the 19 -day experiment involving transfer from long to short days (Fig. 5.8) had continued for as long as the 36 -day experiment involving transfer from short to long days, a decrease in photoperiod might have been observed to have a depressive effect on the concentration of plasma LH.

EVIDENCE FOR THE DEVELOPMENT OF PHOTOREFRACTORINESS FOR PRL SECRETION.

6.1 Introduction

Reproductive behaviour is cyclic in most temperate zone birds. In Spring the avian reproductive system is stimulated by increasing daylengths, but after prolonged exposure to long days, the gonads regress. These photoinduced changes in the reproductive system are reflected in correlated changes in the concentrations of plasma follicle stimulating hormone (FSH) and LH. The condition in which birds become insensitive to photoperiods which were previously stimulatory is termed photorefractoriness (Section 1.3.4) and photorefractoriness is dissipated by exposure to short days (Section 1.5.5). The development of photorefractoriness for FSH and LH release is accompanied by development of photorefractoriness for PRL secretion (Section 1.3.4). The main difference in the development of photorefractoriness for gonadotrophin and PRL secretion is that plasma PRL tends to increase and decrease more slowly than plasma LH after photostimulation and peaks at a time when plasma LH concentrations are falling (Section 1.3.4). Photoinduced PRL secretion and the subsequent development of photorefractoriness for PRL have been described in several wild avian species (Lincoln *et al.*, 1980; Ebling *et al.*, 1982; Sharp *et al.*, 1986a) and in the turkey (Lea and Sharp, 1982; El Halawani *et al.*, 1983, 1984b; Lien and Siopes, 1989) but no studies on recovery of photosensitivity for plasma PRL secretion have been reported. There is evidence for reproductive refractoriness in chickens where it takes the form of relative refractoriness (Section 1.5.4). The development of this condition appears to be associated with a progressive fall in plasma LH but it is unknown whether it is associated with a decrease in PRL release.

The present experiments were done to establish a) whether in castrated bantams prolonged exposure to long days results in a depression of PRL secretion, indicative of the development of photorefractoriness and if so, b) whether exposure to short days results in the recovery of photosensitivity for PRL release. The data in these experiments were subjected to logarithmic transformation to normalise the variations before analysis using split -plot ANOVA. The differences between means were tested using Fisher's protected least significant difference test. Student's *t*-tests were used to compare values between groups of birds.

6.2 Effect of transfer from short days to a range of photostimulatory daylengths for 148 days.

Castrated birds were reared on short days (8 h light/ day) from hatch and, at 16 weeks, groups of 8 birds were transferred to 12, 14, 16, 18 and 20 h light/ day. A control group was retained on 8 h light/ day. Birds were housed in individual cages. Blood samples were collected before transfer to longer photoperiods and thereafter at regular intervals of 7 days for 36 days, and then every 14 days for a further 112 days.

Transfer from short to long days induced a significant ($P < 0.05$) increase in PRL secretion (Fig. 6.1a). Plasma PRL decreased progressively 36 days after transfer from short days to photoperiods of 14 h or more. After 148 days, with the exception of birds exposed to 14 h light/ day, plasma PRL stabilised at low values which were significantly ($P < 0.05$) higher than in short day control birds. Plasma PRL in birds exposed to 14 h light/ day decreased to the same concentration as the short day control birds after 148 days of photostimulation (Fig. 6.1a). Plasma PRL increased more slowly after transfer to 12 h light/ day than after transfer to longer photoperiods. After 148 days, plasma PRL was higher in birds exposed to 12 h light/ day than in birds exposed to longer photoperiods. There was no significant ($P > 0.05$) change in the plasma PRL in control birds exposed to 8 h light/ day during the experiment.

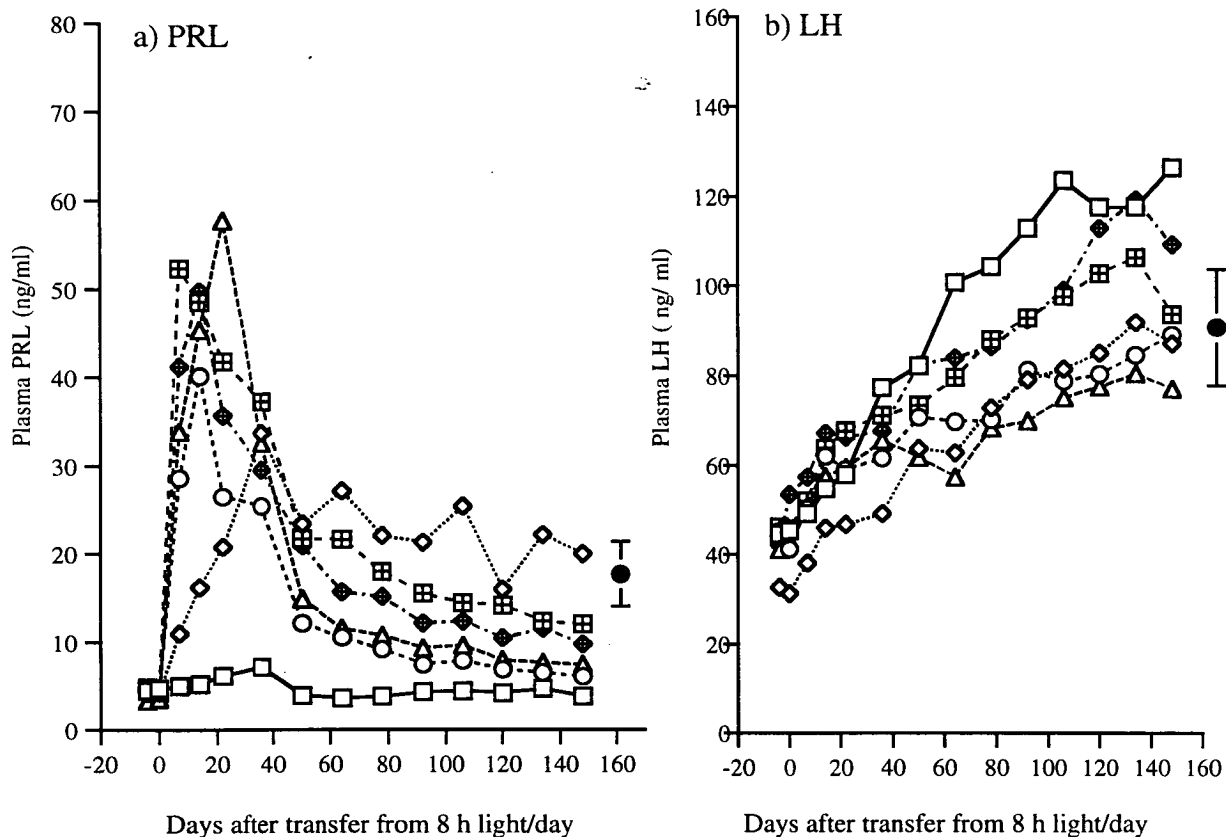


Fig. 6.1 The concentration of a) plasma PRL and b) plasma LH, in castrated somatically mature bantam cockerels, after transfer at 16 weeks of age from 8 h light/ day to range of fixed long days. The birds were castrated at 4 weeks of age and were reared on 8 h light/ day (means \pm sem; n=8). The inserts show the mean and sem for all the hormones measurements in the experiment. The SEMs have otherwise been omitted for clarity.

- 8h light/day
-◇..... 12h light/day
- 14h light/day
- mean and sem for all the values.
- △--- 16h light/day
- 18h light/day
- ◆--- 20h light/day

Plasma LH increased significantly ($P < 0.05$) in both the short day control and photostimulated groups during the experiment (Fig. 6.1b). As a result, there were no significant ($P > 0.05$) differences in plasma LH between the short day control and photostimulated groups.

6.3 Evidence for the development of absolute photorefractoriness for PRL secretion in birds exposed to 16 h but not 12 h light/ day for 148 days.

Castrated bantams which had been reared on short days (8 h light/ day) and transferred to 12 or 16 h light/ day for 148 days (Section 6.2) were given an additional 4 h light/ day. A control group was included which had been transferred from short days to 20 h light/ day for 148 days and was maintained on 20 h light/ day. The birds were transferred to the increased photoperiods for 32 days. Blood samples were collected at the time of transfer and at intervals of 3-4 days thereafter. At the beginning of the experiment, plasma PRL was higher in birds exposed to 12 h light/ day than in birds exposed to 16 or 20 h light/ day (Fig. 6.2).

A 4 h increase in photoperiod resulted in a significant increase ($P < 0.05$) in plasma PRL in birds initially exposed to 12 h light/ day (Fig. 6.2), reaching a peak after 7 days. Thereafter plasma PRL decreased progressively and, after a further 11 days, was not significantly ($P > 0.05$) different from the value before the photoperiod was increased (day 0). After 18 days of photostimulation, plasma PRL concentrations decreased to values seen in control birds exposed to 20 h light/ day and in birds transferred from 16 to 20 h light/ day (Fig. 6.2). The concentration of plasma PRL did not increase in birds transferred from 16 to 20 h light/ day (Fig. 6.2). No significant ($P > 0.05$) change occurred in the concentration of plasma PRL in control birds maintained on 20 h light/ day during the experiment.

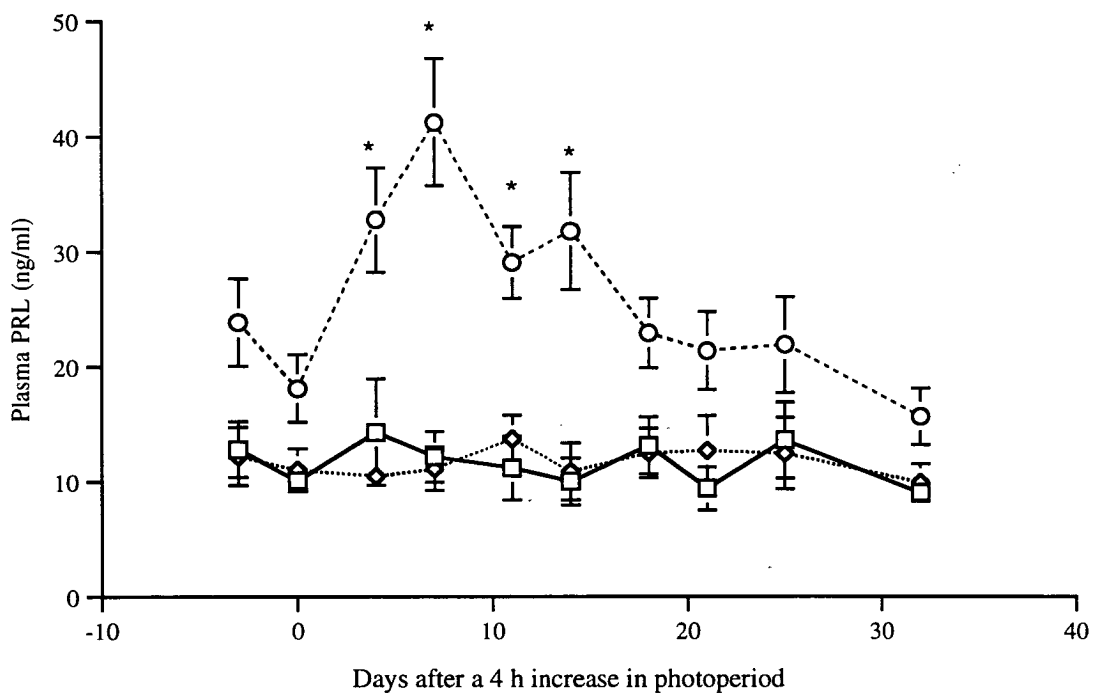


Fig. 6.2 Changes in the concentrations of plasma PRL in castrated bantams previously exposed to 12 or 16h light/ day for 160 days, after increasing the photoperiod by 4 h for 32 days. The control group of birds had been previously exposed to 20 h light/ day for 160 days.* $P < 0.05$ compared with the values before photoperiod was increased (day 0). Values are mean \pm sem; n=6

—□— 20h light/ day (controls)
◇..... increased from 16 to 20h light/ day
 ----○---- increased from 12 to 16h light/ day

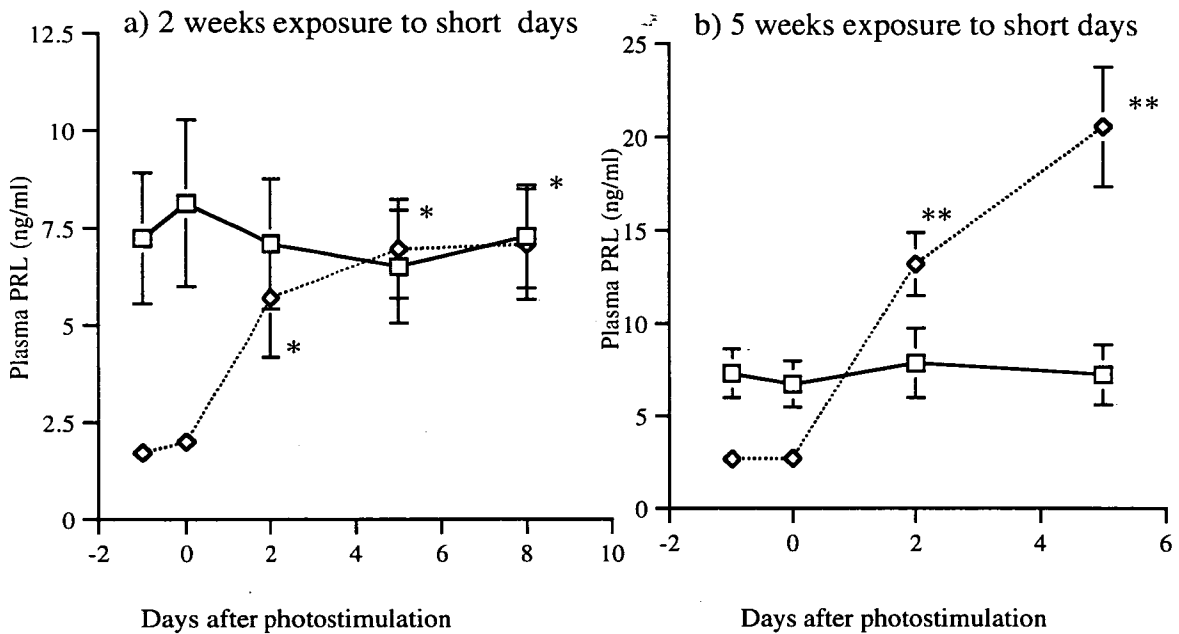


Fig. 6.3. The dissipation of photorefractoriness for PRL secretion after exposure to short days in castrated bantams previously exposed to long days (20L:4D) (.....◇.....). Changes in concentration of plasma PRL after transfer from 20 h light/ day to 8 h light/ day for a) 2 weeks or b) 5 weeks and then back to 20 h light/ day. The control groups (—□—) were maintained on 8 h light/ day.* $P < 0.05$,** $P < 0.01$ compared with the values before photostimulation. Values are mean \pm sem; $n=6$.

6.4 Recovery of photosensitivity for photoinduced PRL secretion after exposure to short days.

Castrated bantams (>45 weeks of age) which had been exposed to 20 h light/ day for 160 days were exposed to 8 h light/ day for 2 or 5 weeks and than transferred back to 20 h light/ day for 8 or 5 days respectively to test for the recovery of photosensitivity for photoinduced PRL secretion. Two groups of castrated bantams (n=6) of the same age, reared and maintained on 8 h light/ day were used as the control groups for the experiments. Blood samples were collected immediately before and after transfer from short days to 20 h light/ day every 2-3 days for 5- 8 days.

The concentration of plasma PRL was significantly lower ($P<0.05$) in birds transferred from 20 h light/ day to 8 h light/ day than in the short day controls (Fig. 6.3). Rephotostimulation with 20 h light/ day significantly ($P<0.05$) increased the concentration of plasma PRL in both the experimental groups (Fig. 6.3). The concentration of plasma PRL in the group exposed to short days for 2 weeks increased to the concentrations seen in the short day controls, whereas the concentration of plasma PRL in the group exposed to short days for 5 weeks increased to higher values, well in excess of those in the short day controls (Fig. 6.3).

6.5 Discussion

The pattern of increased plasma PRL concentrations observed after photostimulation followed by a decrease indicates an apparent development of photorefractoriness for PRL secretion in castrated bantams exposed to long photoperiods (>12 h light/ day). This observation was unexpected because there is no evidence for the development of absolute reproductive refractoriness in chickens. However, Sharp (1988) showed that prolonged exposure of bantam hens to 14 h light/ day resulted in almost complete cessation of egg production. Egg production was

stimulated either by transfer to 20 h light/ day or by transfer to 8 h light/ day for 5 weeks and back to 14 h light/ day. This indicated that the bantams became relatively photorefractory for reproductive function. In earlier studies, photorefractoriness for PRL release has only been observed in species showing absolute reproductive refractoriness (e.g. turkeys, El Halawni *et al.*, 1984a; starlings, Dawson and Goldsmith, 1983; rooks Lincoln *et al.*, 1980 and partridges, Sharp *et al.*, 1986a). In contrast to this study, in quail, there is no fall in plasma PRL concentration after exposure to a prolonged period of long days (Juss, 1993).

A feature of the mechanism involved in the development of photorefractoriness is that it is not readily induced if birds are photostimulated with daylengths that are close to the critical daylength (Nicholls *et al.*, 1988; Sharp, 1993). For example, willow ptarmigan, transferred from short days (6 h light/ day) to 14 h light/ day, remained photosensitive with elevated plasma LH until the end of the study (20 weeks), while the willow ptarmigan transferred to 18 h light/ day developed photorefractoriness after 10 weeks of photostimulation (Stokkan *et al.*, 1982). A further example is provided by the study in dwarf broilers in their second egg laying cycle (Sharp, *et al.*, 1992). Birds transferred from short days (3 h light/ day) to 20 h light/ day showed an early peak in egg production followed by a rapid decrease in egg output. In contrast, birds transferred from 3 h light/ day to 11 h light/ day came into lay slowly, but egg production was more persistent than in the birds transferred to 20 h light/ day. Thus after 32 weeks of photostimulation egg production in hens exposed to 20 h light/ day decreased to the level of the short day controls, while the egg output in birds on 11 h light/ day remained significantly greater. These data suggest that photostimulation with 11 h light/ day, which is close to the critical photoperiod for LH secretion in chickens (Chapter 5), is sufficient to stimulate reproductive function without inducing the development of photorefractoriness (Sharp *et al.*, 1992). In starlings the critical photoperiod for LH release lies between 11 and 13 h light/ day and hence birds held on 11 h light/ day never

become photorefractory (Dawson *et al.*, 1985). Thus, photoperiods close to the critical daylength induce breeding condition slowly and also prolong the breeding condition for an extended period. The critical daylength for plasma PRL secretion for castrated bantam lies between 10 and 12 h light/ day (Chapter 5). The slow progressive increase in plasma PRL levels in the birds transferred to 12 h light/ day, and the prolonged maintenance of high plasma PRL secretion on this photoperiod, shows that, as for photoinduced LH secretion, photorefractoriness for photoinduced PRL secretion does not develop when the birds are exposed to a marginally stimulatory daylength.

The increase in plasma LH in the short day castrates in the absence of photostimulation indicates a photoperiod-independent mechanism and it appears to be due to the removal of the negative feedback effects of steroids on LH synthesis or release and an assumption of a degree of autonomy by the pituitary, as has been discussed previously (Chapter 5).

The increase in plasma PRL in birds exposed to 12 h light/ day, but not 16 h light/ day (an additional 4 h photostimulation), indicates that the birds on 12 h light/ day were photosensitive and those on 16 h light/ day were absolutely photorefractory. The increase in plasma PRL in re-photostimulated birds on 12 h light/ day was less persistent than in birds transferred from 8 h to 12 h light/ day (Section 6.2). This indicates that the process leading to the development of photorefractoriness is set in motion after exposure to 12 h light/ day but is not completed. Increased photostimulation appears to accelerate the inhibitory effect of long days, allowing the complete development of photorefractoriness. These observations contrast with studies on the development of photorefractoriness for plasma LH secretion in chickens. As far as photoinduced LH release is concerned, there is evidence for the development of relative but not absolute photorefractoriness (Sharp, 1988, 1993). It seems that the mechanism controlling the development of photorefractoriness may differ between PRL and LH release in the bantam. This view is further supported by studies in intact male and female bantams in

which photostimulation induced an increase and a subsequent decrease in plasma PRL but not in plasma LH (Chapter 8).

The rate at which photosensitivity is regained under short day lengths, as demonstrated by measuring the concentration of plasma LH, depends on the species and shortness of the short days (Nicholls *et al.*, 1988, Sharp, 1993, 1996). In the present study, the recovery of photosensitivity for photoinduced PRL release after exposure to short days was measured as the magnitude of the increase in concentration of plasma PRL after transfer back to long days (Fig. 6.3). It was observed in photorefractory birds that 2 weeks of exposure to short days resulted in a partial recovery of photosensitivity, with the magnitude of the photoinduced increase in plasma PRL being small. A full recovery of photosensitivity appeared to occur in photorefractory birds photostimulated after 5 weeks of exposure to short days. In these birds, the photoinduced increase in plasma PRL was similar to that observed after photostimulation in castrated bantams reared on short days (Chapter 5, Section 6.2).

Photorefractory starlings regain photosensitivity for LH secretion after exposure to 28-35 short days and it is marked in castrated birds by a spontaneous increase in plasma LH (Dawson, 1991; Boulakoud and Goldsmith, 1994). It remains to be established whether an increase in plasma PRL marks the recovery of photosensitivity in photorefractory birds transferred to short days. Exposure of castrated chickens reared on long days (20 h light/ day) to 19 short days (8 h light/ day, Chapter 5) did not result in a spontaneous increase in plasma PRL. It is possible that such an increase would have been observed if photorefractory birds had been held on short days for a longer period.

In conclusion it was established, in castrated bantams, that photorefractoriness develops for photoinduced PRL secretion after prolonged exposure to photoperiods longer than 12 h light/ day. It was also established that castrated bantams become absolute photorefractory for PRL secretion. A marginally stimulatory photoperiod of 12 h light/ day induces PRL secretion for a prolonged period without inducing

photorefractoriness. Dissipation of this apparent photorefractoriness and recovery of photosensitivity for plasma PRL secretion was achieved after 2 to 5 weeks of exposure to short days.

PHOTOINDUCED PRL RELEASE IN RESPONSE TO A SINGLE LONG DAY.

7.1 Introduction

The photoperiodic response often seems slow because photoinduced changes, such as gonadal growth, take weeks to complete. However, in birds, the underlying neuroendocrine mechanisms may respond within a few hours to a change in photoperiod. For example, in the Japanese quail, an increase in concentrations of plasma LH and FSH is observed 20-22 h after dawn of the first long day (Follett *et al.*, 1977; Nicholls *et al.*, 1983). This increase in LH concentration continues for about 10 days as a 'carry over effect' if quail are returned to short days after exposure to one long day (Follett *et al.*, 1977; Nicholls *et al.*, 1983; Follett and Pearce-Kelly, 1991; Perera and Follett, 1992; Meddle and Follett, 1995). It has not been established whether photoinduced PRL release similarly occurs after exposure to one long day.

The present experiment was undertaken to establish a), whether PRL secretion in the chicken is induced by exposure to one long day b), to determine the time after the 'dawn' of the first long day at which PRL secretion increases and c), to determine whether photoinduced PRL release is associated with a 'carry over effect' after transfer back to short days.

7.2 First -day photoinduced PRL release.

The experiments were carried out on castrated cockerels (65 weeks-old) which had been used in other photoperiodic experiments. Before being used for this experiment, the birds had been held on short days (8 h light/ day) for more than 3 months to allow

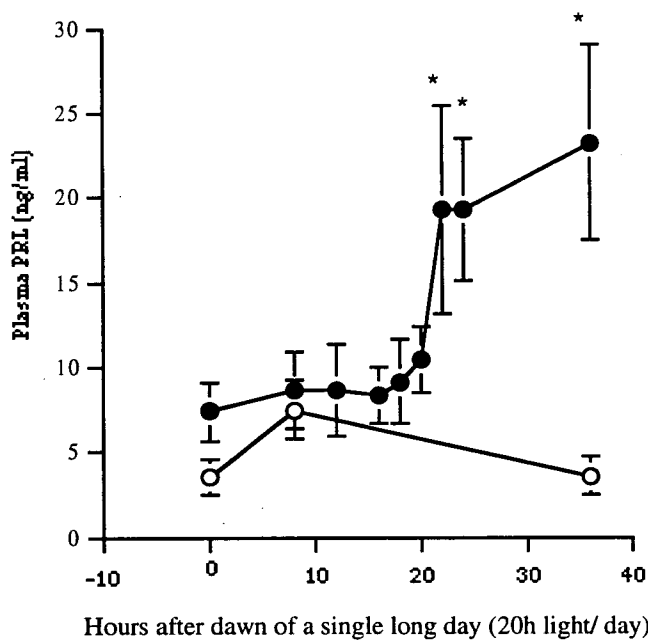
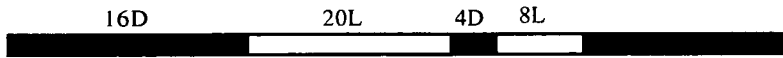


Fig. 7.1 Changes in concentration of plasma PRL in castrated cockerels held on short days (8h light/ day) after exposure to one 20h photoperiod and returned to short days (—●—) or in control birds (—○—) maintained on short days. * $P < 0.05$ compared with values before photostimulation (mean \pm sem; $n=8$).

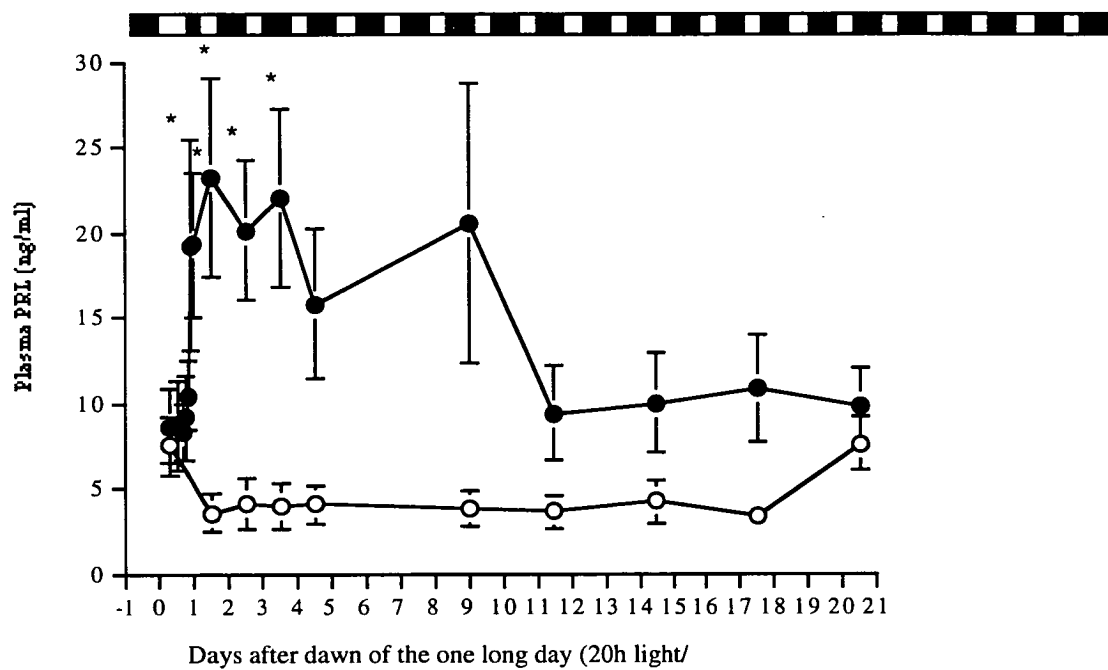


Fig. 7.2 Changes in concentration of plasma PRL in cockerels after exposure to one long day (20h light/ day) and return to short days (—●—). Control birds (—○—) were maintained on short days throughout the study. Plasma PRL shown for 21 days after dawn of the one long day. The bar on the figures indicate the hours of light and darkness during the 21 day experiment. * $P < 0.05$ with values before photostimulation (mean \pm sem; n=8)

full recovery of photosensitivity. The birds were housed in individual cages with free access to feed and water. Groups of 8 birds were transferred to one long day (20L: 4D, experimental) and then returned to short days (8L: 16D), while the control group of 8 birds were kept on short days throughout the experiment. In order to make it more convenient for the collection of blood samples, lights were switched on at 0100 h (dawn). This involved moving the time of 'dawn' back by 8 h whilst the birds were held on 8L: 16D for 2 weeks before photostimulation. Blood samples were collected at the 'dawn' and then at 2 h intervals beginning 8 h later for 24 h. After return to short days, blood samples were taken at 3-4 day intervals for 21 days. Where it was necessary to take blood samples in the dark, the bird was taken to a darkened area outside the room in which it was housed and a dim light was used to locate the wing vein.

Transfer from short days to a single 20 h photoperiod resulted in an increase ($P<0.05$) in plasma PRL beginning 20 -22 h after 'dawn' (Fig. 7.1). The concentration of plasma PRL increased to a peak 36 h after 'dawn', at which time it had increased 3 -fold (Fig. 7.1). After birds were returned to short days (8L: 16D), the concentration of plasma PRL remained significantly elevated ($P<0.05$) for 4 days (Fig. 7.2). Although concentration of plasma PRL remained elevated for a further 5 days (9th day after 'dawn'), it was not statistically significant ($P>0.05$) from PRL values measured at 'dawn'. The concentration of plasma PRL returned unambiguously to pre-photostimulation values 11 days after exposure to one long day.

7.3 Discussion.

The experiment demonstrates that the photoperiodic induction of plasma PRL secretion occurs about 20-22 h after 'dawn' of the single long day. Similar studies on photoinduced gonadotrophin secretion in intact immature male quail indicate that the photoperiodic induction of plasma LH and FSH also occurs 20-22 h after 'dawn' of a single long day (Follett *et al.*, 1977). Studies carried out on castrated quail confirmed that

the photoperiodic induction of LH occurs between 18-24 h or 22-26 h after 'dawn' (Follett and Pearce-Kelly, 1991; Perera and Follett, 1992). Studies *in vitro* on superfused quail hypothalamic explants indicated that the photoinduced increase in LH secretion in the first long day was due to an increase in GnRH release (Perera and Follett, 1992). This observation demonstrates that the mechanism responsible for photoinduced LH release involves brain centres above the level of the anterior pituitary gland. It is well established in birds that VIP is the PRL releasing hormone (Section 1.4.1). Although there is no direct evidence to show that VIP release is increased after exposure to one long day, it is probable that this is the case. This view is supported by the observation that active immunisation against VIP blocks photoinduced PRL release (Chapter 9).

First -day release studies in quail indicate that the magnitude of the photoinduced LH response depends upon the length of the single long day. Castrated quail photostimulated with one 14 h photoperiod did not show a significant rise in plasma LH within 24 h of 'dawn', whereas plasma LH increased at this time in birds exposed to single 17 or 20 h photoperiods (Follett and Pearce-Kelly, 1991). The critical daylength for LH release in quail is between 11-12 h (Urbanski and Follett, 1982). It therefore appears that between this critical daylength and 17 h after 'dawn' of the first long day a cascade of photoinduced neuroendocrine events occurs culminating in the release of GnRH. The reason for the sluggishness of this neuroendocrine response is not known but it may reflect the time required for *de novo* gene transcription and protein synthesis. The critical daylength for photoinduced PRL secretion in the bantam is between 10-12 h (Section 5.2), while an increase in plasma PRL was observed between 20-22h after 'dawn' of the first long day. This interval between the critical daylength and the increase in PRL release is therefore similar to that observed in the quail for the first -day-induced LH release. The similarities in the time courses for first -day PRL and LH release suggest that the neural mechanisms transducing photoperiodic information to VIP or GnRH neurons may be similar. The extent to which a common pathway is involved is

uncertain, partly because knowledge of the avian biological clock and extra-retinal photoreceptor is incomplete (Section 1.5).

A common feature of the 'first day' photoperiodic responses for LH and PRL is the 'carry over effect', where photoinduced hormone release continues for several days in response to one stimulatory photoperiod (Follett *et al.*, 1977; Nicholls *et al.*, 1983; Follett and Pearce-Kelly, 1991; Perera and Follett, 1992; Meddle and Follett, 1995). Experiments on quail shows that the 'carry over' phenomenon is mediated, at least in part, by the central nervous system. Thus, surgical deafferentation of the basal hypothalamus 18 h after the 'dawn' of the first long day completely blocks the photoperiodic increase in LH secretion (Follett *et al.*, 1977). Furthermore, GnRH is released *in vitro* from hypothalamic blocks, taken on the third short day after exposure to a single long day, at a higher rate than from the control hypothalami taken from non - photostimulated birds (Perera and Follett, 1992). It is therefore likely that the 'carry over' effect observed for photoinduced PRL release is also controlled at the level of the hypothalamus, through the release of VIP.

In conclusion, the time-course of the 'first day release' and the 'carry over' phenomena observed for photoinduced PRL release in castrated bantams show similarities with the pattern of first day LH release in quail.

CHANGES IN CONCENTRATIONS OF PLASMA PRL AND LH IN INTACT CHICKENS AND TURKEYS OF BOTH SEXES DURING A PHOTOINDUCED BREEDING CYCLE.

8.1 Introduction.

In chapters 4, 5 and 6 it was shown that an increase in photoperiod stimulates both LH and PRL secretion in intact juvenile and somatically mature castrated bantams. This Thesis demonstrates for the first time (Chapter 6) that photoinduced PRL secretion in the bantam also demonstrates the feature of photorefractoriness. This phenomenon was observed in castrated bantams but it is not known whether this apparent development of photorefractoriness for PRL release also occurs in the presence of the gonads, since gonadal steroids exert a direct stimulatory effect on PRL secretion (Chapter 3).

The present investigation was therefore undertaken to determine whether prolonged exposure of intact bantams and turkeys of both sexes results in the development of photorefractoriness for both photoinduced PRL and LH release. A comparison was made between bantams and turkeys because turkeys are known to become absolutely photorefractory (El Halawani *et al.*, 1984a), whereas bantams become relatively photorefractory (Sharp, 1988). An unimproved breed of turkey was used for the study because it was believed that it was likely to exhibit a clearer development of photorefractoriness than commercial breeds. A comparison was made between males and females to obtain a better understanding of the relative roles of testicular and ovarian steroids in the control of PRL secretion. Since chickens kept on short days for a prolonged period come into lay (Sharp, 1993), a group of female bantams maintained on short days were included in the study so as to provide a better understanding of the

relationship between PRL secretion and photoperiodically-independent and dependent egg production.

8.2 Effect of prolonged exposure to long days on plasma PRL and LH in intact male and female bantams.

Male (n=15) and female (n=11) bantam chicks were reared on a short photoperiod of 8 h light/ day from hatch. Experimental birds comprising 8 males and 6 females were transferred to individual cages at 12 weeks of age and were kept on 8 h light/ day until 16 weeks of age. At this time they were photostimulated by transferring them to 20 h light/ day for 105 days. Control birds comprising 7 males and 5 females were transferred to individual cages at 12 weeks of age and maintained on 8 h light/ day throughout the experiment. Blood samples were collected before photostimulation and at regular intervals of 2 weeks thereafter. Blood samples were taken from the short day control group at the same times. Egg-laying records were kept throughout the study.

The concentration of plasma PRL in photostimulated female bantams was significantly higher ($P<0.001$, ANOVA repeated measure followed by Fishers's protected least squares difference) than in photostimulated males (Fig. 8.1a). The concentration of plasma PRL in the females increased significantly ($P<0.001$) after photostimulation, peaked after 35 days, and remained high until 77 days post-photostimulation; thereafter plasma PRL began to decrease (Fig. 8.1a). At the end of the study, after 105 days photostimulation, the concentration of plasma PRL was lower ($P<0.05$) than the value between 35-77 days of photostimulation but higher ($P<0.05$) than before photostimulation, and higher than in the short day control females (Fig. 8.1a).

After photostimulation, males showed the same pattern of change in plasma PRL concentration as seen in the photostimulated females, but in a less pronounced manner. The concentration of plasma PRL increased significantly ($P<0.01$) after

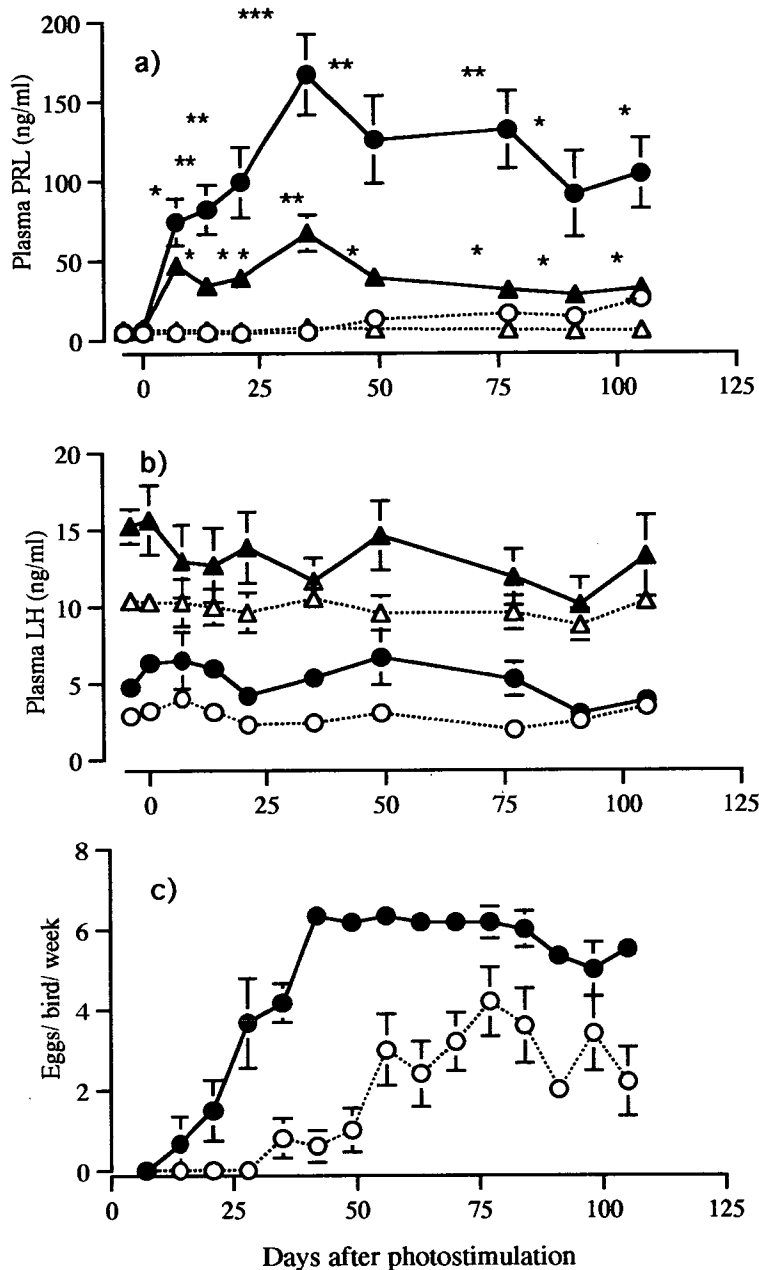


Fig. 8.1 Changes in concentrations of a) plasma PRL and, b) plasma LH in intact male (—▲—, n=8 and female (—●—, n=6) bantams and in c) egg production in the female bantams reared from hatch on 8 h light/ day and transferred to 20 h light/ day at 16 weeks of age. Control intact females (—○—, n=5) and intact males (—△—, n=7) were retained on 8 h light/ day. Values are means ± sem. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ compared with the pre-photostimulation values.

photostimulation, reaching a peak at 35 days, and then decreased ($P<0.01$). However, at the end of the study, after 105 days of photostimulation, the concentration of plasma PRL remained significantly ($P<0.05$) higher than before photostimulation and higher than in the control short day males (Fig. 8.1a)

Control female bantams kept on short days showed a small but significant ($P<0.05$) increase in plasma PRL 75 days after the experimental hens had been photostimulated, and levels remained elevated until the end of the study. Control male bantams kept on short days did not show any significant ($P>0.05$) change in plasma PRL concentration throughout the study (Fig. 8.1a).

Concentrations of plasma LH in bantams transferred to 20 h light/ day at 16 weeks, or maintained on short days, are shown in Fig. 8.1b. Photostimulated females had significantly ($P<0.005$, ANOVA repeated measures) higher concentrations of plasma LH than their short day controls. In contrast, there were no significant differences in LH concentrations between short and long day males. The concentration of plasma LH in photostimulated males was significantly higher ($P<0.001$) than in photostimulated females. Similarly, in the control groups kept on short days, plasma LH concentrations in the males were significantly higher ($P<0.001$) than in the females.

After photostimulation, egg production was stimulated and reached a peak at 42 days; thereafter it remained unchanged ($P>0.05$) until the end of the study. Hens started to lay after 14 days of photostimulation and 50 % were in lay after 21 days. Control birds maintained on short days began to lay 28 days after the photostimulated hens and 50 % were in lay at 22 weeks of age (Fig. 8.1c). All the photostimulated and short day control birds eventually came into lay. The peak of egg production in the short day control hens was lower than that in the photostimulated hens (Fig. 8.1c). Overall mean egg production (Fig. 8.1c) during the 105 day experiment was significantly higher ($P<0.001$) in the photostimulated (4.61 ± 0.44 eggs/ bird/ week) than the non-photostimulated hens (1.76 ± 0.52 eggs/ bird/ week).

8.3 Effect of prolonged exposure to long days on plasma PRL and LH in intact male and female turkeys.

Spotted Nebraska turkeys (8 males and 8 females) were reared from hatch on long days (14 h light/ day) in floor pens. At 16-18 weeks of age the birds were transferred to two separate pens on short days (7 h light/ day), each containing 4 males and 4 females, to dissipate juvenile refractoriness. The photoperiod was increased to 14 h light/ day when the birds were 28-30 weeks of age to induce sexual maturation. Blood samples were collected immediately before photostimulation and then at intervals of 2 weeks for 189 days. Daily egg production records were maintained for each pen throughout the study. Plasma LH and PRL were assayed using chicken LH and chicken PRL radioimmunoassays (Sections 2.4.1 and 2.4.2).

The concentrations of plasma PRL after photostimulation in females were significantly ($p < 0.005$, ANOVA repeated measures) higher than in the males (Fig. 8.2a). Plasma PRL progressively increased after photostimulation and reached peak values after 63 days. After 100 days photostimulation, plasma PRL concentrations began to decrease gradually and were not significantly ($P < 0.05$) different from the pre-photostimulation values after 161 days (Fig. 8.2a). Thereafter, reduced concentrations of plasma PRL were maintained until the end of the study. In males, the concentration of plasma PRL increased after photostimulation and reached a peak after 63 days. Thereafter, plasma PRL remained significantly ($P < 0.01$) elevated for 119 days and then decreased, returning to pre-photostimulation values (Fig. 8.2a).

The concentrations of plasma LH were significantly ($P < 0.005$, ANOVA repeated measures) higher in the male than the female turkeys (Fig. 8.2b). In the males plasma LH increased to a maximum concentration ($P < 0.01$) 21 days after photostimulation and remained unchanged for 49 days. Thereafter the concentration of

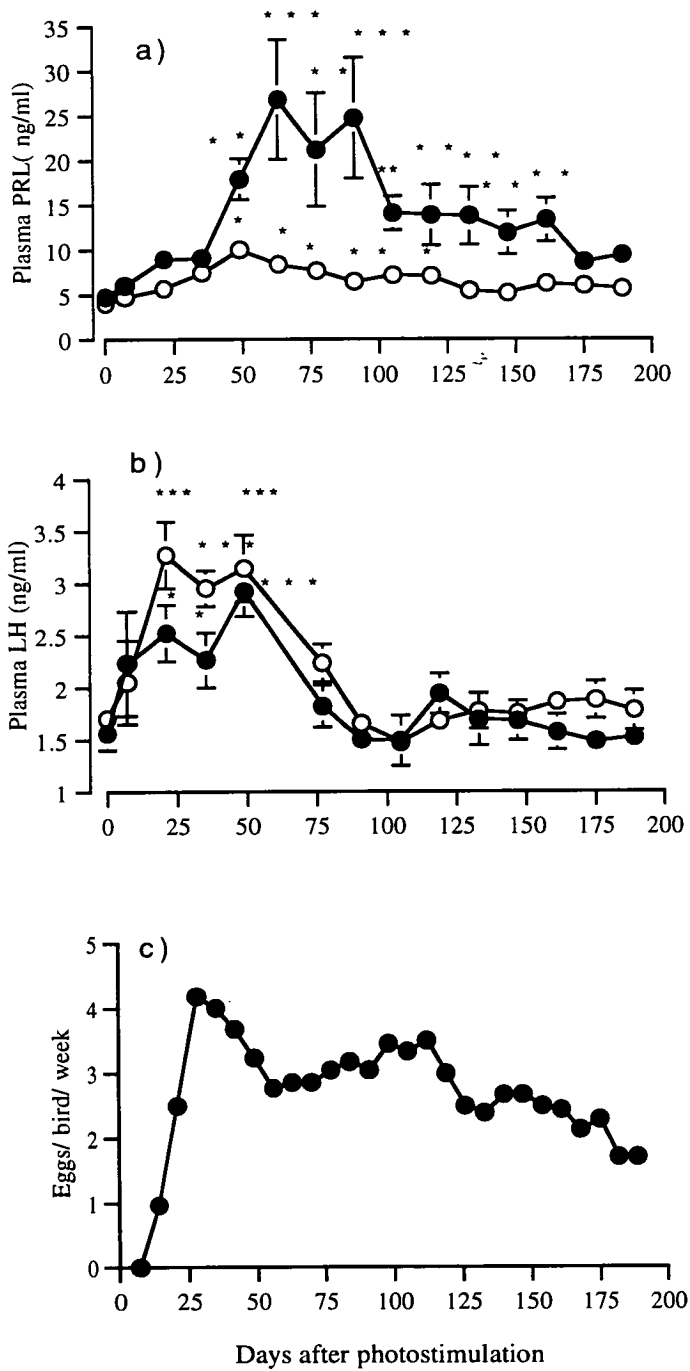


Fig. 8.2 Changes in a) plasma PRL concentration, b) plasma LH concentration and c) egg production in intact turkeys after transfer from 7 h light/day to 14h light/ day (male—○—, n=8 and female—●—, n=8) at 28-30 weeks of age. Values are means \pm sem. * P <0.01, ** P <0.001, *** P <0.0001 significantly different from the pre-photostimulation values.

plasma LH decreased and was not significantly ($P>0.05$) different from the pre-photostimulation values after 77 days of photostimulation. In the females, the concentration of LH increased significantly ($P<0.001$) after photostimulation reaching peak values after 49 days. Thereafter plasma LH decreased to values which were not significantly ($P>0.05$) different from those seen before photostimulation.

Egg-laying started 14 days after photostimulation reaching a peak after 28 days (4.18 eggs/ bird/ week). Thereafter, egg-laying decreased progressively to 1.71 eggs/ bird/ week at 189 days (Fig. 8.2c).

8.4 Discussion.

The increase in concentration of plasma PRL and subsequent decrease after prolonged photostimulation in intact male and female bantams and turkeys suggests the development of photorefractoriness for photoinduced PRL secretion. This view is supported by observations (Chapter 6) in castrated bantams in which the decrease in plasma PRL after prolonged photostimulation is more pronounced.

The sex difference in the magnitude of photoinduced PRL secretion, observed in both bantams and turkeys, with females showing a greater response than males, contrasts with observations on juvenile intact bantams after photostimulation (Chapter 4). In juveniles, there was no sex difference in the magnitude of photoinduced PRL release. This may reflect sex differences in the rate at which ovarian steroidogenesis is induced after photostimulation. The 16-week-old female bantams in the present study came into lay 15 days earlier after photostimulation than the bantams of the same age used for the earlier study (Chapter 4). The reason for this difference is unknown. It is of interest to note that somatically mature bantam hens kept on short days started to lay without a marked increase in plasma PRL, whereas the photostimulated birds showed a pronounced increase in plasma PRL concentration when in lay (Fig. 8.1). This suggests that the increase in concentration of plasma PRL observed at the onset of photoinduced

egg production is mainly due to a photoinduced release of VIP (Chapter 9) and that increased plasma ovarian steroids subsequently enhances the stimulatory action of VIP on PRL release (Chapter 1, Section 1.4.3).

The onset of sexual maturation after prolonged exposure to short days is due to an increase in genotype-dependent, photoperiodically-independent activity of the GnRH-I neurons (review Sharp, 1993). A similar mechanism does not appear to apply to VIP neurons since plasma PRL did not increase in castrated or intact bantams after prolonged exposure to short days. The small increase in concentration of plasma PRL observed in laying bantams exposed for a prolonged period (Fig. 8.1a) to short days might reflect a stimulatory action of prolonged exposure to oestrogen from the fully developed ovary, acting directly on the anterior pituitary gland.

As observed in bantams, there was a marked sex difference in turkeys in photoinduced PRL secretion with the females having a higher values than the males. The five -fold increase in plasma PRL concentration after photostimulation in the females was less than that observed in earlier studies (Etches and Cheng, 1982; Guémené and Williams, 1994 and El Halawani *et al.*, 1995a). The reason for this is uncertain but it could be related to the fact that the early studies were carried out on commercial birds while the present study used an unimproved breed in which the development of photorefractoriness was expected to be more pronounced.

In agreement with the earlier studies of Lien and Siopes (1989), the development of photorefractoriness for PRL and LH release in female turkeys was not associated with the cessation of egg laying (Fig. 8.2). It may be that the process of domestication has resulted in the ovaries of the turkeys becoming more responsive to low concentrations of plasma gonadotrophins. However, domestication does not appear to have altered the neuroendocrine mechanisms responsible for the development of photorefractoriness for LH and PRL secretion. It is of interest to note that in the turkey, the successive peaks of plasma LH and PRL induced by photostimulation, followed by

the development of photorefractoriness for both hormones, shows the same pattern of photoinduced plasma LH and PRL secretion observed in wild birds which become absolutely photorefractory (Chapter 1, Section 1.3.4).

The increase in plasma LH after photostimulation in turkeys was similar to that reported by others (Burke and Dennison, 1980; Lea and Sharp, 1982). It is not solely due to an increase in LH pulse frequency but, in large measure, to an increase in non-pulsatile LH release (Bacon and Long, 1995). The present study showed a sex difference in the photoinduced release of LH in both turkeys and bantams, although this difference was more pronounced in bantams. This is probably due to the fact that ovarian steroids exert a greater inhibitory effect on LH release than do testicular steroids (Liu, 1993).

Unlike turkeys, there was no evidence in bantam hens or cockerels for a decrease in plasma LH after prolonged photostimulation. This is consistent with the observation that in bantams, unlike turkeys, there was no evidence for the development of absolute photorefractoriness (Sharp, 1988). More prolonged exposure to long days may eventually result in decreased plasma LH and egg production although this seems to be due to ageing rather than to the development of relative photorefractoriness (Sharp *et al.*, 1992; Dunn and Sharp, 1992). However, as in turkeys, there was evidence in intact bantams, particularly the males, for a photoinduced increase and subsequent decrease in plasma PRL concentration, indicating the development of photorefractoriness. The finding that the photoinduced peak in PRL secretion was not as well defined in intact bantams as in castrated bantams (Chapter 6) reflects the stimulatory effects of gonadal steroids on PRL release (Chapter 1, Section 1.4.3 and Chapter 3). This is particularly evident in hens in which the photoinduced increase in plasma PRL was 3-4 fold higher than the castrated bantams (Chapter 6). It is likely that the high concentration of plasma PRL in photostimulated hens reflects the potentiating effects of increased plasma oestrogen from the fully developed ovary, on the ability of photoinduced VIP release to stimulate PRL secretion.

In conclusion, prolonged exposure to long days results in the development of photorefractoriness for plasma PRL release in both sexes in the turkey and bantam. The photoinduced release of PRL is sex-dependent with higher values in females than in males. Photoinduced LH release in both chickens and turkeys is higher in males than in females. A photoinduced increase followed by a decrease in plasma LH was seen in male and female turkeys but not in bantams, indicating the development of photorefractoriness, although this was not associated with the cessation of egg laying.

HYPOTHALAMIC CONTROL OF PHOTOINDUCED PRL SECRETION AND ITS FUNCTIONAL SIGNIFICANCE.

9.1 Introduction.

In early Chapters it was established that PRL (Chapters 4 -6) and LH (Chapter 4) secretion in bantams is controlled by changes in daylength. Photoinduced LH secretion is controlled by the release of gonadotrophin releasing hormone (e.g. Perera and Follett, 1992) but it has not been established, in the bantam, which neuropeptide/neurotransmitter controls photoinduced PRL release. The primary candidate is the avian prolactin releasing hormone, vasoactive intestinal polypeptide (VIP, Sharp *et al.*, 1989; Mauro *et al.*, 1989; Cloues *et al.*, 1990; Youngren *et al.*, 1994). Its physiological role in the control of PRL secretion in incubating bantams and turkeys has been demonstrated by showing that passive (Sharp *et al.*, 1989) or active (El Halawani *et al.*, 1995a) immunisation against VIP depresses the concentration of plasma PRL. These studies suggest that a physiological role for VIP in the regulation of photoinduced PRL release might be readily demonstrated by establishing that PRL secretion is not stimulated after photostimulation in birds immunised against VIP.

The functional significance of photoinduced PRL secretion has not been established. Since egg -laying was initiated in short day bantams in the absence of an increase in plasma PRL (Chapter 8), it seems that increased plasma PRL is not necessary for the initiation of reproductive activity.

It is suggested that a major function for the increase in plasma PRL after photostimulation in females may be to encourage incubation behaviour (Sharp, 1989). This view is supported by the finding that active immunisation against PRL in the

bantam, inhibits the development of incubation behaviour (March *et al.*, 1994). It is therefore suggested that, in bantams, active immunisation against VIP, designed to block photoinduced PRL release, should also prevent the development of incubation behaviour.

A further role for the photoinduced release of PRL in birds such as the turkey, which become absolutely photorefractory (Sharp, 1989), may be to participate in the development of reproductive refractoriness. This suggestion is based on the observation in wild birds (Chapter 1, Section 1.3.4), that photoinduced PRL secretion is greatest during the development of absolute photorefractoriness, when the gonads regress. It is therefore suggested that if active immunisation against VIP inhibits photoinduced PRL release, it should also affect the time course of the development of reproductive photorefractoriness

In addition to its role in the control of prolactin secretion, VIP is also produced in the avian anterior pituitary gland where it may act in a paracrine manner to regulate the function of gonadotrophs (Shale, 1996) as has been demonstrated in mammals (Lasaga *et al.*, 1989; Ogwuegbu *et al.*, 1990; Hammond *et al.*, 1993; Lafuente *et al.*, 1995). This function of VIP might be determining whether plasma LH is depressed in birds actively immunised against VIP.

The aim of this study was to establish the effects of active immunisation against vasoactive intestinal polypeptide (VIP) on, a) photoinduced PRL and LH secretion in castrated chickens, b) the development of broodiness in turkeys, and c) photoinduced egg production in turkeys.

9.2 Preparation of immunogen and immunisation procedure.

Two 15 -amino acid polypeptides corresponding to the C-terminal and N-terminal sequences of chicken VIP (cVIP, Nilsson, 1975) were synthesised on a Biosearch 9500 peptide synthesiser (New Brunswick Scientific Ltd) using solid-phase

t-BOC chemistry and the reagents and conditions recommended by the suppliers of the instrument. The peptide sequences were RKQMAVKKYLNSVLT-NH₂ (VIPC) and HSDAVFTDNYSRFRK-NH₂ (VIPN). They were extended at the amino terminus by the addition of cysteine to allow them to be conjugated through the thiol group of cysteine to the purified protein derivative (PPD) of tuberculin (Central Veterinary Laboratory). Conjugation was carried out using sulphosuccinimidyl H-(maleimidomethyl) cyclohexane-1-carboxylate following the protocol indicated by the manufacturer (Pearce and Warriner Ltd). The VIPC/VIPN-PPD conjugates were prepared by N.S. Huskisson (Microchemical Faculty, Babraham Institute, Babraham, Cambridge, U.K)

The active immunisation procedure was based on that described by Lachmann *et al.*, (1986). It was initiated with an intradermal 'priming' vaccination into the comb with BCG vaccine (0.1ml, Evans Medical Ltd) followed after at least 4 weeks, with intramuscular injections of VIPC/VIPN-PPD (1:1, total conjugate, 1 mg/ bird), VIPC-PPD (1 mg/ bird) or PPD (1 mg/ bird) emulsified in Freund's incomplete adjuvant (SIGMA). The VIPC-PPD and VIPC/VIPN-PPD conjugates were supplied in phosphate buffered saline (2 mg/ ml) and were emulsified by sonication with an equal volume of adjuvant. In order to make the emulsion easier to inject, it was homogenised by sonication with an equal volume of physiological saline containing 2% Tween 80 (SIGMA). The control immunogen was prepared following the same procedure, but substituting VIPC-PPD or VIPC/VIPN-PPD with PPD. The birds were immunised by injections into the breast muscle on either side of the keel bone in a total volume of 3 ml for a turkey and 1.5 ml for a bantam.

9.3 Effect of active immunisation against VIP on photoinduced PRL and LH secretion in castrated bantams.

The castrated bantams (91 weeks of age) in this study had been used for previous unrelated photoperiodic experiments. They were held in floor pens on 8 h light/day for at least 12 weeks before initiation of the experiment to ensure they were fully photosensitive, and were vaccinated with BCG 9 weeks before transfer to individual cages on 8 h light/day. The experimental ($n=6$) and control ($n=6$) birds were immunised with 1 mg VIPC/VIPN-PPD and PPD respectively, 5 and 2 weeks before increasing the photoperiod to 20 h light/day. Blood samples were collected to measure VIP antibody titres and changes in plasma PRL and LH concentrations before and after photostimulation. The method used to measure VIP antibody titres is described in Section 2.5

Immunisation against VIPC/VIPN-PPD generated high titres of VIP antibody in the blood before and after the birds were photostimulated (Fig. 9.1a). VIP antibody was not detected in control birds immunised with PPD (Fig. 9.1a). In birds held on short days there was no effect of immunisation against VIP on plasma prolactin levels (Fig. 9.1b). Photostimulation of control bantams immunised with PPD resulted in significant increases in plasma PRL ($P<0.05$ Fig. 9.1b) and LH ($P<0.05$, Fig. 9.1c). A significant increase in plasma LH ($P<0.05$), but not in plasma PRL, was observed after photostimulation in the birds immunised with VIPC/VIPN-PPD (Fig. 9.1b). The incremental change in plasma LH seen after 8 days' photostimulation in PPD - immunised birds (Δ LH, 19.53 ± 3.27 ng/ml) was not significantly different from that in experimental birds immunised with VIPC/VIPN-PPD (Δ LH, 17.60 ± 2.90 ng/ml) after 8 days of photostimulation (Fig. 9.1b). There were no significant differences in concentration of plasma LH or PRL between control and experimental birds at the beginning of the study, before immunisation. In birds exposed to short days,

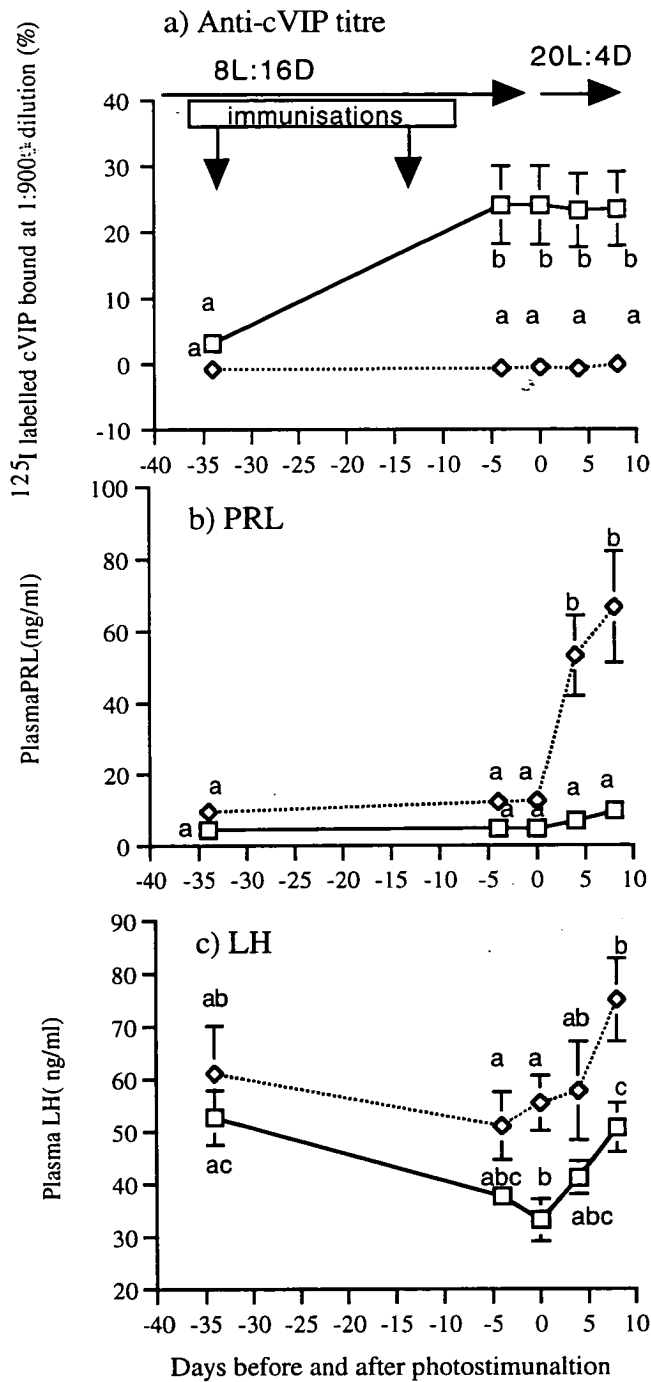


Fig.9.1 Effect of immunisation with VIPC/VIPN-PPD (—□—) or PPD (.....◇.....) on the a) VIP antibody titres b) photoinduced plasma PRL and c) photoinduced plasma LH secretion in 91 week-old castrated cockerels maintained on short days (8L:16D) and photostimulated on 20L:4D. Arrows indicate the times at which the birds were immunised. Values with different superscripts are significantly ($P < 0.05$) different. Values are mean \pm s.e.m; n=6.

immunisation against VIPC/VIPN-PPD but not PPD resulted in a significant decrease in plasma LH ($P<0.05$, one way ANOVA) but not in PRL (Fig. 9.1c).

9.4 Effect of active immunisation against VIP on the development of broodiness in turkeys.

Female Slate turkeys (28-30 weeks old) were reared in groups of five in floor pens on 8 h light/ day. The experimental (n=9) and control (n=10) hens were vaccinated with BCG 4 weeks before the first immunisation with VIPC-PPD conjugate or PPD respectively. The birds were reimmunised with VIPC-PPD and PPD every 4 weeks until the end of the 103 -day study. Two weeks after the second immunisation, the hens were photostimulated by transfer from 8 h to 16 h light/ day and provided with nest boxes each containing 5 hard-boiled eggs to encourage incubation behaviour. The experimental and control birds were kept in separate pens. Blood samples were taken to measure changes in plasma LH, PRL and VIP antibody titres. Egg-laying records were kept for each pen and the birds were observed daily for the development of incubation behaviour.

Immunisation with VIPC-PPD resulted in a high VIP antibody titre ($P<0.001$) while the control group immunised against the carrier protein (PPD) had no antibodies against VIP (Fig. 9.2a). The concentrations of plasma PRL in the PPD immunised birds were significantly ($P<0.001$, ANOVA repeated measures) higher than in the VIPC-PPD immunised birds (Fig. 9.2b). In the VIPC-PPD immunised birds there was a small but significant ($P<0.001$) increase in plasma PRL during the first 23 days of photostimulation. Thereafter, plasma PRL concentrations were not significantly different from the values observed before photostimulation. None of the 9 turkeys immunised with VIPC-PPD showed incubation behaviour at any stage of the experiment.

In the PPD immunised control birds, the concentration of plasma PRL increased progressively after photostimulation, reaching a peak at 19 days in birds which did not subsequently show incubation behaviour. Five of 10 PPD-immunised birds developed

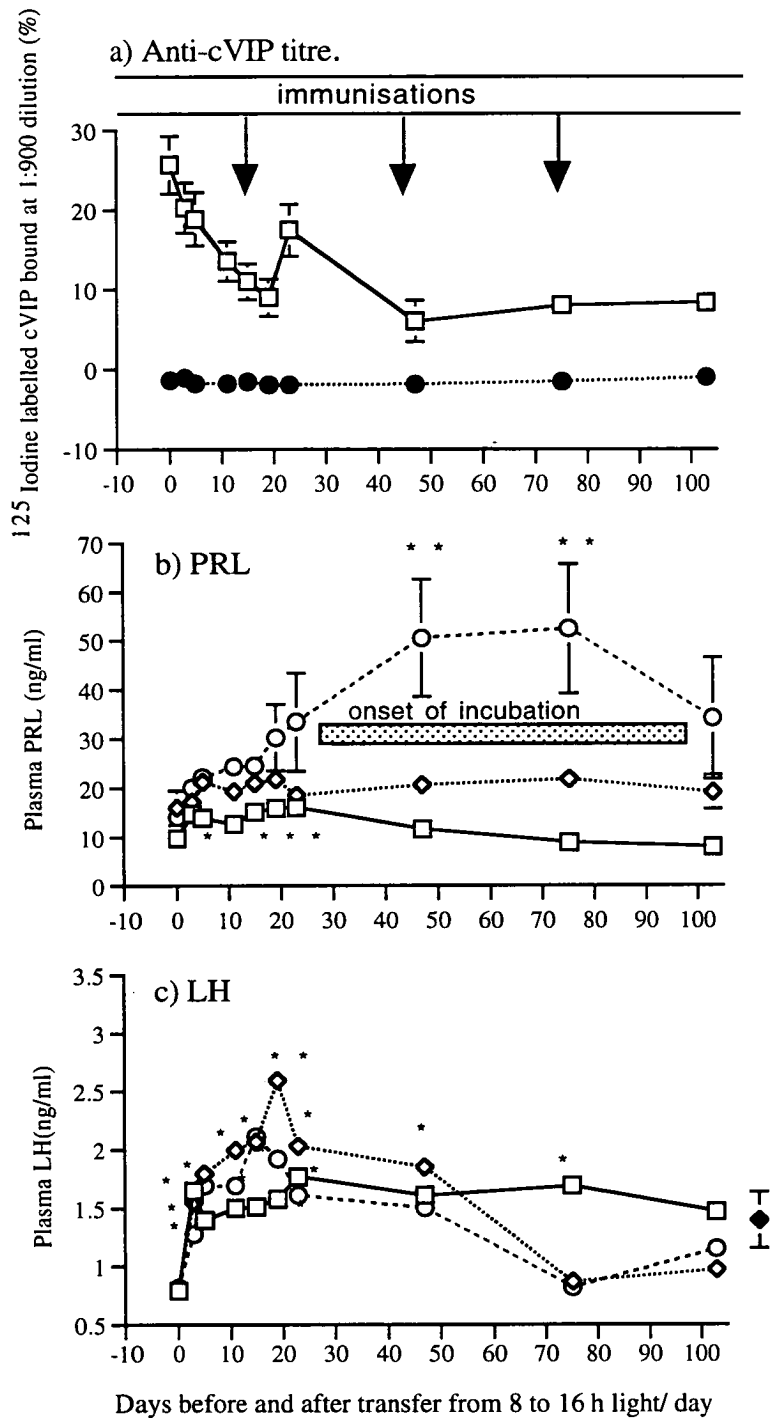


Fig.9.2 Effect of immunisation of female Slate turkeys (28-30 week-old) held in floor pens with nest boxes and eggs to encourage broodiness, against VIPC-PPD conjugate (—□—, n=9) or PPD (---○--- incubating (n=5), ---◇--- none incubating (n=5), —●— incubating and non incubating (n=10)) on, a) cVIP antibody titre, b) plasma PRL and, c) plasma LH. The values with asterisks are significantly (* $P < 0.05$, ** $P < 0.01$) different from the pre-photostimulation values. Arrows indicate the times at which the birds were immunised. The birds were immunised with VIPC-PPD, 6 and 2 weeks before photostimulation. Values are means \pm sem. The inserts (—◆—) show the mean and sem for all plasma LH measurements in the experiment (Fig. 7.2c). The SEMs have otherwise been omitted for clarity.

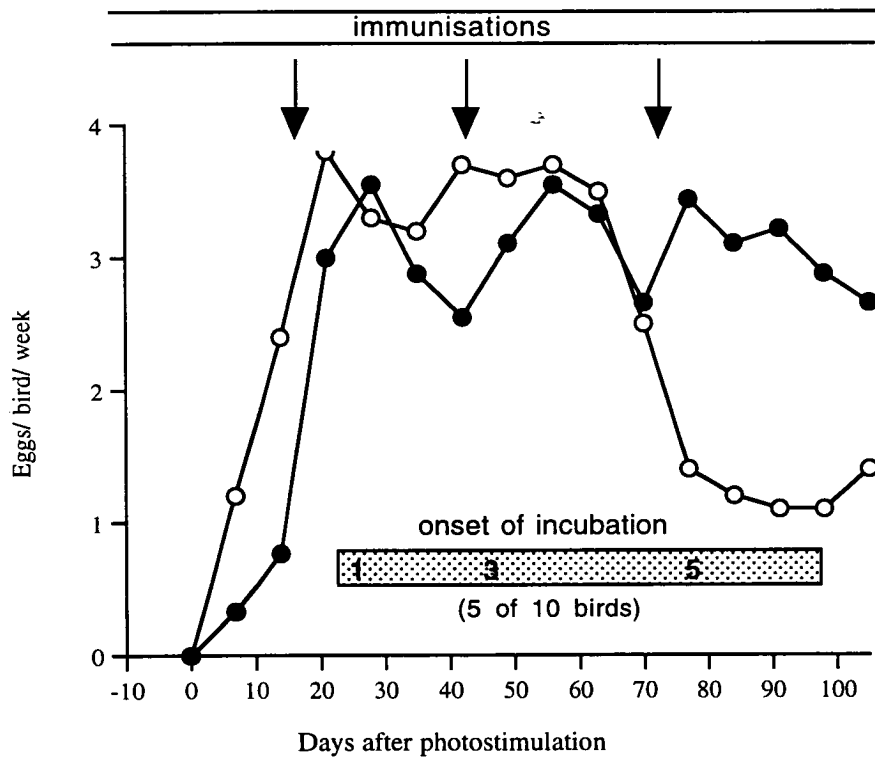


Fig. 9.3 Egg production in female Slate turkeys held in floor pens with nest boxes and eggs to encourage broodiness immunised against VIPC-PPD (—●—, n=9) and PPD (—○—, n=10) during a photoinduced reproductive cycle. None of the nine birds immunised against VIPC-PPD showed incubation behaviour. Five of the ten birds immunised against PPD showed incubation behaviour. Arrows indicate the times at which the birds were immunised. Values are means \pm sem. Number of birds in incubation are shown in the closed horizontal bar.

incubation behaviour. The first bird started incubating 28 days after photostimulation and the last bird began to incubate after 77 days of photostimulation. All the incubating birds maintained incubation behaviour until the end of the study. In these birds, the concentration of plasma PRL was significantly higher ($P < 0.005$) than in non-incubating birds (Fig. 9.2b).

Plasma LH concentrations in PPD-immunised birds, irrespective of whether they subsequently showed incubation behaviour, increased after photostimulation, reaching a peak between 15 and 19 days (Fig. 9.2c). Thereafter, plasma LH decreased and after 75 days was not significantly ($P > 0.05$) different from the pre-photostimulation value (Fig. 9.2c). The concentration of plasma LH increased ($P < 0.05$) after photostimulation in the VIPC-PPD-immunised turkeys, reaching a plateau after 23 days. The overall mean LH concentrations did not show any significant difference ($P > 0.05$, ANOVA repeated measures) between the experimental and the control groups. However, after 75 days of photostimulation, the concentration of plasma LH in the VIPC-PPD-immunised birds was significantly ($P < 0.05$) higher than in the PPD-immunised controls (Fig. 9.2c).

The hens started to lay eggs during the first week of photostimulation and total egg production was not significantly ($P > 0.05$) different between the experimental and control groups up to 70 days in lay (Fig. 9.3). After 77 days in lay there was a significant ($P < 0.001$) drop in the total egg production in the PPD but not in the VIPC-PPD-immunised birds (Fig. 9.3). It was not possible to identify egg production in individual birds because the birds were kept on floor pens with open nest boxes.

9.5 Effect of active immunisation against VIP on photoinduced egg production in turkeys.

The turkeys used in the previous experiment to determine the effect of active immunisation against VIP on the development of broodiness (Section 9.4) were recycled into a second period of egg production after being kept on 8 h light/ day for 3 months in

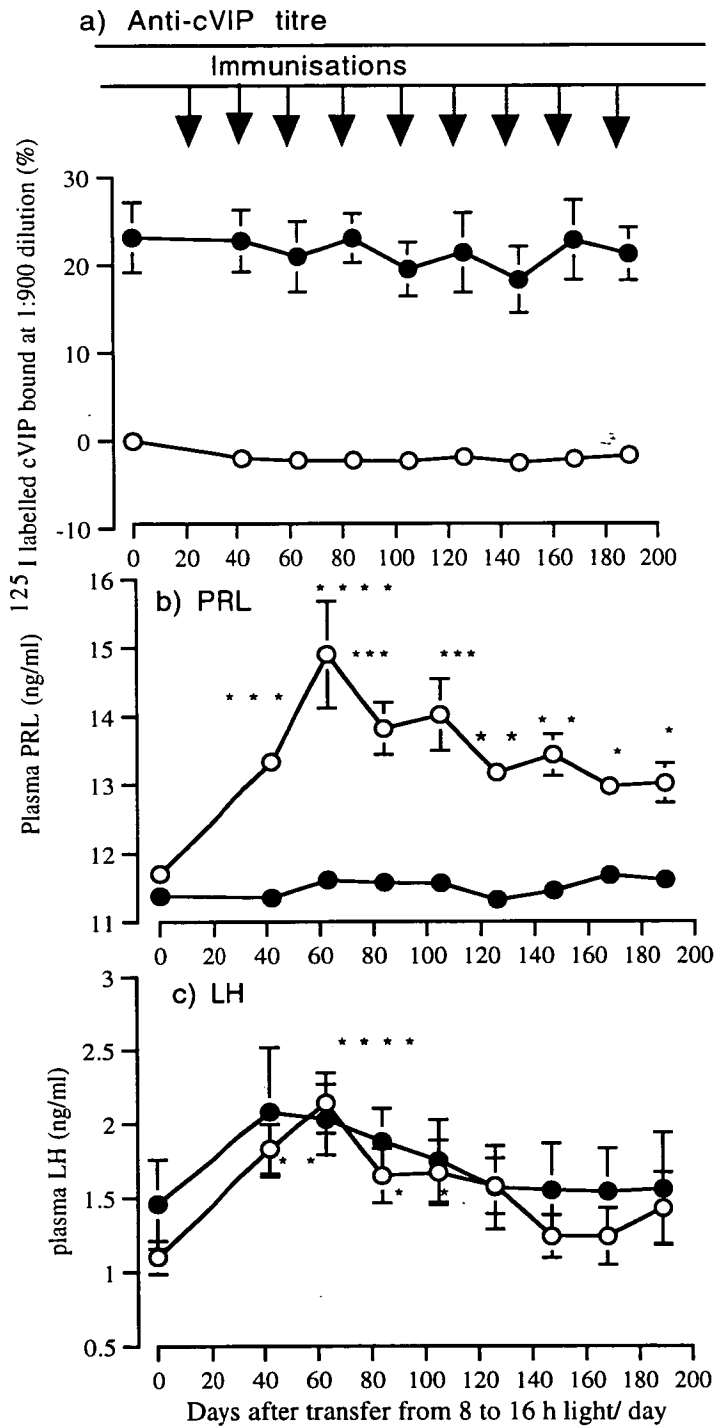


Fig. 9.4 Effect of immunisation of female Slate turkeys (31-33 week-old) held in cages to discourage broodiness against VIPC/VIPN-PPD conjugate (—●— n=9) and PPD —○— n=10) on a) cVIP antibody titre, b) plasma PRL and, c) plasma LH concentrations. The values with asterisks are significantly (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.005$, **** $P < 0.0001$) different from the pre-photostimulated values. Arrows indicate the time at which the birds were immunised. Values are means \pm sem. These birds were previously immunised with VIPC-PPD or PPD in another experiment before being immunised with VIPC/VIPN-PPD or PPD in the present study, a week before photostimulation.

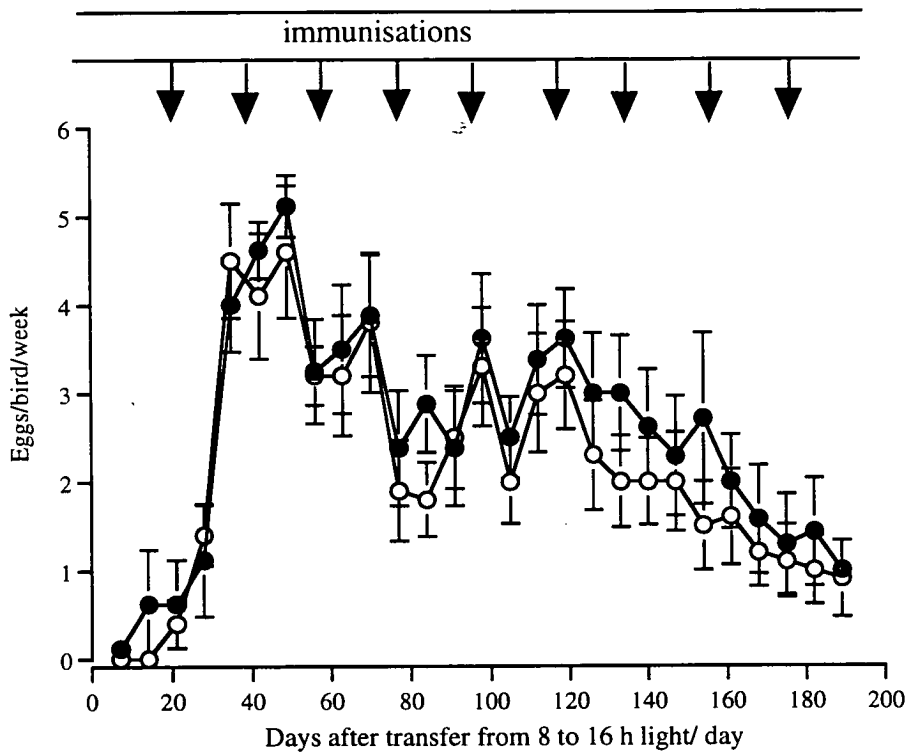


Fig.9.5 Egg production in Slate turkeys held in individual cages to discourage broodiness, immunised with VIPC/VIPN-PPD (—●—, n=9) and PPD (—○—, n=10) during a photoinduced egg cycle. Arrows indicate the times at which the birds were immunised. Values are means \pm sem. The VIP antibody titre and plasma PRL and LH values of these hens are shown in Fig. 7.4.

floor pens. During this period, immunisation against VIPC-PPD or PPD continued at 4 - weekly intervals as described in Section 9.4. The birds were then transferred to individual cages on 8 h light/ day in order to record individual eggs. After a week's acclimatisation, the photoperiod was increased from 8 to 16 h light/ day to stimulate egg production. At this time, the turkeys were 74 -76 weeks-old. Hens which had been immunised with VIPC-PPD in the previous experiment were reimmunised with VIPC/VIPN-PPD at 3-weekly intervals beginning 7 days before photostimulation. The VIPN-PPD was added to the immunogen in an attempt to further increase VIP antibody titre. The frequency of immunisation was increased to ensure VIP antibody titres were uniformly maintained at high values. Control hens, immunised with PPD in the previous experiment, were again immunised with PPD at 3-weekly intervals. Blood samples were taken to measure the changes in plasma LH, PRL and VIP antibody titres. The eggs laid by individual birds were recorded.

Active immunisation with VIPC/VIPN-PPD resulted in high plasma titres of chicken VIP antibody in the experimental group, which were maintained throughout the study (Fig. 9.4a). Immunisation with the control protein (PPD) did not result in the generation of VIP antibody (Fig. 9.4a). Photoinduced PRL secretion was completely blocked in birds immunised with VIPC/VIPN-PPD but not in the control group, immunised with PPD. In the control group, the concentration of plasma PRL increased significantly ($P < 0.005$) after photostimulation, reaching peak values after 63 days, and thereafter decreased (Fig. 9.4b). After 126 days of photostimulation, the concentration of plasma PRL in the control birds remained stable, but was significantly higher ($P < 0.05$) than the values observed before photostimulation (Fig. 9.4b). The concentration of plasma LH in the control PPD-immunised turkeys increased significantly ($P < 0.01$) after photostimulation, reaching a peak after 63 days. Thereafter plasma LH decreased and after 126 days of photostimulation was not significantly ($P > 0.05$) different from values

observed before photostimulation (Fig. 9.4c). No significant increase in plasma LH was seen after photostimulation in turkeys immunised with VIPC/VIPN-PPD.

The egg production peaked after 49 days' photostimulation in both the groups and thereafter decreased progressively (Fig. 9.5). There were no significant differences ($P < 0.05$, ANOVA repeated measures) in egg production between the VIPC/VIPN-PPD and PPD-immunised hens throughout the study (Fig. 9.5).

9.6 Discussion.

The absence of an increase in plasma PRL after photostimulation in the VIPC/VIPN-PPD -immunised bantams and turkeys, and the photoinduced increase in plasma PRL in the control, PPD -immunised bantams and turkeys (Figs. 9.1, 9.2, 9.4) demonstrate that VIP is an essential intermediary in the PRL response to an increase in photoperiod. The VIP neurons mediating the photoperiodic response are probably located in the tuberal hypothalamus with projections to the median eminence (Section 1.4.1). These VIP neurons are known to be involved in stimulating the increase in PRL in incubating birds, but it remains to be established whether the same neurons, or a discrete subset, mediate the PRL photoperiodic response. The neuronal pathways controlling photoinduced VIP release from the hypothalamus are not known. In ring doves, VIP neurons adjacent to the ventral lateral ventricle, are proposed to be encephalic photoreceptors because they contain opsin-like immunoreactivity (Silver *et al.*, 1988) and these might represent photoresponsive VIP neurons controlling PRL release. However, there is no evidence that these VIP neurons project directly to the median eminence.

Active immunisation against VIP depressed basal plasma LH concentration in the castrated bantams (Fig. 9.1c) but not in intact female turkeys. This observation is consistent with the finding in bantam hens that immunisation against VIP suppresses plasma LH and delays the onset of egg production after photostimulation (Sharp *et al.*,

1993). It has been suggested that this effect of active immunisation against VIP on LH release may be due to the immunoneutralisation of VIP produced within the anterior pituitary gland (Shale, 1996). In support of this view, cells containing VIP have been observed in the anterior pituitary gland of bantams which envelop neighbouring gonadotrophs (Shale, 1996). The view that pituitary VIP acts in a paracrine manner to facilitate LH release is supported by the observations that incubation of turkey pituitary glands with porcine VIP increases LH secretion and also enhances GnRH-induced LH release (El Halawani *et al.*, 1990b). Similar observations have been reported in mammals where the administration of anti-VIP serum to ovariectomised rats reduces plasma LH concentrations (Lasaga *et al.*, 1989). Similarly in man, an infusion of VIP into the peripheral circulation, followed by an infusion of GnRH 30 min later, significantly augments the release of LH (Hammond *et al.*, 1993).

The depression of plasma LH observed after VIP immunisation in castrated bantams but not in intact female turkeys held on short days (cf. Figs 9.1 and 9.4c), probably reflects the fact that a decrease in plasma LH is easier to detect in castrated birds. In castrated bantams, immunisation against VIP did not prevent a photoinduced increase in plasma LH. This is consistent with the view that VIP produced in the anterior pituitary, acting in a paracrine manner, rather than photoinduced VIP release from the hypothalamus, plays a role in controlling LH secretion.

Active immunisation of female turkeys against 15 -amino acid fragments of the chicken VIP molecule (VIP-PPD) prevented the development of incubation behaviour and the associated increase in plasma PRL (turkey: El Halawani *et al.*, 1988; El Halawani and Rozenboim, 1993; domestic hen: Sharp *et al.*, 1988). This observation confirms a similar study done on Nicholas White turkeys using whole chicken VIP (cVIP) as immunogen molecule conjugated to keyhole limpet haemocyanin (KLH) which also prevented the development of incubation behaviour (El Halawani *et al.*, 1995a). The present study demonstrates that it is not necessary to use the whole 28 -amino acid

sequence of VIP as an immunogen to generate antibodies capable of blocking the increase in PRL secretion associated with the onset of incubation behaviour

It is of interest to note that the fall in egg production in the 10 control turkeys immunised with PPD occurred several weeks after 5 of the birds had become broody (Fig. 9.3). It seems that in these birds, the initial expression of broody behaviour was not associated with the cessation of egg laying. This phenomenon has been reported in an earlier study in Large White turkeys (Lea and Sharp, 1982). The decrease in egg production in the PPD-immunised control hens may have been due to a combination of persistent broodiness and to the development of photorefractoriness. The development of photorefractoriness is suggested by the decrease in plasma LH observed in PPD-immunised birds, irrespective of whether they become broody after 75 days of photostimulation (Fig. 9.2c). The observation that plasma LH did not decrease at this time in the VIP-immunised birds (Fig. 9.2c) may explain why egg laying was more persistent in these birds than in the PPD immunised controls. It appears that VIP immunisation may have inhibited the development of photorefractoriness in those birds. However, this conclusion was not supported in the follow-up study in which the same turkeys were immunised against VIP and recycled in a second breeding cycle, while held in cages to discourage the development of broodiness (Fig. 9.5).

In turkeys immunised with VIPC-PPD and held in floor pens, a small but significant increase in plasma PRL was observed after photostimulation (Fig. 9.2b). It is possible that the immunisation with the 15 -amino acid at the carboxy-terminal of the VIP molecule and /or frequency of immunisation was not adequate to keep the VIP antibody titre high enough to suppress entirely the photoinduced increase in plasma PRL concentration. This is supported by the second immunisation study on the same turkeys, which were immunised using the entire amino acid sequence in the VIP molecule (VIPC/VIPN-PPD) at an increased frequency. This immunisation procedure resulted in an unchanging high titre of VIP antibody (Fig. 9.4a) which blocked completely the

photoinduced increase in plasma PRL, presumably by immunoneutralising photoinduced VIP release.

In conclusion, photoinduced plasma PRL secretion is mediated through VIP secretion in both bantams and turkeys. VIP may also act in a paracrine manner to regulate basal plasma LH secretion in chickens. The development of broodiness in turkeys was prevented by immunisation against a 15 amino acid sequence at the C-terminal of cVIP sequence and this increased egg production. Immunisation against VIP did not affect the development of photorefractoriness in female turkeys as measured by decreased egg production.

GENERAL DISCUSSION

The overall aim of the Thesis was to increase understanding of the photoperiodic control of prolactin secretion, and its functional significance in the chicken. At the onset of this work, it was known that PRL secretion is controlled by changes in photoperiod in several species, although no information was available for the domestic chicken (Section 1.3.4). It was also known that gonadal steroids, particularly oestrogen, exert a stimulatory effect on PRL secretion (Section 1.4.3.1). This was confirmed in domestic cockerels held on short days (Section 3.2). The presence of testes after 4 weeks of age resulted in higher concentrations of plasma PRL than in castrated birds. It appears that in birds older than 4 weeks, testicular development, which occurs on short days, results in a sufficient increase in concentrations of testicular steroids to stimulate PRL secretion. However, this effect of testicular steroids is small compared with the effect of increased photoperiod (Chapters 4, 5 6). In females, which become sexually mature on short days, the presence of high concentrations of oestrogen results in only a modest increase in plasma PRL (Chapter 8). These observations are consistent with the findings *in vitro* (Hall *et al.*, 1984a) showing that the direct stimulatory effects of oestrogen on PRL release from the anterior pituitary are modest compared with the combined action of oestrogen and hypothalamic extract/ PRL releasing hormone. It therefore appears that the major component of the stimulatory action of oestrogen on PRL release is via a synergism with PRL releasing factor, which is assumed to be released in increased amounts after photostimulation. The observation that the increase in plasma PRL in somatically mature males was less than in somatically mature females (Chapter 8) further supports the view that increased plasma oestrogen rather than an increase in

other gonadal steroids (Section 1.4.3) is the primary steroidal factor enhancing the effects of PRL releasing factor on PRL release.

The observation that the changes in ambient temperature and fasting for one day had no effects on the concentration of plasma PRL (Chapter 3) rules out the possibility that changes in plasma PRL reported in this Thesis can be accounted for by either of these environmental factors.

In view of the stimulatory effects of gonadal steroids on photoinduced PRL secretion, castrated cockerels were used to investigate the PRL photoperiodic response to avoid confounding the stimulatory effects of increase in photoperiod and of increase in plasma gonadal steroids. It was anticipated when this Thesis was being planned, that use of castrated birds would facilitate comparison with photoinduced changes in plasma LH secretion. In other birds, such as quail and white-crowned sparrow, castration amplifies the effects of changes in photoperiod on LH secretion, making it easier to interpret LH photoperiodic responses (Urbanski and Follett, 1982; Mattocks *et al.*, 1976). Unfortunately, in the bantam, long-term castration results in a partial loss of hypothalamic control of LH secretion (Chapter 5) which made it difficult to use changes in plasma LH as precise indicators of the changes in photoinduced GnRH release.

The neuroendocrine pathways controlling photoinduced PRL and LH secretion were observed to be functional at 4 weeks of age in both sexes. The observation on the age at which the neuroendocrine pathways controlling photoinduced gonadotrophin release became mature confirms earlier findings in female broilers (Dunn *et al.*, 1990). Although it has not been established when the photoperiodic responses for PRL and LH release first develop, the presence of photoperiodic responses for both hormones during early posthatch somatic growth suggests that a common maturational mechanism might be involved. This could involve the maturation of neural pathways from the extra-retinal photoreceptor or maturation of neural outputs from the biological clock to GnRH/ PRL releasing factor neurons (Sections 1.5.1, 1.5.2). The juvenile maturation of the

reproductive photoperiodic response is suggested to be central to the development of juvenile reproductive refractoriness. This is the mechanism which prevents unimproved breeds of chicken, exposed to natural lighting from coming into breeding condition in the year in which they are hatched (review, Sharp, 1992). The significance of the juvenile development of a photoperiodic response for PRL release is uncertain. However, since plasma PRL concentrations were very low in somatically mature castrated cockerels reared on 20 h light/ day (Chapter 5), it is likely that juvenile photorefractoriness also develops for photoinduced PRL release. The functional significance of this is uncertain since, as demonstrated in intact bantams reared and maintained on short days (Section 8.2), the onset of sexual maturation does not depend on an increase in concentration of plasma PRL.

The critical daylength for PRL secretion, between 10-12 h, was difficult to define precisely because the incremental changes in photoinduced PRL secretion at the lower end of the photoperiodic response curve were small (Chapter 5, Fig. 10.1). A direct comparison with the critical daylength for LH release was impossible because of the poor photoperiodic LH response in castrated bantams (Chapter 5). However, the reported critical daylength is between 10-12.75 h in intact female domestic chickens (Sharp, 1988; Dunn and Sharp, 1990). It is therefore concluded that the critical daylengths for PRL and LH secretion are similar.

The saturation daylength for photoinduced PRL release, between 14 -16 h was unambiguously greater than the 10.25 -12.75 h observed for LH (Dunn and Sharp, 1990). It is therefore concluded that the most important difference between the photoperiodic responses for PRL and LH release is in the slopes of the photoperiodic response curves. The slope of the photoperiodic response curve for LH is much steeper than that for PRL (Fig. 10.1).

A major consequence of the difference in slopes of the photoperiodic response curves is most likely to be seen in birds exposed to natural changes in daylength. As

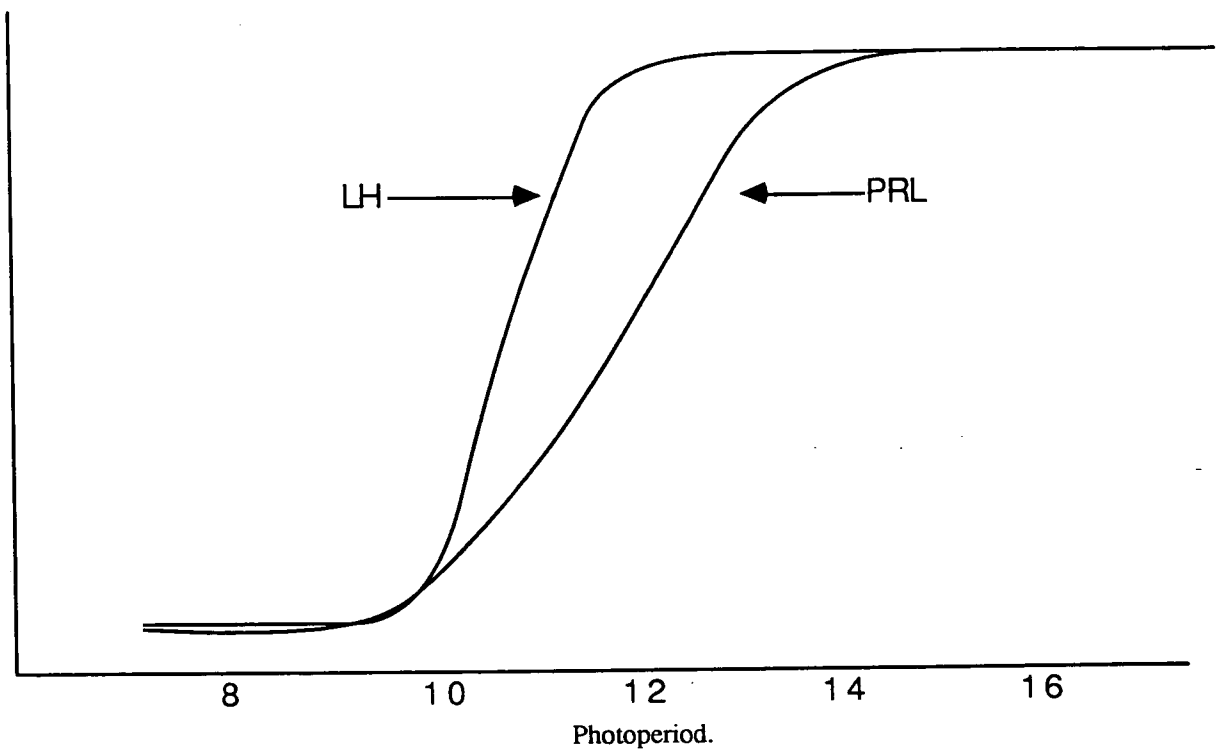


Fig 10.1 An interpretation of the photoperiodic response curves for plasma prolactin (PRL) and luteinising hormone (LH) release in castrated cockerels transferred from a short non-photostimulatory (8 h light/day) to range of fixed longer photoperiods. (Based on data from Chapter 5)

daylength increases in Spring the concentration of plasma LH would be expected to reach maximum values much more rapidly than concentrations of plasma PRL. This sequence of photoinduced changes in concentrations of plasma LH and PRL has been observed in several avian species exposed to natural changes in daylength (Section 1.3.4). Unfortunately there are no observations on domestic chickens exposed to natural changes in daylength. However, the observations in the castrated bantams on the difference in the slopes of the photoperiodic response curve for LH and PRL secretion provides an explanation, in other birds exposed to increasing spring-like daylengths, for the rapid increase in plasma LH followed by slow increase in plasma PRL (Section 1.3.4).

In birds which develop relative reproductive photorefractoriness such as the quail (Urbanski and Follett, 1982; Robinson and Follett, 1982) and chicken (Sharp, 1993), the long-day induced inhibitory input to GnRH-I neurons (Section 1.5) partially counteracts the long-day stimulatory input. As a result there is an apparent upward shift in the critical daylength required for gonadotrophin release (Urbanski and Follett, 1982; Robinson and Follett, 1982, Sharp, 1984). Evidence has been presented in the domestic hen for an increase in the critical daylength for LH release as a consequence of the development of relative refractoriness (Sharp, 1993). In Chapter 5, it was demonstrated that the critical daylength for photoinduced PRL release is also increased from 10-12 h to >14 h light/ day as a consequence of prolonged exposure to 20 h light/ day. These observations suggests that in the chicken, prolonged exposure to long days results in the development of photorefractoriness for PRL secretion. This observation contrasts with findings in quail, where a decrease in daylength from 20 h light/ day to marginally stimulatory 13 h light/ day does not result in a decrease in PRL secretion (Juss, 1993). This further supports the view (Section 1.3.4) that in quail, unlike the chicken photorefractoriness does not develop for photoinduced PRL release.

An unexpected finding in this Thesis was that in castrated bantams exposed to photoperiods of 14 h or more, a photoinduced increase in PRL secretion is followed by a

photoinduced decrease (Chapter 5). This sequence of events is characteristic of the development of 'absolute' photorefractoriness. However, this form of 'absolute' photorefractoriness differs from that seen for photoinduced LH release (Follett, 1984; Nicholls *et al.*, 1988). Even when PRL concentrations are very low, they still tend to be higher than in short day controls (Chapter 5). In birds showing absolute reproductive photorefractoriness, the concentration of plasma LH is not depressed further after transfer from long to short daylengths (Dawson, 1991; Boulakoud and Goldsmith, 1994). In other respects, however the expression of 'absolute' photorefractoriness for PRL release in bantams is similar to that observed for 'absolute' photorefractoriness for LH release (Nicholls *et al.*, 1988). The development of 'absolute' reproductive photorefractoriness is characterised by the inability of a further increase in daylength to stimulate LH release. This characteristic was also a feature of absolute photorefractoriness for PRL release in castrated bantams exposed for a prolonged period to 16 h light/ day (Chapter 6). In these birds an increase in photoperiod to 20 h light/ day failed to stimulate PRL release.

A further feature of 'absolute' reproductive photorefractoriness is that it does not develop in birds transferred from short days to photoperiods close to the critical daylength (Section 1.3.4). This was also a feature of development of photorefractoriness for PRL release. Birds transferred from short days to a marginally stimulatory 12 h photoperiod showed no tendency for plasma PRL concentration to decrease after prolonged exposure to this photoperiod (Chapter 6). Evidence that birds exposed to 12 h light/ day for prolonged periods remain photosensitive was obtained by demonstrating an increase in plasma PRL after such birds were transferred to 16 h light/ day (Chapter 6).

The final test for the development of photorefractoriness for PRL release was to demonstrate, as predicted by the model for avian photoperiodic response (Section 1.5.4), that exposure to short days leads to the recovery of photosensitivity. As shown in

Chapter 6, exposure of long day castrated bantams with low concentrations of plasma PRL to short days for 5 weeks resulted in a full recovery of photosensitivity for PRL release.

The neural pathways controlling the photoinduced release of LH in the quail are fully activated after exposure to one long day (Follett *et al.*, 1977) and mediate photoinduced release of GnRH-I (Perera and Follett, 1992). The neural pathways concerned may include a group of neurons in the tubero-infundibular hypothalamus, shown using *c-fos* immunocytochemistry to be activated after exposure to a single long day (Meddle and Follett, 1995). An interesting feature of the 'first day' release of LH is that it is initiated 22-23 h after 'dawn' which is 11-12 h longer than the critical daylength for LH release. In Chapter 7, it was demonstrated that a 'first day' release also occurs for photoinduced PRL secretion and that this follows a temporal pattern which is similar to that observed for 'first day' LH release in quail. The 11-12 h lag between the critical daylength for an increase in PRL or LH release after exposure to one long day implies that the mechanisms controlling the release of both hormones may be similar. The induction of LH secretion in quail in response to one long day is associated with a 'carry over effect' of increased LH secretion after transfer back to short days. In castrated bantams, a similar phenomenon was also observed for PRL secretion (Chapter 7). The similarities between the time courses for the 'carry over effect' for LH release in quails and PRL release in bantams again implies that mechanisms controlling photoinduced PRL and LH release may be similar.

The development of a form of absolute refractoriness in castrated bantams for photoinduced PRL release prompted experiments to determine whether the same phenomenon occurs in intact birds (Chapter 8). The presence of ovarian or testicular steroids amplifies the initial photoinduced PRL response, particularly in females. However, in both males and females there was evidence that prolonged exposure to long days resulted in a decrease in plasma PRL indicative of an underlying trend towards the

development of photorefractoriness for PRL release. The presence of gonadal steroids, particularly from the fully-developed ovary, tended to obscure this long-term reduction in the concentration of plasma PRL. In a parallel study in male and female turkeys, the photoinduced pattern of increased PRL release followed by the development of photorefractoriness for plasma PRL was more evident than in intact bantams. However, in both turkeys and bantams, the development of photorefractoriness for PRL release was not associated with reproductive refractoriness as it is in many wild birds (Lincoln *et al.*, 1980; Dawson and Goldsmith, 1982; Sharp *et al.*, 1986a; Stokkan *et al.*, 1988). Of particular interest was the observation that, in the intact turkeys, the development of photorefractoriness for PRL release was preceded by the development of photorefractoriness for LH release as observed in many wild birds (Section 1.3.4, Nicholls *et al.*, 1988). In contrast, in the intact bantams, there was no evidence for the development of photorefractoriness for LH release. This may be reflection of the fact that bantams develop 'relative' but not 'absolute' reproductive photorefractoriness (Sharp, 1988), while some breeds of turkeys develop 'absolute' photorefractoriness (Lien and Siopes, 1989). Unlike the development of 'absolute' refractoriness, the development of 'relative' refractoriness has not been reported to be associated with a depression in plasma LH (Robinson and Follett, 1982, Lien and Siopes, 1989).

The observation that immunisation against VIP blocked photoinduced PRL secretion in castrated bantams demonstrated that VIP release is controlled by changes in photoperiod and that it transduces the PRL photoperiodic response (Chapter 9).

The physiological significance of photoinduced PRL secretion was investigated by actively immunising turkey hens against VIP. This treatment suppressed photoinduced PRL release and, as observed by El Halawani *et al.* (1995a), inhibited the development of broodiness. It has been suggested for wild birds such as the starling that increased PRL secretion after photostimulation may be involved in the initiation but not the maintenance of reproductive refractoriness (Section 1.3.4). Unfortunately, in the

'unimproved' breed of turkey used in the present studies, the low concentrations of LH observed after prolonged exposure to long days, indicative of the development of reproductive photorefractoriness, were sufficient to maintain full ovarian function. It was therefore not possible to determine whether the peak in PRL secretion observed 63 days after photostimulation plays any role in the regulation of reproductive function.

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APPENDIX 1

Addresses of suppliers of reagents and equipments used in the Thesis.

Abacus concepts Inc
Berkley
CA, USA

Amersham International plc
North European Region
Lincoln Place, Green End
Aylesbury
Buckinghamshire

BDH Chemicals Co
Poole
Dorset

BIOSOFT
22 Hill Road
Canbridge

Brunswick Scientific (UK)Ltd
Wartford
Herts

Central Veterinary Laboratory
New Haw
Weybridge
Surrey

Cynamid
Animal Health Divison
Gosport
Hampshire

Denley-Luckham Limited
Victoria Gardens
Burgess Hill
West Sussex

Dupont (UK) Ltd
Wedgwood way, Stevenage
Hertfordshire

Ethicon Limited
Bankhead Avenue
Edinburgh

Evans Medical Limited
Langhurst,
Horsham

Fisons
Bishop Meadow Road
Loughborough
Leicestershire

GIBCO Life Technologies Limited
Trident House
PO Box 35
Renfrew Road
Paisely

Hichrom
1 The Markham Centre
Reading,
Berkshire

How & Co Limited
12-14 St Ann's Crescent
London

ISA Poultry Service
Forfar
UK

LKB-Pharmacia
351 Midsummer Boulevard
Central Milton Keynes

Millipore Corporation
34 Maple Street
Massachusetts
USA

New Brunswick Scientific Ltd
Wartford
Herts

Peninsula Laboratories plc
PO Box 62
17K Westside Industrial Estate
Jaskson Street, St Helens
Merseyside

Pierce and Wariner Ltd
Chester
Cheshire

Scottish Antibody Production Unit
Carluke
Lanarkshire

SIGMA Chemicals Company Ltd
Fancy Road
Poole
Dorset

