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# THE EFFECTS OF FUNGAL-INFESTED FESCUE ON HORMONAL SECRETIONS AND OVARIAN DEVELOPMENT IN THE BEEF HEIFER

A Thesis

Presented for the

Master of Science

Degree

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# ABSTRACT

A fungal endophyte, Acremonium coenophialum, associated with tall fescue is known to produce factors which are toxic to grazing livestock. Cattle which ingest the affected fescue may exhibit depressed physical condition, reduced reproductive performance as well as decreased prolactin (PRL) levels. Decreased PRL release has been shown to delay puberty in some species and may be the cause of reduced reproductive performance in animals grazing infested fescue.

Four experiments were conducted on heifers grazing tall fescue pastures with varying degrees of infestation to determine the effect of treatment (fungal endophyte level) on the ability of the hypothalamus, pituitary and ovary to respond to hormonal challenges.

An intramuscular injection of Gonadotropin releasing hormone (GnRH) analog was administered when the heifers were approximately 3 and 5 months of age. Jugular blood was collected at 0, 1, and 2 hours after the injection of GnRH and plama was assayed for gonadotropins, prolactin and 17β-estradiol ($E_2$). An injection of 1 mg of 17β-estradiol benzoate at 7 months of age was administered to test the ability of the hypothalamus to produce a preovulatory-like LH surge. Samples were collected 14.5, 15.5 and 18.0 following the injection of estrogen. To test the effects of fungal-infested fescue on the growth and functional ability of the ovarian follicle, a superfollicular response was induced with 1,000 IU of Pregnant Mare Serum Gonadotropin (PMSG) when the heifers were 9-months-old. Two 12 ml blood samples were collected 5 days post-injection of PMSG and heifers were ovariectomized immediately following

the bleedings. Ovaries were quartered, sectioned (10 microns) and representative sections were evaluated for level of follicular development, germ-cell survival, follicular atresia, number of ovulations and signs of luteinization.

GnRH, $E_2$ and PMSG did not significantly affect PRL release ($p > 0.10$) however PRL levels were significantly less ($p < 0.01$) in the treatment groups as compared to Controls, except when ambient temperature was high.

Follicle-stimulating hormone (FSH) and luteinizing hormone (LH) were not significantly affected by treatment ($p > 0.10$); however, the LH levels of heifers in the infested fescue groups were lower than those of Controls in all experiments except for the $E_2$ challenge. Those heifers which did respond to 17β-estradiol benzoate stimulation with LH levels greater than 5.0 ng/ml were either in the Control or 60% infestation group. Therefore, factors other than toxicants from the fungus must have delayed the maturtion of the hypothalamo-hypophysial axis. Germ-cell survival was adversely affected by the ingestion of fungal-infested fescue ($p < 0.02$) and an apparent dose-dependent relationship existed. Vesicular-follicle response to PMSG was also adversely affected in all infestation levels as compared to the Controls, however, the difference was not significant ($p > 0.10$). The results indicate that the reduction in PRL and/or LH levels in heifers grazing fungal-infested fescue may be responsible for decreased germ-cell survival and impaired follicular development and these factors would probably cause a reduction in reproductive performance.

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# CHAPTER I

## INTRODUCTION

Tall fescue is a commonly used perennial grass grown in the South and Central regions of the United States where it occupies approximately 35 million acres of land (Ball, 1984). This grass is popular for many of its qualities such as excellent seed production, adaptability, persistence and high forage yield (Ball, 1984). However, a fungal endophyte, Acremonium coenophialum, has been associated with tall fescue and is known to produce toxic factors which are detrimental to grazing livestock (Hemken et al., 1984). Animals which ingest the infested-fescue may exhibit depressed physical condition, reduced reproductive performance as well as decreased prolactin (PRL) levels (Ball, 1984; Bolt et al., 1983).

Researchers suggest that the toxic substances produced by the fungus are ergot alkaloids and related ergopeptide species (Lyons et al., 1986; Bacon et al., 1986). Experiments indicate that treatment of animals with ergot alkaloids will also result in reduced PRL release (Floss et al., 1973).

Reduced reproductive performance may be related to low prolactin levels or hypoprolactinemia. Advis et al. (1981) suggest that PRL may play an important role in the development of the ovary. In the rat, PRL appears to determine the number of LH receptors on the follicle thereby influencing the response of the follicle to gonadotropin stimulation (Advis et al., 1981). Hypoprolactinemia, induced with an ergot

alkaloid, Bromoergocriptine, has been shown to delay puberty as well as reduce serum levels of progesterone ($P_4$), luteinizing hormone (LH) and the release of ovarian progesterone in the rat (Shaban et al., 1986).

These results suggest that endophyte-infested fescue has negative effects on reproductive development and performance. Therefore, the main objective of this study is to determine the extent to which ingestion of endophyte-infested fescue affects germ-cell survival, ovarian development and the maturation of the hypothalamo-pituitary axis, and secondarily, to consider the degree of treatment effects between infestation levels.

# CHAPTER II

# LITERATURE REVIEW

## The Effects of Fungal-Infested Fescue on Prolactin Release

Animals grazing fescue infested with the fungus Acremonium coenophialum are generally in poor condition and exhibit low performance. This fungal endophyte has been known to produce toxic factors, such as ergot alkaloids or other ergopeptide species, which are detrimental to those animals which ingest it (Lyons et al., 1986; Hemken et al., 1984). Lyons et al. (1986) reported that ergot alkaloids, as well as the ergopeptide species, are present in all aboveground parts infested tall fescue thereby increasing availability when grazing. "Fescue toxicosis," "summer syndrome" and "fescue foot" are conditions which result from the ingestion of fungus-infested fescue. Conditions associated with poor performance are decreased body weight, rough hair coat carried through the summer, increased body temperature, and respiration, excessive salivation and reduced reproductive performance (Ball, 1984; Hemken et al., 1979). Problems associated with reproduction include agalactia (milk deficiency following parturition), failure to maintain pregnancy, and weak to stillborn young (Daniels et al., 1983).

Endophyte-infested tall fescue seed was fed to female Sprague-Dawley rats to determine its effect on reproductive performance (Varney et al., 1987). Those rats fed seed with a 40% infestation level failed to maintain a normal estrous cycle and become pregnant. Their body weight was decreased as well as the weight of their uterus. Rats fed

seed with a 20% infestation level conceived but experienced a 2.2 day extension of their estrous cycle. Lower levels of infestation did not affect the reproductive performance of rats in the remaining test groups.

Zavos et al. (1987) conducted a study to determine the effect of a toxic fescue seed, Kentucky-fescue 31 (Ky-31), with an 80% level of infestation on the reproductive performance of both male and female mice by combination crosses. Combination crosses consisted of: (1) males and females fed Diet 1 (50% noninfested fescue and 50% mouse chow); (2) males fed Diet 2 (50% infested fescue and 50% mouse chow) and females fed Diet 1; (3) Diet 1 males and Diet 2 females; (4) Diet 2 males and females. The pregnancy rate, average number of pups per litter, and the weights of pups born per litter were compared and measured. The pregnancy rate of group 1 was 100% compared to 83.3%, 75.0% and 54.5% for groups 2, 3, and 4 respectively. The average number of pups born per litter were 11.5, 8.0, 9.7 and 7.5 with an average birth weight per litter of 17.5 gm, 12.6, 12.4 and 9.8 respectively. Therefore, toxic fescue adversely affected the reproductive performance of both the male and female, however, the negative effects were more severe in the female.

Garrett et al. (1980) conducted a survey to identify the incidence of reproductive problems in mares grazing fescue. Data received from 298 Missouri farms showed that the major reproductive problems associated with fescue were agalactia, prolonged gestation, abortion, and thick placentas. They also reported that foal losses were 3 times

greater in mares grazing fescue than on other forages with the incidence of agalactia being 12 times greater.

A study was conducted by Bond et al. (1982) to determine whether the cause of reduced fertility in ewes grazing GI-307 fescue (a highly infested fescue) was due to increased occurrence of fertilization failure or embryonic death. Examination of the ova revealed that all ova had cleaved within each of the test groups, therefore fertilization had occurred. The remaining ewes were observed for return to estrous. Of those ewes fed GI-307 fescue, 5 out of 14 returned to estrus within 19 days of mating. Bond et al. (1982) concluded that reduced fertility may be due to embryonic death.

Piper and Henson (1983) fed crossbred ewes a high alkaloid fescue hay, Kentucky-fescue 31 (Ky-31), during the last trimester of gestation to determine its effect on prolactin (PRL) levels and lambing weights. Results indicated that peripheral PRL concentrations were suppressed and the lambing weights in twins were reduced as compared to those of controls.

Bolt et al. (1982) found reduced plasma PRL levels in ewes grazing GI-307. Five ewes each were placed on orchardgrass or GI-307 and the resulting mean plasma PRL levels were 349 ± 79 and 1 ± 0.1 ng/ml, respectively. The ewes were then switched to the other pasture. The plasma PRL levels after 24 hours were 38 ± 5 and 47 ± 4 ng/ml and after 72 hours PRL levels had changed to 1.3 ± 0.2 and 117 ± 3 ng/ml. The response of the ewes' pituitary to exogenous stimulation with thyrotrophin-releasing hormone (TRH), a PRL releasing factor, was also

studied and the PRL levels in both test groups were elevated. They concluded that the reduction in PRL concentration was a result of inhibited pituitary secretion rather than lowered pituitary concentrations.

Hurley et al. (1981) conducted a study to determine serum concentrations of PRL, thyrotropin (TSH), triiodothyronine ($T_3$), and thyroxine ($T_4$) between Holstein calves fed a highly toxic fescue (GI-307) and a less toxic fescue (GI-306) at different temperatures. Temperature or infection level did not affect basal TSH levels or TRH-induced (15 µg/100 kg body weight) TSH release. As temperature increased basal $T_3$ levels decreased, but TRH-induced $T_3$ release increased linearly with an increase in temperature. Neither $T_3$ nor $T_4$ were affected by forage. Basal levels of PRL in calves fed the more toxic fescue, G1-307, were lower than those of calves fed G1-306. Calves fed G1-306 had a greater response to the TRH injection than those fed G1-307 with PRL concentrations of 35.7 ± 5.2 ng/ml and 5.6 ± 0.6 ng/ml respectively. Basal levels of PRL in calves fed G1-306 increased as the temperature increased but this response did not occur in calves fed G1-307 (Hurley et al., 1981). Temperature also positively affects TRH-induced PRL release in calves fed G1-306 but not in those fed G1-307. Hurley et al. (1981) suggested that some factor in the fescue limits the ability of the animal to adapt to an increase in temperature.

Henson et al. (1984) noted that feeding fescue hay to lactating dairy cows resulted in suppressed PRL concentrations more so than feeding Bermuda grass. However, milk production was not affected. In

another study using primiparous Holstein heifers fed toxic fescue, serum PRL levels declined 5 minutes after milking but not within one-half hour before or after the 5 minute period (Jackson et al., 1982). Milk levels were also not affected by the depressed PRL levels.

Ergot alkaloids, the apparent toxic factor in infested fescue, have been implicated in the reduction of PRL secretion in the rat and bovine. Zeilmaker and Carlson (1962) demonstrated a direct effect of ergocornine, an ergot alkaloid, on the rat pituitary resulting in decreased PRL secretion. Smith et al. (1974) demonstrated the ability of ergocryptine to inhibit PRL release from bovine anterior pituitary cells in primary culture. However, hypothalamic extracts of ergocornine-treated rats cultured with pituitaries resulted in reduced PRL and luteinizing hormone (LH) levels as compared to controls (Wuttke et al., 1971). Thus suggesting that the ergocornine treatment acts directly on the hypothalamus to increase the prolactin inhibiting factor (PIF) and decrease the luteinizing hormone-releasing hormone (LHRH) (Wuttke et al., 1971).

Since a hypothalamic-ergot alkaloid interaction has been implicated in the reduction of PRL release, many studies have been conducted to determine the effect of dopamine antagonists on PRL release in animals fed fescue. Dopamine (DA), a catecholamine, is released by the hypothalamus and is believed to be an inhibiting factor involved in PRL control.

Bolt et al. (1983) conducted a study testing the effects of two different DA antagonists on PRL secretion in beef heifers grazing fescue. The nondrug treated heifers grazing fescue exhibited lowered

PRL levels but those heifers treated with either Domperidone or Spiperone responded with elevated PRL levels. Grazing behavior was also examined as DA is known to be involved in the thermoregulatory mechanism. Those animals grazing the fescue spent most of their grazing time under the shade as did the heifers treated with the DA antagonists. These results suggest that DA is involved in the PRL response to infected fescue but that other mechanisms must also be involved (Bolt et al., 1983).

Henson et al. (1987) tested the response of PRL and DA in wethers fed Kentucky-fescue 31 (Ky-31) or Rye-orchardgrass for 8 days. One-half of each test group was also administered Spiperone, a DA antagonist. Plasma DA levels were elevated ($p < 0.05$) following day 8 in nondrug treated wethers grazing infected fescue compared to those fed Rye-orchard grass, while plasma PRL levels were reduced ($p = 0.08$). The Spiperone was successful in depressing DA release and allowed for an increase in PRL secretion. These results may explain the mechanism by which toxic fescue exerts its negative influence over PRL secretion (Henson et al., 1987).

## Control of Prolactin Secretion

Prolactin (PRL) is a polypeptide hormone released from the lactotropes of the anterior pituitary gland. It is thought largely to be controlled by an inhibiting factor released by the hypothalamus (Karsch, 1984).

Experiments where the hypothalamic effect on the anterior pituitary was removed resulted in elevated prolactin levels and depressed

gonadotrophin levels. This indicates that the gonadotrophic hormones are under a stimulatory influence and prolactin is controlled by inhibiting factors (Karsch, 1984).

Dopamine (DA), a catecholamine released by the tuberoinfundibular neurons (TIDA) of the hypothalamus, is believed to be involved in prolactin control. The cell bodies of the TIDA neurons are located in the arcuate and periventricular hypothalamic nuclei with their axons found adjacent to the primary capillary plexus of the hypophysial portal vessel. The location of their axons near the vascular link between the hypothalamus and anterior pituitary gland may explain the mechanism by which DA exerts its control over prolactin.

In addition, in vitro studies have shown the presence of high affinity DA receptors on the prolactin secreting cells or lactotropes of the anterior pituitary (Foord et al., 1983). Rosenzweig et al. (1982) demonstrated the internalization and intracellular distribution of [$^{3}$H]Dopamine in the lactotropes as well as the somatotropes of the female rat anterior pituitary.

It has been proposed that DA may exert its effect through the release of a prolactin inhibiting factor (PIF) or that DA itself is the PIF influencing PRL secretion. Gibbs and Neill (1978) conducted a study utilizing DA infusions at levels mimicking that in the hypophysial stalk plasma. Their results supported the hypothesis that DA itself is a physiological PIF acting directly at the level of the adenophypophysis.

Hypoprolactinemia and hyperprolactinemia can be induced through the use of the DA agonist Bromocryptine and the antagonist Haloperidol,

respectively (Arita et al., 1986). Bromocryptine administered to women in the immediate postpartum period completely inhibited the breast engorgement and puerperal lactation normally induced by elevated PRL levels (del Pozo, 1972).

The dopamine precursor, L-dopa, administered i.v. has also been shown to inhibit PRL secretion in normal and stalk sectioned rhesus monkeys (Diefenbach et al., 1976). L-dopa also suppressed the TRH-induced release of PRL in both of these test groups, thus indicating direct action on the anterior pituitary (Diefenbach et al., 1976).

Prolactin itself has been implicated in its own regulation via a negative feedback process. High levels of PRL have been shown to increase DA turnover in the median eminence and resulted in elevated DA concentrations in the hypophysial portal blood (Gudelsky et al., 1976). Subsequently the high DA levels suppressed further release of PRL.

There has been experimental evidence suggesting that the control of PRL secretion may be under the influence of a prolactin releasing factor (PRF). For example, Thyrotrophin releasing hormone (TRH), when injected in large doses, can stimulate PRL release in vivo and in vitro as well as stimulate its own target cells, the thyrotropes, to secrete TSH. TRH is known to stimulate PRL in a variety of species including humans, sheep, cattle, and rats (Hadley, 1984).

Other peptides which may be involved in stimulatory control of PRL secretion are the opioids. Ragavan et al. (1981) demonstrated that β-endorphin is specifically involved in both the basal and stress-induced release of PRL by experiments utilizing anti-β-endorphin anti-serum injected into the cerebral ventricles. However, anti-β-endorphin

antiserum injected intravenously had no effect on PRL release in rats. Intravenous injections of Naloxone, the opiate receptor antagonist, into male primates did reduce the basal serum PRL (Gold et al., 1979; Gosselin et al., 1983). A study by Dalip et al. (1985) demonstrated Naloxone's ability to suppress the spontaneous proestrus, estrus and cervicovaginal stimulus-induced PRL surges in rats. In addition, persons addicted to the opiates for as long as two years and then placed on a Methadone maintenance program exhibited high PRL levels, 43.2 ± 14.5 ng/ml, compared to controls, 5.49 ± 0.59 ng/ml (Vescovi et al., 1985). They concluded that the opiate's effect on PRL release may occur through a variety of mechanisms: (1) a decrease in DA turnover resulting in decreased DA levels, (2) opiates may allow for an increase in serotonin availability to the CNS, or (3) there is a decrease in the DA/serotonin ratio. Wehrenberg et al. (1981) suggest however that the mechanism by which opiates increase PRL levels is through a dopaminergic pathway and not a serotonergic pathway.

Prolactin levels are known to vary under certain physiological conditions. They increase during sleep, suckling, proestrus in the rat, and during cervical stimulation or sexual intercourse. Experiments using antagonists of the neurotransmitter serotonin prevented the suckling-induced PRL release as well as the nocturnal rise in PRL in humans (Crosignani et al., 1979).

The presence of oxytocin (OT) in the hypophysial portal blood of rats in concentrations up to fifty times greater than that in systemic blood (Fink et al., 1984) suggests that OT may play a role in regulating

PRL secretion. Lumpkin et al. (1983) demonstrated that OT could stimulate PRL release in a dose-dependent fashion during in vitro studies using cells of the anterior pituitary. The in vivo studies using conscious male rats injected intravenously with OT also demonstrated an elevation in PRL levels in a dose-dependent manner (Lumpkin et al., 1983).

The administration of an antiserum specific for OT in lactating female rats and estrogen primed-ovariectomized rats delayed and significantly reduced the expected PRL surge (Samson et al., 1986). This evidence suggests that OT, during certain physiological states, could play an important role in the control of PRL release.

The estrogenic steroids have been shown to increase PRL levels in the rat, hamster and ewe. Gudelsky et al. (1981) showed that treatment of ovariectomized rats with estradiol benzoate for five consecutive days resulted in increased serum PRL concentrations. Cultured rat pituitary cells could be stimulated to release PRL when treated with 17β-estradiol (Haug and Gautvik, 1976). Single injections of either estradiol benzoate (70 µg) or 17β-Estradiol (50 µg) increased PRL levels in ovariectomized ewes (Howland et al., 1984). In vitro studies by Padmanabhan and Convey (1979) demonstrated the ability of bovine pituitary cells in primary culture to respond to 17β-estradiol treatment and induce PRL secretion (both basal and TRH-induced). Swanson and Hafs (1971) demonstrated that serum PRL concentrations increased ($p \cong .08$) at estrus in Holstein heifers, but Beck et al. (1976) found that 17β-estradiol implants, at concentrations similar to that found in the normal estrous

cycle, had no effect on PRL release in the Holstein heifer. In a study by Schams and Karg (1972) involving dairy cows, infusions of 2-6 mg of 17β-estradiol resulted in a 2-5 fold increase in plasma PRL levels following an initial depressed plasma PRL response. Infusion of 12 mg of 17β-estradiol caused a sustained initial depression and a subsequent slight elevation in PRL. However, serum PRL levels in the lactating cow did not change significantly during the estrous cycle in a study conducted by Koprowski and Tucker (1973). In addition, Baxter and Gorski (1981) found that estrogen administration did not alter the PRL synthesis in male calves or in ovariectomized or adrenalectomized cows but did stimulate a two-fold increased in PRL release in male weanling rats.

In the rat, a PRL peak occurs simultaneously with the LH surge. In sheep, PRL levels rise at the time of luteal regression and continue to increase until the end of the ovulatory LH surge. It has been suggested that the elevated PRL levels associated with the LH surge are a result of the increase in estrogen (Neill, 1971; Kann and Denamur, 1974). Injection of an antiserum specific for estrogen can prevent the proestrous rise in PRL levels in the rat (Neill, 1971). However, McNeilly (1980) states that in sheep the increase in PRL occurs prior to any rise in estrogen concentration therefore, in sheep, estrogen may not be a stimulatory factor involved in the increase of PRL.

Women exhibit high PRL levels in association with the preovulatory LH surge and this response is directly related to an increase in estrogen (Robyn et al., 1973). It has also been suggested that the increase in PRL may be the result of a decrease in dopamine (DA) release which occurs around the time of ovulation in women and sheep (McNeilly, 1980).

Reproductive Functions of Prolactin

Prolactin is most commonly known for its role in lactation however autoradiographic studies in the rat have revealed that many tissues in the body have receptors specific for PRL and therefore PRL may affect the physiological processes that occur within that tissue. Some of the tissues found to contain receptors specific for PRL are: the epithelial cells of the mammary gland, the secretory epithelium of the prostate gland, and specific steroid-producing cells found within the gonads of both the female and male (Costlow and McGuire, 1977).

In the ovary, receptors for PRL have been found on the granulosa cells of large follicles in rats (Wang et al., 1979) and also on rat luteal cells (Richards and Williams, 1976). Prolactin receptor number in the granulosal cells is apparently determined by follicle stimulating hormone (FSH) (Wang et al., 1979).

Ovarian development and steroidogenesis appear to be affected by the level of PRL in the rat (Advis et al., 1981). Chronic hyperprolactinemia results in precocious puberty in the rat by involving progesterone and estradiol responsiveness to gonadotrophin stimulation (Ojeda et al., 1980). Prolactin has been implicated in determining the number of luteinizing hormone (LH) receptors and thereby enhancing the ovarian response to gonadotrophin stimulation prior to puberty (Advis et al., 1981).

In the rat and ewe, PRL is considered to be luteotrophic in that it enhances the growth of the corpus luteum (C.L.) and the secretion of progesterone. Prolactin achieves this luteotrophic activity through its

modulation of the number of LH receptors on cells of the C.L. of the ovary (Richards and Williams, 1976). Grinwich et al. (1976) demonstrated that treatment with the dopamine agonist, Bromocriptine, resulted in a loss of LH receptors on the C.L. Prolactin was able to maintain luteal activity in the ewe for up to 12 days at a reduced capacity whereas LH administered alone did not have this ability (Kann and Denamur, 1974).

Through regulation of the LH receptor, PRL can control the response of the corpus luteum to the luteolytic action of Prostaglandin F2α (PGF2α) in the rat (Grinwich et al., 1976). The loss of LH receptors due to exposure to PGF2α was reduced when the cells were pretreated with PRL, thus indicating a specific role for PRL in the maintenance of LH receptors (Grinwich et al., 1976).

Murphy and Rajkumar (1985) suggest that another mechanism by which PRL maintains LH receptors may be through the regulation of membrane viscosity. More specifically, PGF2α induces degeneration of the C.L. by decreasing the fluidity of the membrane and the destruction of the LH receptors. Prolactin prevents this loss of receptors by increasing membrane viscosity (Buhr et al., 1983).

Experiments indicate that PRL plays a role in steroidogenesis. Fortune and Vincent (1986) demonstrated the ability of PRL, in culture, to stimulate progesterone secretion from mature rat PMSG-differentiated granulosa cells but not from immature rat DES-treated undifferentiated granulosa cells. In addition PRL enhanced the stimulatory effect of FSH and testosterone on both types of granulosa cells (Fortune and Vincent,

1986). Wang et al. (1979) concluded from their studies that FSH-induced PRL receptors were capable of stimulating progesterone production by the granulosa cells in vivo and in vitro. They noted, however, that the ratio of progesterone to 20-α-dihydroprogesterone by the granulosa cells was increased. These results indicate that PRL acts to inhibit the action or formation of the enzyme 20-α-hydroxysteroid dehydrogenase (Wang et al., 1979). This may explain one of the mechanisms by which PRL exerts its luteotrophic effects. Murphy and Rajkumar (1985) suggest that PRL may increase progesterone secretion through the binding of high density lipoproteins (HDL) thereby providing substrate for progesterone synthesis.

Wang et al. (1979) demonstrated that FSH-induced PRL receptors are associated with progestin secretion but not estrogen synthesis. Studies conducted on rat granulosa cells in culture indicate that PRL inhibits estrogen secretion by its prevention of FSH-induced aromatization of androgen to estrogen (Dorrington and Gore-Langton, 1982). Experiments using graded doses of PRL on rat granulosa cells in culture showed suppressed estrogen secretion under conditions in which progesterone synthesis would be either stimulated or inhibited (Fortune et al., 1986).

Dusza et al. (1986) demonstrated that PRL infusions during the luteal phase and follicular phase of the estrous cycle of gilts had no effect on progesterone or LH secretion. They concluded that PRL does not play a major role in the regulation of the estrous cycle in the pig. However, Veldhuis et al. (1980) demonstrated that PRL could enhance progesterone secretion in porcine granulosal cells in culture if the cells had been retrieved from mature follicles.

Prolactin exhibits no measurable luteotrophic effect in the bovine. Studies were conducted by Hoffmann et al. (1974) to test the luteotrophic ability of endogenous LH and PRL. Antisera or inhibitors specific for these hormones were administered and C.L. function was measured. There was no change in progesterone after treatment with PRL antiserum or inhibitor, but C.L. function ceased in all heifers and cows treated with the LH antiserum. Therefore LH, not PRL, appears to be the major luteotrophic hormone in the bovine.

The literature did not contain any information on the effects of PRL on folliculogenesis in the bovine.

Prepuberal Development

Puberty is "the state at which the gonads come to maturity relative to their endocrine and gametogenic potential for reproductive function" (Hadley, 1984). The initiation of puberty appears to be dependent on the maturation of the hypothalamic-pituitary axis (McLeod et al., 1984; Gonzalez-Padilla et al., 1975). The age at which puberty occurs is influenced by a number of factors including: weight, species, breed, nutritional status and photoperiod. The ranges in age at puberty are 8-18 months in heifers, 5-10 months (seasonal) in ewes, and 5-7 months in gilts (Hunter, 1980).

Desjardins and Hafs (1968) studied the pituitary content of the gonadotropins in heifers from birth to 12 months. They found FSH to be highest at 1 month of age, decreasing at 2 months of age and then constant until 12 months old. Gonzalez-Padilla et al. (1975) conducted a

study in the beef heifer to determine the relationship between pituitary, hypothalamic and ovarian hormones starting 30 to 68 days prior to puberty through the middle of the first cycle. Puberty is described in this study as the age at which the LH peak or surge results in the formation of the first corpus luteum detectable by rectal palpation (Gonzalez-Padilla et al., 1975). In this study, FSH also remained relatively constant except for a period of decrease which occurred between days 38 and 20 prior to ovulation (Gonzalez-Padilla et al., 1975). Levels of FSH had a tendency to be high between 5 days before and the day of ovulation in some of the heifers (Gonzalez-Padilla et al., 1975).

In the heifer, levels of LH increased from 1 to 4 months of age and then concentrations fluctuated until 12 months of age (Desjardins and Hafs, 1968). Gonzalez-Padilla and associates (1975) described two LH peaks occurring prior to puberty. The first peak occurred between 8 and 10 days prior to ovulation and was referred to as the "priming LH peak" (Gonzalez-Padilla et al., 1975). The second peak or surge termed "the pubertal LH peak" occurred on the day of ovulation with concentrations ranging from 11-52 ng/ml. The concentration of the "priming LH peak" was similar in magnitude to that of the "pubertal LH peak" (Gonzalez-Padilla et al., 1975).

Gonadotropin-releasing hormone levels were high up until 40 days prior to ovulation then decreased during the period between days 40 and 12 before ovulation (Gonzalez-Padilla et al., 1975). GnRH levels increased between day 11 and the day of ovulation but did not reach the values which were present prior to day 40. Gonzalez-Padilla et al.

(1975) did not observe any relationship between GnRH and the release of LH or FSH ($p > .05$) during the prepuberal period or the first half of the first estrus cycle. In addition, there were no elevations in GnRH associated with either of the LH peaks (Gonzalez-Padilla et al., 1975). However, other studies indicate that the LH release depends upon hypothalamic stimulation and removal of that stimulation will result in inhibition of the LH pulse (Anderson et al., 1981).

Studies conducted in Holstein heifers demonstrated an increase in pituitary PRL by 333% between birth and 3 months postnatal, however, peak levels of PRL occurred at 9 months of age (Sinha and Tucker, 1968). Prolactin levels appeared to be lowest 40 days prior to ovulation when GnRH and estrogen levels were highest (Gonzalez-Padilla et al., 1975). The greatest sustained concentrations of pituitary PRL occurred within 3 days of ovulation but levels were minimal at the time of ovulation in the dairy heifer (Sinha and Tucker, 1968).

Prolactin, GnRH, LH and FSH levels in blood collected at 20 minute intervals fluctuated greatly indicating that hormone release occurs in an episodic pattern in the beef heifer (Gonzalez-Padilla et al., 1975). Anderson et al. (1981) also demonstrated an episodic release pattern in their study of prepuberal heifers. Gonzalez-Padilla et al. (1975) noted that the frequency of episodic bursts were higher in PRL, GnRH, and FSH than in LH but the magnitude of the LH burst was always greater. However, in the female sheep, as puberty approaches the frequency of LH pulses increases in order to stimulate follicular development and estrogen production (Foster et al., 1985).

Gonzalez-Padilla noted that progesterone levels were low during the 20 days that precede ovulation but every heifer experienced two phases of elevated progesterone release which lasted 2-5 days. The first period of elevated progesterone occurred between days 18 and 11 prior to the "priming LH peak." The second rise in progesterone was higher in magnitude, lasted longer, and occurred between the two LH peaks (Gonzalez-Padilla et al., 1975). The concentration of progesterone during the first luteal phase was higher than at any other time observed up to that point.

Progesterone and PRL were negatively correlated during the prepuberal phase but became positively correlated after the puberal LH peak. Progesterone and FSH were always positively correlated.

There were no distinct elevations in 17β-estradiol associated with the LH peaks (Gonzalez-Padilla et al., 1975). Estrogen levels were high 39 days before ovulation and then gradually declined to remain constant at a low level until the middle of the first cycle (Gonzalez-Padilla et al., 1975).

Prolactin levels were high in prepuberal gilts between 15 and 39 days of age with average concentrations of 16.2 ± 0.8 ng/ml (Camous et al., 1985). Levels dropped to 10.8 ± 0.8 ng/ml at 40 days of age and rose gradually to 14.4 ± 0.8 ng/ml at day 192. The drop at 40 days in PRL coincided with weaning. Camous et al. (1985) suggest that the high levels of PRL prior to weaning may have been the result of intestinal absorption of milk PRL. The LH levels were high in gilts before day 15, constant between days 54 to 125, and then dropped between days 137 and

192 (Camous et al., 1985). Amplitude of the LH pulses was greatest at 15 days, however, the LH pulse frequency was higher between days 83 and 125 than at any other period up to 200 days (Camous et al., 1985). Camous and associates suggest that the drop in LH levels after day 125 may be related to the development of the negative feedback due to more ovarian activity in these gilts. FSH remained relatively constant throughout the study (birth to first estrus) unlike the pulsatile changes which occurred in LH (Camous et al., 1985). FSH secretion was at a maximum at 54 days with concentrations of 11.1 ± 1.0 ng/ml and remained high until day 125. Then FSH levels decreased linearly from day 125 (7.4 ± 1.4 ng/ml) until day 192 (2.3 ± 0.3 ng/ml) (Camous et al., 1985). The increase in FSH around 50 days may have stimulated antral follicle development. This decrease in FSH at day 125 parallels that of LH and as stated above may be the result of development of the negative feedback process (Camous et al., 1985).

Estrogen levels, measured as the metabolite Estrone, increased steadily from day 40 until peak values at first estrus (>200 days) (Camous et al., 1985). The first rise in Estrone, with a concentration range of 1.3 to 2.5 µg/24 hours, coincided with the appearance of antral follicles at an age between 68-110 days. Three more levels of increased estrone were measured with ranges of 2.7 to 5.7 µg/24 hours, 9.2 to 10.9 µg/24 hours, and 17.9 to 21.2 µg/24 hours occurring respectively at age ranges of 96-152 days, 116-199 days and >200 days for those gilts who reached puberty (Camous et al., 1985).

McLeod et al. (1984) demonstrated that in the heifer "the prepubertal period is associated with the lack of pituitary stimulation rather

than the inability to respond to GnRH." Therefore puberty depends upon the maturation of the hypothalamus. In the beef heifer, McLeod et al. (1984) demonstrated the ability of the pituitary to respond to exogenous GnRH as early as 4 months of age. GnRH injections could induce LH release in 4 and 10 month old heifers in a dose dependent manner (0.5, 2.0, 5.0 μg GnRH) but no significant difference was detected between the age groups. Staigmiller et al. (1979) and Schillo et al. (1983) showed that the pituitary can initiate an LH surge in response to estrogen stimulation in some heifers as young as 3-5 months of age. However, at this age, the LH surge would not result in ovulation.

The 19 week old lamb also has the ability to produce an LH surge in response to exogenous estrogen long before the first ovulation would normally occur (31 weeks of age) (Foster, 1984). Foster (1984) suggests that the anovulatory prepuberal period in the sheep is the result of low levels of estrogen and not the inability of the surge mechanism to respond to estradiol-positive feedback.

Ovarian Development

In most mammals, the population of primary oocytes is established before birth (Hunter, 1980) and the bovine is not an exception to the rule (Erickson, 1966).

In the human female, the embryonic ovary is initially populated with 1,000-2,000 primordial germ cells which in turn give rise to 2 to 4 million primary oocytes (oocyte with a single layer of granulosal cells) (Hadley, 1984). At birth, this number is reduced as a result of cell death. At puberty the number of primary oocytes has dropped to

approximately 250,000 per ovary and only a small percentage of these will ovulate.

In the bovine, the average number of primordial follicles (133,000 per individual) remained relatively stable until approximately 4 years of age at which time the number declined and reached near zero in ovaries from cows 15-20 years old (Erickson, 1966). Germ cell quality in primordial follicles was high until 180 days postnatal with a subsequent decrease in quality occurring at age 4 years and older when nearly all had reached a degenerative state (Erickson, 1966).

The number of growing follicles (oocytes with 2 or more layers of granulosal cells) increases rapidly between 50-80 days and continues to rise up until day 120. At this time vesicular follicle number is also increasing. By day 180, vesicular follicle number had reached 63, a lifetime high. A decrease in the number of vesicular follicles occurred near puberty with no further significant reduction until 10-14 years of age.

The majority of follicles developing during the prepuberal period up until 10 years of age were normal. In the prepuberal female there appeared to be a positive correlation between the primordial and more advanced follicles (Erickson, 1966).

The literature does not contain any information regarding the direct effects of the toxins produced by the fungal endophyte in infested fescue on germ cell survival and ovarian development. Studies, however, have been conducted to determine the chronic and acute effects of radiation and chemicals on the ovary. During prenatal development,

the primordial germ cells are apparently more susceptible to the detrimental effects of radiation and alkylating chemicals than the oocytes of the primary or growing follicle (Erickson, 1985). However, the oocytes in the growing follicle or oocytes in later stage of meiosis are more sensitive to the toxicants than the oocytes of primary follicles (Erickson, 1985). Reduction of the germ cell population may result in reduced reproductive capacity but chronic prenatal exposure would be required to severely affect reproduction (Erickson, 1985).

# CHAPTER III

# MATERIAL AND METHODS

## Experimental Design

Forty cows and their calves were placed on eight 4-ha tall fescue (Festuco aruninacia Schreb.) pastures infested to varying degrees with the fungal endophyte Acremonium coenophialum. Levels of fungal infestation were estimated using the ELISA and microscopic procedures. Treatments consisted of (1) two control pastures containing tall fescue with 0% infestation, (2) two pastures containing tall fescue with moderately low fungal infestation (15% and 30%), (3) two pastures containing moderately high fungal infestation (45% and 60%), and (4) two pastures with high fungal infestation (75%). Each pasture was grazed by five cows and their calves. The cows were mature Angus x Hereford crosses and their calves (born in spring) were sired by Simmental bulls. Only the heifer calves were considered in this study as the bull calves were implanted with Ralgro, a synthetic estrogen.

Four experiments were conducted to determine the effect of treatment (fungal endophyte level) on the ability of the hypothalamus, pituitary and ovary to respond to hormonal challenges. The first experiment was conducted in May when the average age of the heifers was 90 days. An intramuscular injection (20 µg) of gonadotropin releasing hormone (GnRH) analog, des-Gly, [D-Ala]-GnRH-ethylamide was administered. A 12 ml blood sample was collected prior to and 1 and 2 hours after the injection. In August at an average age of 160 days old, heifers were

again tested for their response to GnRH. The same procedure was followed except the dose 10 μg instead of 20 μg was employed.

When the heifers reached an average age of 202 days, the ability of their hypothalamus to produce a preovulatory-like LH surge was tested. Heifers were injected with 1 mg of 17β-estradiol-3-Benzoate in 1 ml of Sesame oil and a 12 ml blood sample was collected at 14.5, 15.5 and 18.0 hours postinjection.

Two basal level blood samples were collected in October when heifers were about 245 days old. No challenge was given and samples were collected approximately 1 hour apart.

To test the effects of fungus-infested fescue on the growth and functional ability of the ovarian follicle, a superfollicular response was induced with 1000 IU of Pregnant Mare Serum Gonadotropin (PMSG) when heifers were an average age of 281 days. Two 12 ml blood samples were collected 5 days postinjection.

At collection all blood samples were placed on ice and within 12 hours thereafter centrifuged at 3000 rpm for 20 minutes. Plasma was removed and stored at -20°C until assayed for gonadotrophins, prolactin and estrogen. For estrogen analysis, plasma samples were pooled.

Heifers were ovariectomized immediately following the bleedings associated with the PMSG challenge. A local anesthetic was used and access to the ovaries was obtained by means of an incision in the left paralumbar fossa. Ovaries were quartered, sectioned (10 microns) and representative sections were evaluated microscopically for level of follicular development, germ-cell survival, number of ovulations and

signs of luteinization. Mature follicles were categorized as atretic or nonatretic.

Radioiodination Procedures

Luteinizing hormone was radioiodinated by the Cloramine-T method. The procedure followed was a modification of the method described by Brown et al. (1983). Dr. L. H. Reichert of the Albany Medical School, New York, provided both the iodination and reference grades of LH.

Follicle-stimulating hormone was radioiodinated by a modified version of the Iodogen method described by Dr. D. J. Bolt (1983 unpublished). Dr. Bolt of the USDA, Beltsville, MD, provided the iodination and reference grades of FSH.

Prolactin was also radioiodinated by a modified version of the Iodogen method described by Dr. Bolt (1983 unpublished). The procedure was identical to that of FSH except for the starting amount of protein (see Appendix A). The purified and reference grades of PRL were also supplied by Dr. Bolt.

The detailed protocol for the LH, FSH and PRL radioiodination procedures and tracer verification (% labelled protein) are in Appendix A.

Radioimmunoassay Techniques

Plasma LH was measured using the method of Niswender et al. (1969) as modified by Bolt (unpublished). The LH antibody (#15 antiovine LH) was provided by Dr. Gordon Niswender, Colorado State University. The antibody did not significantly cross-react with FSH, TSH or growth

hormone (Niswender et al., 1969). An antibody dilution of 1:40,000 and a tracer activity of 18,000 cpm LH-$I^{125}$ was used. There was an average total binding capacity of 43.0%. The reference curve consisted of eight concentrations, in triplicate, of the standard (LER 1056) ranging from 31 to 4,000 pg. In all assays (LH, FSH and PRL), unknowns were compared to the standard curve to determine plasma levels of hormone. Nonspecific binding was < 2.0%. Intra- and interassay variation was determined by the measure of a standard sample of pooled plasma from steers in each assay. Intraassay variation of the pooled samples was < 5.0% and interassay variation was < 7.0%. Addition of tracer (18,000 cpm) to stock antibody provided a further measure of tracer quality, giving an average maximum binding of 60%. Variation among sample replicates was < 3.5%. Details of the LH RIA protocol are in Appendix B.

Plasma FSH levels were determined by the RIA procedure developed by Bolt and Rollins (1983) with modification. FSH reference standard (USDA-bFSH-B1) and FSH antibody (USDA-5-0122) were supplied by Dr. D. J. Bolt. Cross-reactivity of this antibody with bovine LH, growth hormone and bovine prolactin was < 0.09% and bovine TSH had a 0.5% cross-reactivity (Bolt and Rollins, 1983). A reference curve (1-128 ng) was constructed using triplicate samples of the standard (USDA-bFSH-B1). FSH antibody concentration was 1:10,000 and the tracer activity was about 20,000 cpm of FSH-$I^{125}$ to provide an average binding capacity of 20%. Nonspecific binding was < 2.0%. Intra- and interassay variation was determined in the same manner described for the LH assay.

Intraassay variation was < 5.0% and interassay variation was < 9.0%. The average maximum binding capacity was 40.0%. Variation among sample replicates was < 3.5%. See Appendix B for details of RIA protocol.

Measurement of plasma PRL was accomplished using the RIA method described by Bolt and Rollins (1983) (modified). PRL reference standard (USDA-bPRL-B-1) and PRL antibody (DJB 7-0330) were also supplied by Dr. D. J. Bolt. Cross-reactivity with other protein hormones was not significant (personal communication). The reference curve consisted of ten concentrations, in triplicate, of the standard (DJB 7-0330) ranging from 0.25 to 12.8 ng. An antibody dilution of 1:200,000 and a tracer activity of 20,000 cpm PRL-I125 was used to obtain an average total binding capacity of 50.0%. Nonspecific binding was < 3.0%. Intraassay variation was < 5.0% and interassay variation was < 11.0%. The average maximum binding capacity was 75%. Variation among sample replicates was < 10.0%. Details of the RIA protocol for PRL are included in Appendix B.

Estradiol was measured using the RIA protocol developed by Thompson et al. (1978). A reference curve (2.5 to 250 pg) was constructed using duplicate samples of the standard. Estradiol was measured by comparison to the curve. The reference standard was obtained from the Sigma Chemical Co. The first antibody (#244 anti-E-6-BSA serum) was supplied by Dr. Gordon Niswender. An antibody dilution of 1:50,000 and a tracer activity of 20,000 cpm $E_2$-$^3H$ was used to obtain an average total binding capacity of 60%. See Appendix B for details of the Estrogen assay.

Statistical Analysis

In all experiments conducted, the effect of treatment (fungal-infestation) on Prolactin, FSH, LH and estrogen release was tested for significance using a one-way analysis of variance and Student-Newman-Keuls (SNK). Effects between treatment levels and differences between the control and treated populations were tested using orthogonal contrasts.

Correlations (Pearson's product-moment correlation coefficient) were calculated between PRL and the gonadotropins and estrogen. Correlations between follicle classes and hormone levels were also calculated.

The means, standard deviations, standard errors and correlations were computed with the programs provided by the Statistical Analysis Systems (SAS, 1984a). Analysis of variance, SNK and orthogonal contrasts were completed using Statistical Analysis Systems (SAS, 1984b).

# CHAPTER IV

## RESULTS

The level of fungal infestation was not precisely known and due to deficiencies in available assays, the pasture infestation levels originally specified were used with the exception of one pasture. The two heifers of pasture 5 were considered as part of the Control or untreated group instead of part of the 75% infestation level because of their consistently high levels of prolactin (PRL) possibly due to high weed concentrations in the pasture.

### Effects of a GnRH Challenge in Heifers 3.0 ± 0.5 Months of Age

Prolactin release was not affected by GnRH stimulation therefore sample means across time were averaged and that value was used in analysis of treatment effects. Means and standard errors of PRL levels are listed in Table I. Mean PRL levels in the untreated (0% infestation) group were significantly greater ($p < 0.0001$) than the treated population. Overall, there was a tendency for mean PRL levels to decrease as infestation levels increased.

GnRH significantly increased FSH release over time ($p < 0.0001$). Data concerning the GnRH induced FSH release are shown in Table II. Ingestion of fungal-infested fescue did not significantly affect basal, 1 hour or 2 hour levels of FSH ($p > 0.1$). Mean FSH levels of the untreated group at 1 hour (time postinjection) and 2 hours were not

Table I. Effects of Fungal-infested Fescue on Prolactin Levels in the Beef Heifer of 3 Months of Age*

| Level of Fungal Infestation | Number of Heifers | Prolactin (ng/ml) |
|---|---|---|
| 0% | 5** | 194.35 ± 5.65[a] |
| 15% | 3 | 124.16 ± 45.42[a,b] |
| 30% | 5 | 45.43 ± 14.92[b] |
| 45% | 1 | 122.30[a,b] |
| 60% | 3 | 17.91 ± 7.82[b] |
| 75% | 2 | 20.63 ± 13.97[b] |

*Mean ± standard error

**Based on pasture condition and prolactin levels, 2 of the 5 heifers were moved from the 75% infestation group to the Control (0%) group.

[a,b]Means in the same column with different letters are significantly different ($p < 0.0001$)

Table II. Effects of Fungal-infested Fescue on Gn-RH-induced Release of FSH in the Beef Heifer of 3 Months of Age*

| Level of Fungal Infestation | Number of Heifers | FSH (ng/ml)** Hours after Gn-RH | | |
|---|---|---|---|---|
| | | 0 hr | 1 hr | 2 hr |
| 0% | 5 | 11.82 ± 1.66 | 149.06 ± 50.09 | 267.42 ± 51.24 |
| 15% | 3 | 12.02 ± 1.33 | 86.56 ± 30.92 | 202.42 ± 114.71 |
| 30% | 5 | 10.88 ± 1.53 | 116.42 ± 50.89 | 175.44 ± 62.90 |
| 45% | 1 | 10.65 | 73.55 | 113.55 |
| 60% | 3 | 12.40 ± 4.22 | 124.42 ± 29.05 | 239.97 ± 30.05 |
| 75% | 2 | 9.04 ± 0.49 | 30.71 ± 21.58 | 81.49 ± 73.96 |

*Mean ± standard error

**No significant difference between treatment groups ($p > 0.10$)

statistically greater ($p > 0.10$) than FSH levels of the treated population. The very low levels of FSH at 1 and 2 hours in the 75% infestation group indicate a possible treatment effect. However, heifer number is limited and any difference which may occur is not likely to be significant (Table II). There was a modest dose-dependent response at 2 hours after GnRH (Figure 1) and except for the heifers in the 60% infestation group, FSH levels decreased as infestation levels increased.

LH release also increased greatly over time under GnRH stimulation ($p < 0.0001$). Means and standard errors of basal, 1 hour, and 2 hour levels of LH are shown in Table III. Fungal infestation did not significantly affect basal or stimulated levels of LH ($p > 0.10$). Control LH levels (0%) at 1 hour after GnRH were greater than that of the treated population although the difference was not significant ($p > 0.10$). LH levels at 1 and 2 hours after GnRH in the 75% infestation group were very low compared to the control group as well as the rest of the treated population.

Prolactin was positively correlated ($r = .51$; $p < 0.05$) with the 2 hour level of FSH but not with LH at any time. Basal FSH and LH levels were correlated ($r = .65$; $p < 0.01$) as were levels at 1 hour after GnRH ($r = .80$; $p < 0.01$).

Effects of a GnRH Challenge in Heifers 5.3 ± 0.5 Months of Age

The August GnRH challenge resulted in effects similar to those seen in May with all heifers responding to GnRH stimulation. Prolactin release was not significantly ($p > 0.10$) affected by GnRH. Means and

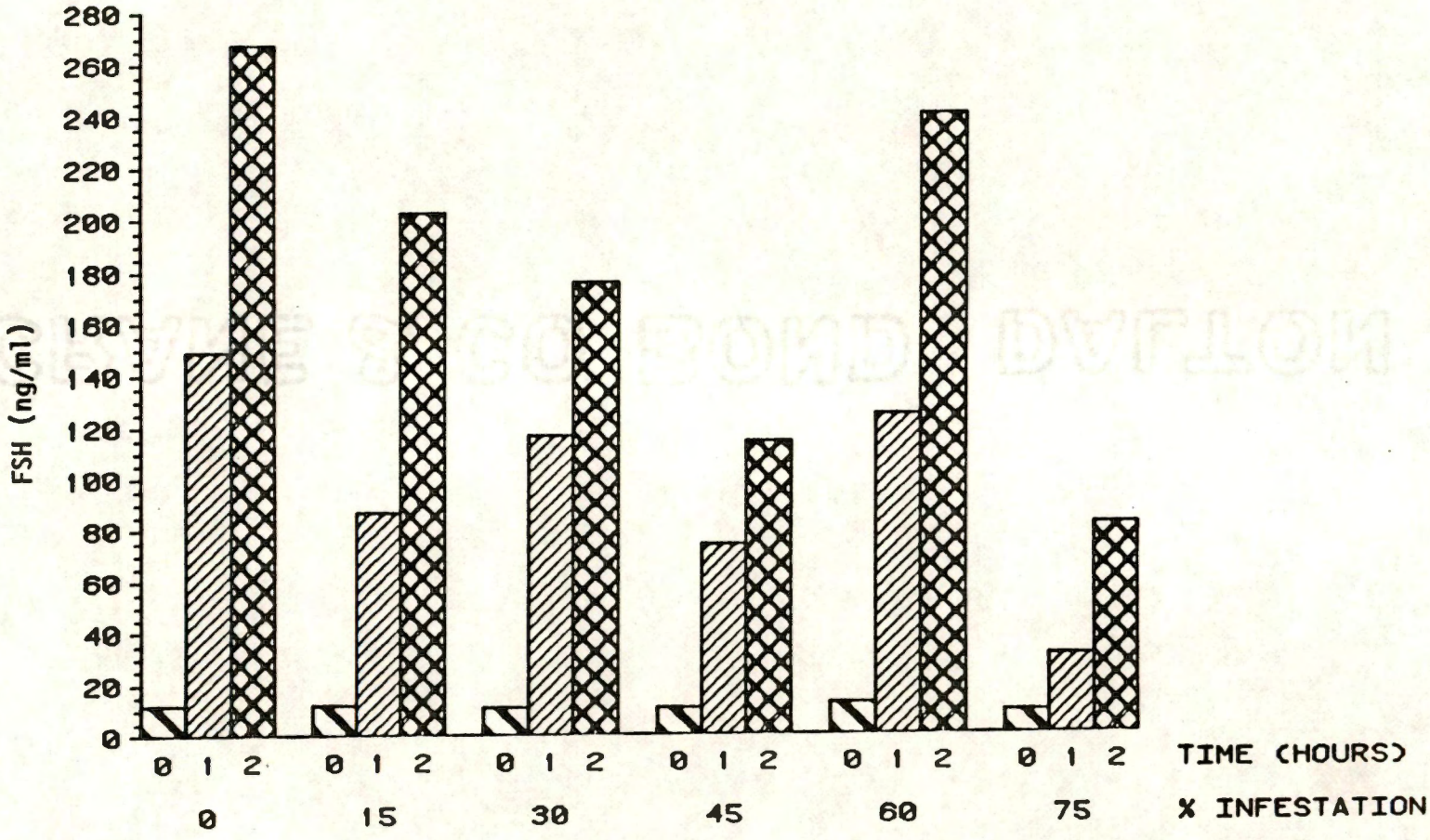


Figure 1. Effect of Fungal-infested Fescue of FSH Response To GnRH in Beef Heifers of 3 Months of Age

Table III. Effects of Fungal-infested Fescue on Gn-RH-induced LH Release in the Beef Heifer of 3 Months of Age*

| Level of Fungal Infestation | Number of Heifers | LH (ng/ml)** Hours after Gn-RH | | |
|---|---|---|---|---|
| | | Basal | 1 hr | 2 hr |
| 0% | 5 | 0.59 ± 0.03 | 27.34 ± 6.26 | 29.66 ± 4.86 |
| 15% | 3 | 0.56 ± 0.02 | 13.25 ± 3.49 | 21.68 ± 9.91 |
| 30% | 5 | 0.54 ± 0.07 | 25.50 ± 6.25 | 30.90 ± 9.80 |
| 45% | 1 | 0.63 | 9.84 | 9.81 |
| 60% | 3 | 0.51 ± 0.13 | 20.90 ± 2.86 | 21.80 ± 2.60 |
| 75% | 2 | 0.54*** | 7.54*** | 14.80*** |

*Mean ± standard error

**No significant difference between treatment groups ($p > 0.10$)

***Missing values

standard errors of PRL levels are listed in Table IV. There was no significant ($p > 0.10$) treatment affect on PRL levels although mean PRL levels in the untreated group are greater than the PRL levels within the treated group. Basal PRL levels were not significantly different ($p > 0.10$) than PRL levels in the younger heifer although the higher infestation levels resulted in higher means PRL levels than was seen in the 3 month old heifer (Figure 2).

Treatment did not affect FSH levels ($p > 0.10$) at either time after GnRH (Table V). Unlike the response described in the younger heifers, there was no dose-related trend between FSH and infestation levels. Mean basal FSH levels of the 5 month old heifer were significantly greater ($p < 0.05$) than basal FSH levels in the 3 month old heifer (Figure 3).

Table VI lists the means and standard errors of the LH levels over time within treatment. The basal levels of LH in the Control group were significantly greater than the 15% ($p < 0.03$), 30% and 75% infestation groups ($p < 0.05$) but not the 45% or the 60% infestation group, but the results are questionable due to too few animals per group. The 1 hour LH levels in the control heifers were greater than the treated population yet the difference was not significant ($p > 0.10$). Fungal infestation has no significant effect on 2 hour LH release ($p > 0.10$). LH levels in the 5-month-old heifer were significantly lower ($p < 0.005$) than those of the 3 month old heifer (Figure 4).

Prolactin was not significantly correlated with either of the gonadotropins at this age. Basal levels of FSH and LH were correlated

Table IV. Effect of Fungal-infested Fescue on Prolactin Levels in the Beef Heifer of 5 Months of Age*

| Level of Fungal Infestation | Number of Heifers | Prolactin (ng/ml)** |
|---|---|---|
| 0% | 5 | 153.25 ± 11.88 |
| 15% | 3 | 119.17 ± 32.70 |
| 30% | 5 | 114.34 ± 25.41 |
| 45% | 1 | 115.97 |
| 60% | 3 | 127.57 ± 22.85 |
| 75% | 2 | 106.35 ± 53.32 |

*Mean ± standard error

**No significant difference between treatments ($p > 0.10$)

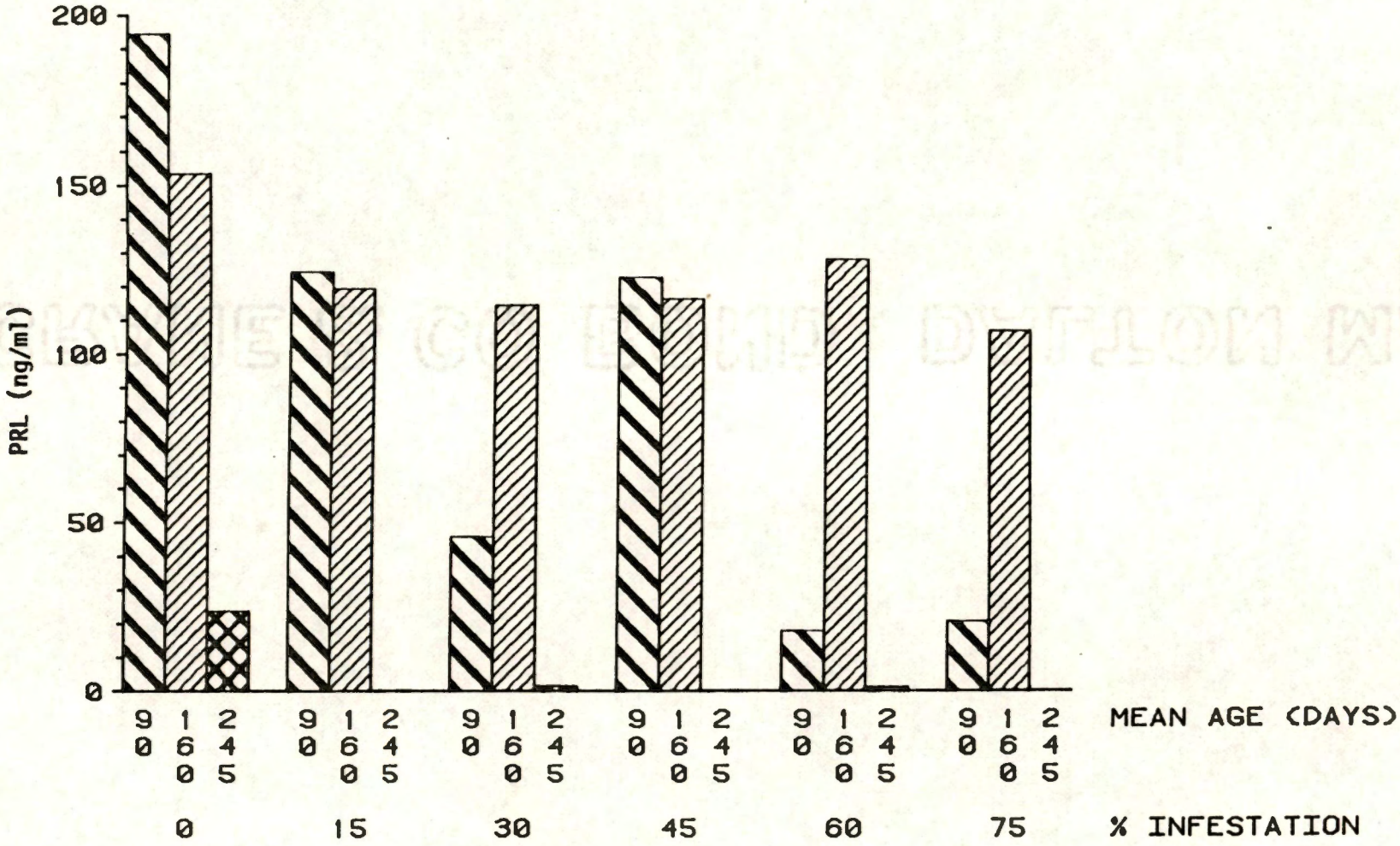


Figure 2. Effect of Fungal-infested Fescue on Basal Levels of PRL in Beef Heifers at Varying Ages

Table V. Effects of Fungal-infested Fescue on FSH Response to Gn-RH in the Beef Heifer of 5 Months of Age*

| Level of Fungal Infestation | Number of Heifers | FSH (ng/ml)** Hours after Gn-RH | | |
|---|---|---|---|---|
| | | 0 hr | 1 hr | 2 hr |
| 0% | 5 | 19.70 ± 2.01 | 86.55 ± 21.76 | 165.46 ± 57.53 |
| 15% | 3 | 19.28 ± 0.66 | 80.32 ± 11.45 | 122.96 ± 18.62 |
| 30% | 5 | 20.73 ± 1.83 | 77.83 ± 12.62 | 172.29 ± 59.09 |
| 45% | 1 | 14.40 | 49.95 | 56.78 |
| 60% | 3 | 17.12 ± 1.64 | 79.21 ± 2.83 | 161.79 ± 29.29 |
| 75% | 2 | 19.65 ± 0.10 | 96.43 ± 4.92 | 123.85 ± 6.70 |

*Mean ± standard error

**No significant differences among treatments at any time ($p > 0.10$)

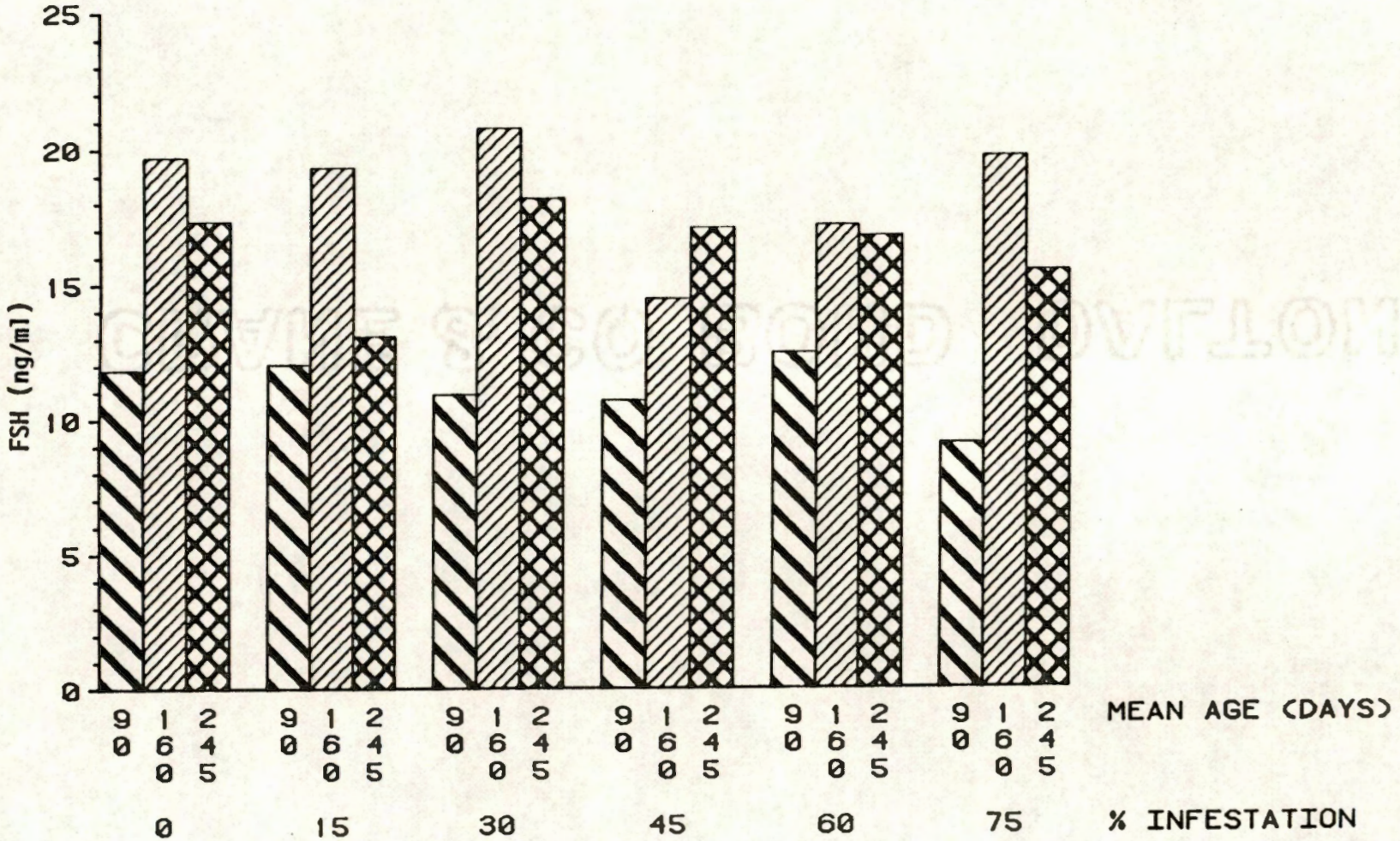


Figure 3. Effect of Fungal-infested Fescue on Basal Levels of FSH in Beef Heifers at Varying Ages

Table VI. Effect of Fungal-infested Fescue on LH Response to Gn-RH in the Beef Heifer of 5 Months of Age*

| Level of Fungal Infestation | Number of Heifers | LH (ng/ml) Time (Post-injection) 0 hr | 1 hr** | 2 hr** |
|---|---|---|---|---|
| 0% | 5 | 0.14 ± 0.03[a] | 14.53 ± 3.88 | 21.49 ± 3.84 |
| 15% | 3 | 0.06 ± 0.0[b] | 10.27 ± 1.57 | 17.79 ± 5.02 |
| 30% | 5 | 0.08 ± 0.02[b] | 8.53 ± 1.50 | 13.49 ± 3.57 |
| 45% | 1 | 0.06[a,b] | 5.04 | 6.10 |
| 60% | 3 | 0.12 ± 0.03[a,b] | 11.27 ± 1.81 | 24.49 ± 7.30 |
| 75% | 2 | 0.06 ± 0.00[b] | 10.73 ± 1.10 | 14.39 ± 1.44 |

*Mean ± standard error

[a,b]Means in the same column with different letters are significantly different ($p < 0.05$)

**No significant differences among treatments at 1 and 2 hours ($p > 0.10$)

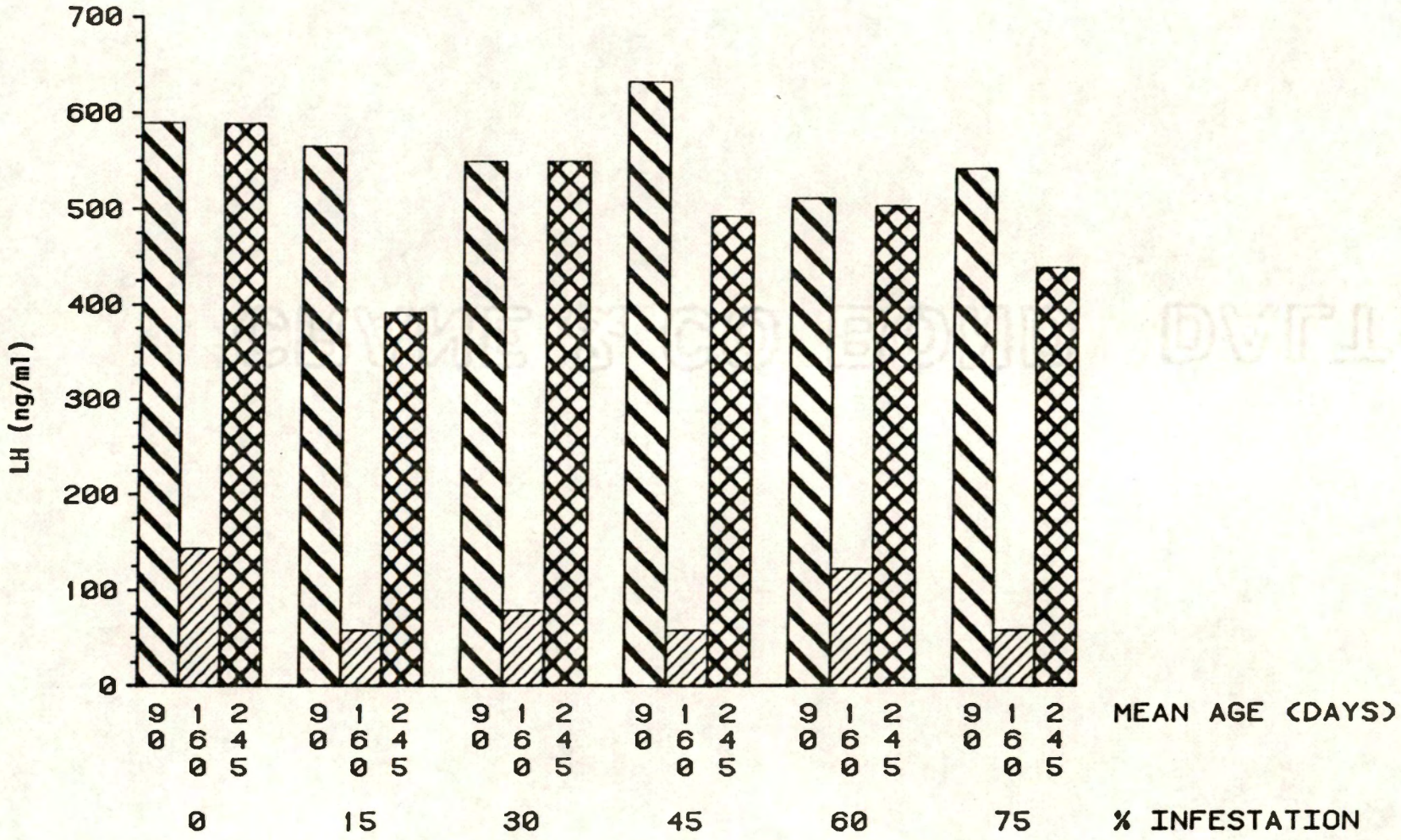


Figure 4. Effect of Fungal-infested Fescue on Basal Levels of LH in Beef Heifers at Varying Ages

($r = .43$; $p < 0.05$). FSH and LH were correlated at both 1 hour ($r = .78$; $p < 0.05$) and 2 hours after GnRH ($r = .62$; $p < 0.05$). Basal and peak levels of FSH were correlated ($r = .53$; $p < 0.05$). Basal LH levels were not correlated with peak LH levels.

17β-estradiol concentrations were also measured in the plasma samples obtained from the August GnRH challenge. Samples collected before and 1 hour after the injection were pooled and analysis was performed. Estradiol levels did not differ among treatments ($p > 0.10$) (Table VII) however treatment severely reduced release in the 75% infestation group. The estrogen levels within the sample were higher in the 0%, 15% and 30% infestation levels than the rest of the population although when contrasted the difference was not significant ($p > 0.10$).

Effects of 17β-Estradiol Challenge in Heifers 6.7 ± 0.5 Months of Age

Pituitaries of three heifers in the 60% infestation group and one heifer in the Control group released a preovulatory-like surge of LH at 14.5 hours after an injection of 1 mg of benzoate (Table VIII). For a heifer to be categorized as "responsive" her LH levels at some point after the injection of estradiol must equal or exceed 5 ng/ml. Differences between Control and treated groups in number of responders was not significant. As expected, the FSH response was similar to that of LH in that the 14.5 hours FSH levels of heifers in the 60% infestation group were significantly higher ($p < 0.0002$) than the rest of the population (Table IX). FSH levels of the 60% group at 15.5 hours were also significantly greater ($p < 0.04$) than those of the other groups.

Table VII. Effect of Fungal-infested Fescue on 17 β-estradiol Levels in the Beef Heifer of 5 Months of Age*

| Level of Fungal Infestation | Number of Heifers | Estrogen (pg/ml)** |
|---|---|---|
| 0% | 5 | 8.8 ± 4.5 |
| 15% | 3 | 7.9 ± 5.6 |
| 30% | 5 | 9.5 ± 7.2 |
| 45% | 1 | 3.0 |
| 60% | 3 | 5.0 ± 2.1 |
| 75% | 2 | 1.8 ± 0.3 |

*Mean ± standard error

**No significant differences among treatment groups ($p > 0.10$)

Table VIII. Effect of Fungal-infested Fescue on the Response of LH to an $E_2$ Challenge in the Beef Heifer of 7 Months of Age*

| Level of Fungal Infestation | Number of Heifers | LH (ng/ml) Hours after 17β-estradiol | | |
|---|---|---|---|---|
| | | 14.5 hr | 15.5 hr** | 18.0 hr** |
| 0% | 5 | 1.70 ± 1.09[b] | 0.45 ± 0.22 | 0.25 ± 0.10 |
| 15% | 3 | 0.12[b] | 0.17 | 0.13 ± 0.04 |
| 30% | 5 | 0.43 ± 0.20[b] | 0.21 ± 0.07 | 0.09 ± 0.01 |
| 45% | 1 | 0.10[b] | 0.10 | 0.11 |
| 60% | 3 | 7.95 ± 2.03[a] | 2.92 ± 1.65 | 1.83 ± 1.23 |
| 75% | 2 | 0.38[b] | 0.33 ± 0.26 | 1.36 |

*Mean ± standard error

[a,b]Means in the same column with different letters are significantly different ($p < 0.01$)

**No significant difference among treatments ($p > 0.10$)

Table IX. Effect of Fungal-infested Fescue on the Response of FSH to an $E_2$ Challenge in the Beef Heifer of 7 Months of Age*

| Level of Fungal Infestation | Number of Heifers | FSH (ng/ml) Hours after 17β-estradiol | | |
|---|---|---|---|---|
| | | 14.5 hr | 15.5 hr | 18.0 hr** |
| 0% | 5 | 22.57 ± 1.78[b] | 16.41 ± 1.88[b] | 18.07 ± 1.80 |
| 15% | 3 | 17.59 ± 2.63[b] | 12.92 ± 2.21[b] | 12.72 ± 1.92 |
| 30% | 5 | 21.62 ± 1.83[b] | 18.87 ± 1.92[b] | 15.88 ± 1.03 |
| 45% | 1 | 15.48[a,b] | 20.05[a,b] | 12.43 |
| 60% | 3 | 71.84 ± 15.05[a] | 44.76 ± 15.43[a] | 36.42 ± 13.78 |
| 75% | 2 | 11.20 ± 3.30[b] | 13.26 ± 5.23[b] | 13.86 ± 4.03 |

*Mean ± standard error

[a,b]Means in the same column with different letters are significantly different ($p < 0.01$)

**No significant difference among treatment groups ($p > 0.10$)

Prolactin release was apparently not affected by estrogen stimulation but it is possible that PRL was released at a time prior to the bleedings effected to detect the LH release (Table X). There was no significant difference between collection times (bleedings) in PRL levels at 14.5, 15.5 or 18.0 hours after estradiol, therefore the results for each time were combined. Prolactin levels in the Control group were significantly greater ($p < 0.01$) than those of the treated populations and a dose-response relationship existed (Table X). Prolactin levels were not correlated with the gonadotropins at any time.

Basal Hormonal Levels in Heifers
8.2 ± 0.5 Months of Age

Within a heifer there was no significant difference in hormone levels in samples taken at an interval of one hour; therefore the values were combined and listed in Table XI. Again, prolactin levels in the untreated group were significantly higher ($p < 0.004$) than those of the treated group. Prolactin levels were markedly reduced in all treatment groups with the 75% group apparently most affected therefore suggesting an age effect on PRL release. PRL levels of the 8-month-old heifer were significantly less ($p < 0.05$) than those of heifers at 3 and 5 months of age (Figure 2).

There was no treatment effect on basal FSH levels (Table XI). Basal FSH levels of the 8-month-old heifer were greater ($p > 0.10$) than those of the 3-month-old heifer but not significantly different ($p > 0.10$) from those heifers of 5 months of age.

The LH levels of the Control group were significantly greater ($p < 0.05$) than the treated group but there was no dose-response relationship

Table X. Effect of Fungal-infested Fescue on PRL Levels Following an $E_2$ Challenge in the Beef Heifer of 7 Months of Age*

| Level of Fungal Infestation | Number of Heifers | Prolactin (ng/ml) |
|---|---|---|
| 0% | 5 | 58.42 ± 12.53[a] |
| 15% | 3 | 34.12 ± 13.49[a,b] |
| 30% | 5 | 28.42 ± 12.07[a,b] |
| 45% | 1 | 7.33[a,b] |
| 60% | 3 | 29.14 ± 12.29[a,b] |
| 75% | 2 | 1.75 ± 0.92[b] |

*Mean ± standard error

[a,b]Means in the same column with different letters are significantly different ($p < 0.0175$)

Table XI. Effect of Fungal-infested Fescue on Basal PRL, FSH & LH Levels in the Beef Heifer of 8 Months of Age*

| Level of Fungal Infestation | Number of Heifers | Basal Level Hormone (ng/ml) | | |
|---|---|---|---|---|
| | | PRL*** | FSH** | LH** |
| 0% | 5 | 23.72 ± 9.69 | 17.33 ± 0.75 | 0.59 ± 0.03 |
| 15% | 3 | 0.10 ± 0.05 | 13.06 ± 1.38 | 0.39 ± 0.02 |
| 30% | 5 | 1.55 ± 1.02 | 18.15 ± 1.88 | 0.55 ± 0.05 |
| 45% | 1 | 0.15 | 17.02 | 0.49 |
| 60% | 3 | 1.27 ± 1.27 | 16.73 ± 0.42 | 0.50 ± 0.05 |
| 75% | 2 | 0.0 ± 0.00 | 15.43 ± 0.78 | 0.44 ± 0.00 |

*Mean ± standard error

**No significant difference among treatments ($p > 0.10$)

***Untreated group significantly greater than treated group ($p < 0.004$)

(Table XI). Luteinizing hormone levels of 8-month heifers were significantly higher ($p < 0.05$) than those of the 5-month-old heifers, however, the difference ($p > 0.10$) between 8 months and 3 months was not significant ($p > 0.10$).

Basal levels of FSH and LH were correlated ($r = .60$; $p < 0.01$) and there was a correlation between PRL and LH ($r = .52$; $p < 0.05$).

Effects of a PMSG Challenge in Heifers 9.4 ± 0.5 Months of Age

Mean PRL levels were significantly higher ($p < 0.004$) in plasma samples collected at 1 hour than at 0 hour as a probable consequence of stress associated with presurgical preparation (Table XII). Five days after PMSG, intertreatment differences in PRL levels were not statistically significant ($p > 0.10$) but PRL levels in the higher infestation groups were lower than those in the lower treatment groups.

FSH and LH levels did not differ ($p > 0.10$) between times of collection or between treatments (Table XIII). LH levels were again higher in the control group than in the treated population as was seen in heifers 3, 5 and 9 months of age, but this difference was not significant ($p > 0.10$). All heifers in the Control group had LH values greater than 1.0 ng/ml. However, as a probable consequence of the estrogen generated in response to PMSG one of the heifers experienced an LH surge of 10.714 ng/ml, thus explaining the high LH values in the Control group.

Estrogen levels did not differ between infestation groups ($p > 0.10$) (Table XIII). As reflected by number of vesicular follicles

Table XII. Effects of Fungal-infested Fescue on PRL Levels After PMSG Stimulation in the Beef Heifer of 9 Months of Age*

| Level of Fungal Infestation | Number of Heifers | Prolactin (ng/ml) 5 Days Post-injection | |
|---|---|---|---|
| | | T = 0** | T = 1 hr |
| 0% | 5 | 13.60 ± 8.76 | 39.46 ± 26.99[b] |
| 15% | 3 | 25.10 ± 16.79 | 14.80 ± 6.53[b] |
| 30% | 5 | 14.18 ± 6.85 | 59.20 ± 35.52[b] |
| 45% | 1 | 10.60 | 190.00[a,b] |
| 60% | 3 | 10.80 ± 1.91 | 157.63 ± 16.97[a] |
| 75% | 2 | 1.70 ± 0.40 | 36.95 ± 30.95[b] |

*Mean ± standard error

[a,b]Means in the same column with different letters are significantly different ($p < 0.05$)

** No significant differences among treatments ($p > 0.10$)

Table XIII. Effects of Fungal-infested Fescue on FSH, LH & Estrogen Response to PMSG Stimulation in the Beef Heifer of 9 Months of Age*

| Level of Fungal Infestation | Number of Heifers | Gonadotrophin and Estrogen Levels 5 Days Post-injection | | |
|---|---|---|---|---|
| | | FSH (ng/ml)** | LH (ng/ml)** | $E_2$ (pg/ml)** |
| 0% | 5 | 20.63 ± 3.02 | 2.39 ± 0.99 | 202.57 ± 77.33 |
| 15% | 3 | 22.84 ± 2.54 | 1.41 ± 0.17 | 16.98 ± 3.49 |
| 30% | 5 | 23.21 ± 1.87 | 1.66 ± 0.07 | 58.95 ± 40.12 |
| 45% | 1 | 20.56 | 1.57 | 24.84 |
| 60% | 3 | 26.95 ± 3.1 | 1.36 ± 0.39 | 26.71 ± 13.50 |
| 75% | 2 | 21.97 ± 4.47 | 1.68 ± 0.10 | 226.39 ± 223.14 |

*Mean ± standard error

**No significant difference among treatments ($p > 0.10$)

(Table XIV), one heifer in the 75% infestation group responded very well to PMSG stimulation and consequently her estrogen concentrations (449.53 pg/ml) were much higher than those of her pasture mate (3.25 pg/ml) which had no large vesicular follicles.

Ovary Morphology in Heifers
9.4 ± 0.5 Months of Age

As evidenced by low plasma estradiol levels and no vesicular follicles greater than 3 mm in diameter, the ovaries of 5 heifers in the treated groups did not respond to PMSG stimulation. All Control heifers responded to PMSG and exhibited large stimulated follicles. Controls had the highest number of primary, secondary, and vesicular follicles (Table XIV). Between individual treatments, there was no significant difference ($p > 0.10$). However, when 0% levels were compared with the whole treated population the difference was significant ($p < 0.02$). There also appeared to be a dose-dependent tendency for number of primary follicles to decrease as infestation levels increased (Table XIV). Number of secondary follicles, atretic and number of small and total vesicular follicles were not affected by treatment ($p > 0.10$). However, the number of follicles in each of these classes was greater in the Control group than the rest of the population. The heifer in the 75% infestation group with the high $E_2$ levels also had a high number of primary follicles (248,000) and total vesicular follicles (69) as compared to her pasture mate (1,440 and 9 respectively).

Correlations between follicle classes and hormone levels are shown in Table XV. Number of primary and secondary follicles were highly

Table XIV. Effect of Fungal-infested Fescue on Follicular Survival & Development in the Beef Heifer*

| Levels of Fungal Infestation | Primary[a] Follicles (x $10^3$) | Secondary[b] Follicles (x $10^3$) | Vesicular Follicles | | | |
|---|---|---|---|---|---|---|
| | | | Normal[c] | Atretic[d] | Small[e] | Total |
| 0% (n = 5) | 326 ± 76.7 | 3.6 ± 0.5 | 43.0 ± 6.3 | 12.4 ± 4.1 | 26.8 ± 9.3 | 82.2 ± 15.1 |
| 15% (n = 3) | 169 ± 53.7 | 2.0 ± 0.6 | 34.0 ± 16.0 | 7.3 ± 3.2 | 20.0 ± 6.8 | 61.3 ± 19.0 |
| 30% (n = 5) | 161 ± 47.4 | 2.3 ± 0.8 | 27.2 ± 6.8 | 6.0 ± 3.3 | 13.0 ± 4.7 | 46.2 ± 11.2 |
| 45% (n = 1) | 105 | 1.7 | 19.0 | 15.0 | 18.0 | 52.0 |
| 60% (n = 3) | 145 ± 54.7 | 3.0 ± 1.1 | 29.0 ± 11.2 | 8.0 ± 6.0 | 22.7 ± 6.9 | 59.6 ± 21.3 |
| 75% (n = 2) | 125 ± 12.3 | 1.4 ± 1.2 | 23.5 ± 21.5 | 1.5 ± 0.5 | 14.0 ± 8.0 | 39.0 ± 30.0 |

*Mean ± standard error

[a]Oocyte with one layer of granulosal cells

[b]Oocyte with two layers of granulosal cells

[c]Follicle with a greater than 2 mm vesicle

[d]Follicle with absent or necratic granulosal cells

[e]Follicle with a less than 2 mm vesicle

Table XV. Correlation Coefficients Among Follicular Classes in the Beef Heifer

| | Primary Follicles | Secondary Follicles | Normal | Atretic | Small | Total |
|---|---|---|---|---|---|---|
| Primary | 1.00 | 0.78 | 0.75 | 0.21 | 0.75 | 0.80 |
| Secondary | 0.78 | 1.00 | 0.70 | 0.11 | 0.53 | 0.66 |
| Normal Vesicular | 0.75 | 0.70 | 1.00 | 0.42 | 0.51 | 0.89 |
| Atretic Vesicular | 0.21 | 0.11 | 0.42 | 1.00 | 0.32 | 0.61 |
| Small Vesicular | 0.75 | 0.53 | 0.51 | 0.32 | 1.00 | 0.80 |
| Estrogen (PMSG Challenge) | 0.69 | 0.34 | 0.51 | 0.08 | 0.53 | 0.54 |

correlated with all other types of follicles except atretic follicles (Table XV). Estrogen was correlated with normal ($r = .51$; $p < 0.05$), small ($r = .53$; $p < 0.05$) and total vesicular follicles ($r = .54$; $p < 0.05$). No correlations of significance were found between gonadotropin levels and number of follicles.

# CHAPTER V

# DISCUSSION

## Effects of Endophyte-Infested Fescue on Cow PRL Levels

Fungal-infested fescue also significantly inhibited ($p < 0.0001$) PRL release in the dams of the heifer calves used in this study. Prolactin levels of controls were 80% greater than the average of the treated group. Differences in PRL levels between infestation levels were, however, not significant ($p > 0.10$) and beyond the 15% level of infestation there was no dose-related trend. Prolactin levels of cows grazing Pasture 5 (75% infestation) were also significantly higher ($p < 0.01$) than the PRL levels of cows in Pasture 1, also a 75% infestation group. These results coincide with the PRL levels of the calves in both Pasture 5 and Pasture 1 respectively. Cow PRL levels and PRL levels of the 3-month-old heifers were correlated ($r = .63$; $p < 0.05$).

## Effects of a GnRH Challenge in Heifers 3.0 ± 0.5 Months of Age

The administration of GnRH resulted in increased LH and FSH release within all treatment levels but there was not affect on PRL release. These results differ from studies conducted on human pituitary cell cultures which indicated that GnRH had the ability to induce PRL release (Blackwell et al., 1986).

The ingestion of fungal-infested fescue reduced plasma PRL levels in all treatment groups (Table I, p. 32). These results coincide with the studies conducted by Hurley et al. (1981) on Holstein calves in

which basal and TRH-stimulated levels of PRL were reduced compared to controls. Bolt et al. (1983) suggested that a possible mechanism by which the toxic factors in infested fescue reduce PRL is through an increase in DA release. The use of DA antagonists such as Domperidone and Spiperone resulted in increased PRL levels but additional mechanisms may be responsible for the symptoms associated with the toxic effects of infested fescue.

Although the differences between the treated groups were not significant, the difference between control and treated was significant ($p < 0.05$) and as infestation levels increased (Table I, p. 32).

Basal and stimulated levels of FSH were not affected by treatment ($p > 0.10$). The literature did not contain any findings relative to the effects of toxic fescue or ergot alkaloids on FSH release. However, there was a modest dose-related response in FSH levels 2 hours after GnRH (Table II, p. 33). A tendency for FSH levels to decrease as infestation levels increase is shown in Figure 1 (p. 35).

Basal LH was also not significantly affected ($p > 0.10$) by treatment (Table III, p. 36). Studies conducted with ergocornine-treated hypothalami indicate however that the ergot alkaloid, ergocornine, may reduce LH levels via a reduction in GnRH (Wuttke et al., 1971). The 1 hour and 2 hour postinjection LH levels of the Control group were higher than most of the treated groups although this difference was not significant ($p > 0.10$). These results suggest, however, that a more extensive study may reveal a gonadotropin-suppressing effect of fungal-infested fescue. Prolactin levels were not correlated with LH and only slightly correlated with FSH ($r = .51$; $p < 0.05$).

Effects of a GnRH Challenge in Heifers 5.3 ± 0.5 Months of Age

Results of this GnRH challenge were similar to those obtained in the younger heifers with the exception of a significantly increased release of FSH ($p < 0.004$) in response to the GnRH challenge.

There did not appear to be any treatment effect ($p > 0.10$) on PRL release at this age although Control levels of PRL were still higher than those of the treated groups (Table IV, p. 38). A possible explanation for this discrepancy is that high ambient temperatures, like those occurring in August, will increase PRL release. This phenomenon has been demonstrated in both the heifer (Wettemann and Tucker, 1974) and the steer (Smith et al., 1977). Hurley et al. (1981) has demonstrated, however, that cows grazing infested-fescue were limited in their ability to secrete more PRL in response to increased temperature. Other factors could, therefore, be involved in the response of the 5 month old heifer.

Due to extremely poor pasture conditions occurring in middle and late summer, the pastures were supplemented with hay of little or no fungal infestation. Ball (1984) reports that any hay/feed supplementation will reduce the detrimental toxic effects associated with tall fescue and these would probably include PRL secretion.

Infested fescue did not affect FSH levels in this experiment (Table V, p. 40) and levels within the 75% infestation group were comparable to those of the Controls. Overall, concentrations of FSH were greater at 5 months than those seen in the younger heifers (Figure 3, p. 41).

There was a significant treatment effect ($p < 0.05$) on basal LH levels (Table VI, p. 42) even though there was not a statistically significant treatment effect on PRL release. LH levels at 1 hour after GnRH in the control group were also higher ($p > 0.10$) than the LH levels in the treated population indicating that the toxic factors in infested fescue may have the ability to affect the LH response to GnRH stimulation. Correlations between PRL and the gonadotropins were not significant, therefore, it appears that the level of PRL is not related to gonadotropin release in the bovine. Ergot alkaloids have been shown to reduce LH levels by inhibiting GnRH release (Wuttke et al., 1971). Another study indicates that the mechanism of action through which the toxic fescue toxins suppress PRL and possibly LH release is through the regulation of Dopamine (DA) (Bolt et al., 1983). Dopamine infusion has been shown to reduce LH levels in humans with no significant changes in TSH, GH or FSH (LeBlanc et al., 1976). This could explain why there has been an apparent negative effect on LH release and not FSH release.

Secretion of 17β-estradiol was apparently not significantly affected ($p > 0.10$) by treatment, although the estrogen levels in the 0%, 15%, and 30% groups were greater than the estrogen concentrations of the higher infestation levels (Table VII, p. 45). The literature does not contain any information regarding the effects of toxic fescue on 17β-estradiol secretion. However, the severely depressed estrogen levels within the 75% infestation group suggest that the toxic factors in infested-fescue may be detrimental to estrogen secretion and hence, follicular development.

Effects of 17β-Estradiol Challenge in Heifers 6.7 ± 0.5 Months of Age

A high level of 17β-estradiol will induce a preovulatory-like surge of LH in the responsive heifer. A heifer was considered responsive if her LH concentration was greater than 5.0 ng/ml. Of the four heifers that responded to estrogen, three were in the 60% infestation group and one was in the Control group. Samples were collected at 14.5, 15.5 and 18.0 hours postinjection and the response was initiated in all four heifers before the 14.5 hour bleeding. Acute release of LH occurs in crossbred beef heifers between 12 and 14 hours following an injection of 500 μg of 17β-estradiol and the LH levels remained high for 6 to 8 hours (Staigmiller et al., 1979). In other breeds the average time of response is 18 hours (Staigmiller et al., 1979). Apparently, only the last part of the response to estradiol was detected in this study since concentrations of LH were below surge level at 15.5 hours.

These results differ from the findings of Staigmiller et al. (1979) who found that the hypothalamic-hypophyseal mechanism responsible for the estrogen-stimulated LH release becomes functional between 3-5 months of age in crossbred beef heifers that do not ovulate until 12 to 16 months of age. Four of the six heifers in their study responded at 5 months of age with LH surges ranging from 39 to 146 ng/ml. The average age of the heifers which responded in this study was 6.7 ± 0.5 months of age with LH responses ranging from 5.8 to 11.9 ng/ml. The difference in the results of these two studies indicates that the maturation of the mechanism responsible for estrogen-stimulated LH release was delayed in our heifers.

Undernutrition can delay puberty in sheep by impairing the mechanism which controls GnRH secretion (Foster and Olster, 1985). The ingestion of toxic fescue has been shown to reduce animal performance and decrease average daily gain (Ball, 1984). Since a low nutritional level induced by infested-fescue may delay puberty, it is possible that the maturation process of the hypothalamic-hypophyseal mechanism was also delayed. However, all of the heifers in the 60% group responded to the estrogen challenge; therefore, factors other than infested fescue were involved.

Breed of calf and breed of sire is also known to influence the age at which heifers reach puberty (Dow et al., 1982; Grass et al., 1982). Straightbred Hereford calves reached puberty at a later age than Holstein calves (Grass et al., 1982) or Angus x Hereford crossbreds (Dow et al., 1982). Calves sired by Simmental bulls also reached puberty later than crossbred calves sired by Angus or Hereford bulls (Grass et al., 1982). The heifers used in this study are known to reach puberty at a later age possibly due to their breed combination of (Angus x Hereford) x Simmental.

In this study, FSH and LH levels were highly correlated ($r > .70$; $p < 0.05$) with each other at all times. FSH levels also increased in response to estrogen stimulation in those heifers which produced an LH surge (Table IX, p. 47). Karsch (1984) indicates that FSH release is influenced by the positive feedback mechanisms of estrogen. Rzepkowski et al. (1982) demonstrated that peak FSH levels coincide with the preovulatory LH surge in the heifer. Toxic fescue did not affect FSH release in this experiment ($p > 0.10$).

The ingestion of infested fescue significantly reduced PRL levels ($p < 0.02$) and there was a slight dose-dependent response (Table X, p. 49). Apparently 17β-estradiol does not affect the release of PRL ($p > 0.10$) as indicated by Pabmanabhan and Convey (1979) in bovine pituitary cultures. However, the results of this experiment agree with the findings of Baxter and Gorski (1981) who demonstrated that in vivo administration of estrogen does not affect PRL synthesis in the bovine. It is possible that any response of PRL to estrogen stimulation may have occurred at a time prior to the collection of the samples.

Basal Hormonal Levels in Heifers
8.2 ± 0.5 Months of Age

Differences in PRL levels between treatment groups were not significant ($p > 0.10$) but Control levels of PRL were significantly greater than the treated population ($p < 0.004$). There was no dose-dependent relationship between infestation levels as all treatment groups had drastically reduced PRL levels (Table XI, p. 50). PRL levels in heifers 8 months of age were lower ($p > 0.10$) than those of heifers of either 3 or 5 months of age (Figure 2, p. 39). Pituitary PRL concentrations increased 333% between birth and 3 months of age in the dairy heifer and reached maximum levels at 9 months (Sinha and Tucker, 1968). The results of this study correspond in part with the findings of Sinha and Tucker (1968) in that PRL levels were high in heifers of 3 months of age in both studies. Cause of the interstudy difference in prolactin levels in heifers of more advanced age is, however, unknown.

Once again, FSH levels were not affected by treatment (Table XI, p. 50). FSH levels in heifers of 8 months of age were lower ($p > 0.10$) than those of 5-month-old heifers but higher ($p > 0.10$) than those of 3-month-old heifers (Figure 3, p. 41). These results differ from the findings of Gonzalez-Padilla and associates (1975) which indicate that FSH levels during the prepuberal period remain constant except for decreased levels of FSH occurring between 20 and 38 days prior to puberty. Results of another study showed that FSH levels were high between birth and 10 weeks after birth, low for the succeeding 15 weeks and high during the remainder of the prepubertal period (Dodson et al., 1985). The interstudy difference in the pattern of FSH secretion may be due, among other factors, to the differences in breeds of beef heifers and level of nutrition.

Groups did not differ significantly in basal LH levels although levels of LH in the controls were higher than that in the treated population. The difference, however, was not significant ($p > 0.10$). As proposed earlier there was a trend for treated heifers to have decreased LH levels as compared to controls. Basal LH levels of 8-month-old heifers were significantly higher ($p < 0.05$) than those of 5-month-old heifers but not significantly different ($p > 0.10$) from the LH levels of heifers of 3 months of age (Figure 4, p. 43). Although the drastic drop in LH levels in heifers of 5 months of age corresponds to a time when pastures were in extremely poor condition, pastures were supplemented with hay and FSH and PRL levels were apparently not affected; therefore other factor(s) must have affected LH release at 5 months.

Effects of a PMSG Challenge in Heifers 9.4 ± 0.5 Months of Age

Mean PRL levels were significantly higher ($p < 0.004$) at the 1 hour collection than they were at the 0 hour collection (Table XII, p. 52) possibly due to the stress associated with surgical preparation. Although the heifers experienced some degree of stress in each of the experiments conducted, the stressful stimulus was of short duration compared to the presurgery period. Stress-induced PRL release has also been demonstrated in other species. Male patients with hypertension exhibit hyperprolactinemia and stressful stimuli applied to the rat will result in a rapid rise in PRL levels (Zamir et al., 1983).

There were no significant differences ($p > 0.10$) in PRL levels between infestation levels at the 1 hour collection, however, PRL levels were lower in the 75% group as compared to the Control group at the first collection time. In terms of a relative response to stress, heifers in the Control and lower infestation groups did not respond to the same extent as heifers grazing the higher infested-fescue (Table XII, p. 52).

Gonadotropin levels did not vary significantly ($p > 0.10$) between collection times or between treatment levels, although LH concentrations were higher in the Control group. All heifers in the Control group responded to the PMSG challenge with LH levels greater than 1.0 ng/ml however, one heifer experienced an LH surge of 10.714 ng/ml probably as a consequence of high estrogen concentrations generated by the PMSG. Therefore, the high levels of LH in the Control group may be the result

of one heifer's response to PMSG stimulation. It is also possible that the lower LH levels in the treatment groups were the result of the ingestion of infested-fescue as LH levels in the Control groups have been consistently higher than LH levels in the treatment groups throughout the entire study.

There was no significant differences between estrogen levels of the Control and treated population ($p > 0.10$) although, with the exception of one of the two heifers in the 75% infestation group, 17β-estradiol levels were higher in the Control and low-dose groups.

Ovary Morphology in Heifers 9.4 ± 0.5 Months of Age

Toxic fescue adversely affected germ cell survival and follicular development in the beef heifer. Although the differences between treatment levels were not significant ($p > 0.10$) (Table XIV, p. 55), primary follicle number was significantly greater ($p < 0.02$) in the Control group than in the treated population with a suggestion of a dose-dependent relationship. The loss of primary follicles in the treated groups may result in reduced reproductive performance as the heifers reach maturity. Since the population of primary oocytes is established before birth (Hunter, 1980), the loss of primary follicles may have occurred during prenatal development. The low number of primary follicles may be the result of oogonia or oocyte death possibly due to the presence of too few granulosal cells required to sustain the oocyte. The mechanism by which this loss of primary follicle population may occur is unknown. However, it is not known whether the low levels of

ergot alkaloids produced by the toxic fescue would have cytotoxic effects.

Loss of primary follicle populations may have occurred postnatally, however, in our assessment of primary follicle number, atresia of the primary follicle was not considered.

Total vesicular follicle number was not statistically greater ($p > 0.10$) in the Control group than in the treated population and five heifers within the treated population did not respond to the PMSG.

In the rat, receptors for PRL have been found on the granulosa cells of large follicles (Wang et al., 1979) and on the luteal cells of the corpus luteum (Richards and Williams, 1976) thus indicating a specific role for PRL in follicle development. PRL has also been implicated in determining the number of LH receptors in the rat thereby enhancing ovarian response to gonadotropin stimulation (Advis et al., 1981). These findings suggest that a reduction in PRL concentrations, like that which occurs following the ingestion of infested-fescue, could be detrimental to follicular development. However, it is not known whether prolactin is important in follicular development in the bovine, but it is known that it is not the luteotropic hormone in this species (Hoffman et al., 1974).

Throughout the study, there have been indications that toxic fescue also reduces LH levels although not significantly. Increased DA concentrations associated with toxic fescue (Henson et al., 1987) have been shown to inhibit LH release in the human (LeBlanc et al., 1976). Ergot alkaloids associated with toxic fescue have also been shown to decrease

LH levels in the rat (Shaban and Terranova, 1986). Since LH is required for normal follicular development, reduced LH levels may adversely affect the ability of vesicular follicles to respond to PMSG stimulation.

In conclusion, PRL levels were significantly reduced within all treatments as compared to Controls except when ambient temperatures were high. FSH levels were not affected by treatment but LH concentrations were reduced in treated animals although not significantly. 17β-estradiol secretion was ($p > 0.10$) inhibited probably due to reduced follicular development in the treatment groups. Germ cell survival was adversely affected ($p < 0.02$) by the ingestion of toxic fescue with an apparent dose-dependent relationship. Vesicular follicle response to PMSG was also adversely affected in all infestation levels as compared to the Controls with no increase in follicle atresia. The results indicate that either reduced levels of PRL or LH in heifers grazing fungal-infested fescue may be responsible for decreased germ-cell survival and impaired follicular development and these factors would probably cause a reduction in reproductive performance.

# BIBLIOGRAPHY

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Advis, J. P., Richards, J. S. and S. R. Ojeda. 1981. Hyperprolactinemia-induced precocious puberty: Studies on the mechanism(s) by which prolactin enhances ovarian progesterone responsiveness to gonadotropins in prepubertal rats. Endocrinology 108:1333.

Advis, J. P., Smith White, S. and S. R. Ojeda. 1981. Delayed puberty induced by chronic suppression of prolactin release in the female rat. Endocrinology 109:1321.

Anderson, L. L., Hard, D. L., Carpenter, L. S., Awotwi, E. K. and M. A. Diekman. 1981. Neuroendocrine regulation of luteinizing hormone secretion in beef calves. Biol. of Reprod. 24:795.

Arita, J. and F. Kimura. 1986. Characterization of in vitro dopamine synthesis in the median eminence of rats with Haloperidol-induced hyperprolactinemia and Bromocriptine-induced hypoprolactinemia. Endocrinology 119:1666.

Bacon, C. W., Lyons, P. C., Porter, J. K. and J. D. Robbins. 1986. Ergot toxicity from endophyte-infected grasses: A review. Agron. J. 78:106.

Baird, D. T. 1972. The ovary. In: C. R. Austin and R. V. Short (Eds.) Reproduction in Mammals. Book 3, 2nd ed., pp. 91-114. Cambridge University Press, New York.

Ball, D. M. 1984. An overview of fescue toxicity research. Agri-Practice 5:31.

Baxter, L. A. and J. Gorski. 1981. Prolactin synthesis in bovine pituitary: No evidence for regulation by estrogen in vivo. Endocrinology 109:576.

Beck, T. W., Smith, V. G., Seguin, B. E. and E. M. Convey. 1976. Bovine serum LH, GH and prolactin following chronic implantation of ovarian steroids and subsequent ovariectomy. J. Anim. Sci. 42:461.

Blackwell, R. E., Rogers-Neame, N. T., Bradley Jr., E. L. and R. H. Asch. 1986. Regulation of human prolactin secretion by gonadotropin-releasing hormone in vitro. Fertil. Steril. 46:26.

Bolt, D. J., Bond, J. and T. Elsasser. 1983. Changes in plasma prolactin and grazing behavior in heifers treated with dopamine antagonist while grazing tall fescue and orchardgrass pasture. J. Anim. Sci. 56:48 (Suppl. 1).

Bolt, D. J., Bond, J., Lynch, G. P. and T. Elsasser. 1982. Concentrations of PRL, LH, FSH, GH and TSH in plasma and pituitary ewes grazing tall fescue and orchardgrass pastures. J. Anim. Sci. 55:4 (Suppl. 1).

Bolt, D. J. and R. Rollins. 1983. Development and application of a radioimmunoassay for bovine follicle-stimulating hormone. J. Anim. Sci. 56:146.

Bond, J., Hawk, H. W., Lynch, G. P. and C. Jackson Jr. 1982. Lower fertility in ewes grazing tall fescue pasture. J. Anim. Sci. 55:46 (Suppl. 1).

Brown, N. S., Abbott, S. R. and J. E. T. Corrie. 1983. Some observations on the preparation, purification and storage of radioiodinated protein hormones. In: W. M. Hunter and J. E. T. Corrie (Eds.) Immunoassays for Clinical Chemistry. 2nd ed., pp. 167-276. Churchill Livingston, New York.

Buhr, M. M., Carlson, J. C. and J. E. Thompson. 1983. A new perspective on the mechanism of corpus luteum regression. Endocrinology 105:1330.

Camous, S., Prunier, A. and J. Pelletier. 1985. Plasma prolactin, LH, FSH and estrogen excretion patterns in gilts during sexual development. J. Anim. Sci. 60:5.

Caron, M. G., Beaulieu, M., Raymond, V., Gagne, B., Drouin, J., Lefkowitz, R. J. and F. Labrie. 1978. Dopaminergic receptors in the anterior pituitary gland. J. Biol. Chem. 253:2244.

Costlow, M. and W. L. McGuire. 1977. Automradiographic localization of the binding of (125)I-labelled prolactin to rat tissues in vitro. J. Endocr. 75:221.

Crosignani, P. G., Lombroso, G. C., Mattei, A., Caccamo, A. and L. Trojsi. 1979. Effect of three seratonin antagonists on plasma prolactin release to suckling in puerperal women. J. Clin. Endocrinol. Metab. 48:335.

Dalip, J. S. S. and A. R. Audsley. 1985. Endogenous opiod peptides participate in the modulation of prolactin release in response to cervicovaginal stimulation in the female rat. Endocrinology 117:549.

Daniels, L. B., Nelson, T. S., Piper, E. L., Beasley, J. N., Stidham, D. and C. J. Brown. 1983. Physiological and reproductive performances of cattle and horses grazing fescue. Proc. Tall Fescue Toxicosis Workshop.

del Pozo, E. 1972. The inhibition of prolactin secretion in man by CB-154 (2-Br-α-ergocryptine). J. Clin. Endocrinol. Metab. 35:768.

Desjardins, C. and H. D. Hafs. 1968. Levels of pituitary follicle stimulating hormone and luteinizing hormone in heifers from birth through puberty. J. Anim. Sci. 27:472.

Diefenbach, W. P., Carmel, P. W., Frantz, A. G. and M. Ferin. 1976. Suppression of prolactin secretion by L-Dopa in the stalk-sectioned Rhesus monkey. J. Clin. Endocrinol. Metab. 43:638.

Dorrington, J. H. and R. E. Gore-Langton. 1982. Antigonadal action of prolactin: Further studies on the mechanism of inhibition of follicle-stimulating hormone-induced aromatase activity in rat granulosa cell cultures. Endocrinology 110:1701.

Dow Jr., J. S., Moore, J. D., Bailey, C. M. and W. D. Foote. 1982. Onset of puberty in heifers of diverse beef breeds and crosses. J. Anim. Sci. 55:1041.

Dusza, L., Tilton, J. E., and R. M. Weigl. 1986. Prolactin administration during the luteal and follicular phase of the oestous cycle of gilts. J. Reprod. Fert. 76:519.

Erickson, B. H. 1966. Development and senescence of the postnatal bovine ovary. Agricultural Research Laboratory.

Erickson, B. H. 1985. Effects of ionizing radiation and chemicals on mammalian reproduction. Vet. Hum. Toxicol. 27:409.

Fink, G., Horn, A. M. and I. A. C. F. Robinson. 1984. Vasopressin and oxytocin in hypophysial portal blood. J. Physiol. 346:124.

Floss, H. G., Cassady, J. M. and J. E. Robbers. 1973. Influence of ergot alkaloids on pituitary prolactin and prolactin-dependent processes. J. Phar. Sci. 62:699.

Foord, S. M., Peters, J. R., Dieguez, C., Scanlon, M. F. and R. Hall. 1983. Dopamine receptors on intact anterior pituitary cells in culture: Functional association with the inhibition of prolactin and thyrotropin. Endocrinology 112:1567.

Fortune, J. E. and S. E. Vincent. 1986. Prolactin modulates steroidogenesis by rat granulosa cells: I. Effects on progesterone. Biol. Reprod. 35:84.

Fortune, J. E., Wissler, R. N. and S. E. Vincent. 1986. Prolactin modulates steroidogenesis by rat granulosa cells: II. Effects on estradiol. Biol. Reprod. 35:92.

Foster, D. L. 1984. Prevulatory gonadotropin surge system of prepubertal female sheep is exquisitely sensitive to the stimulatory feedback action of estradiol. Endocrinology 115:1186.

Foster, D. L. and D. H. Olster. 1985. Effect of restricted nutrition on puberty in the lamb: Patterns of tonic luteinizing hormone (LH) secretion and competency of the LH surge system. Endocrinology 116:375.

Foster, D. L., Yellon, S. M. and D. H. Olster. 1985. Internal and external determinants of the timing of puberty in the female. J. Reprod. Fert. 75:327.

Garrett, L. W., Heimann, E. D., Pfander, W. H. and L. L. Wilson. 1980. Reproductive problems of pregnant mares grazing fescue pastures. J. Anim. Sci. 51:237 (Suppl. 1).

Gibbs, M. and J. D. Neill. 1978. Dopamine levels in hypophysial stalk blood in the rat are sufficient to inhibit prolactin secretion in vivo. Endocrinology 102:1895.

Gold, M. S., Redmond Jr., D. E. and R. K. Donabedian. 1979. The effects opiate agonist and antagonist on serum PRL in primates: Possible role for endorphins in PRL regulation. Endocrinology 105:284.

Gonzalez-Padilla, E., Wiltbank, J. N. and G. D. Niswender. 1975. Puberty in heifers. 1. The interrelationship between pituitary, hypothalamic and ovarian hormones. J. Anim. Sci. 40:1091.

Gosselin, R. E., Blankstien, J., Dent, D. W., Hobson, W. C., Fuller, G. B., Reyes, F. I., Winter, J. S. D. and D. Faiman. 1983. Effects of nalaxone and an enkephalin analog on serum prolactin, cortisol, and gonadotropins in the chimpanzee. Endocrinology 112:2168.

Grass, J. A., Hansen, P. J., Rutledge, J. J. and E. R. Hauser. 1982. Genotype X envirionmental interactions on reproductive traits of bovine females. I. Age at puberty as influenced by breed, breed of sire, dietary regimen and season. J. Anim. Sci. 55:1441.

Grinwich, D. L., Hichens, M. and B. D. Murphy. 1976. Control of the LH receptor by prolactin and prostaglandin F2$\alpha$ in rat corpora lutea. Biol. Reprod. 14:212.

Gudelsky, G. A., Nansel, D. D. and J. C. Porter. 1981. Role of estrogen in the dopaminergic control of prolactin secretion. Endocrinology 108:440.

Gudelsky, G. A., Simpkins, J. Mueller, G. P., Meites, J. and K. E. Moore. 1976. Selective actions of prolactin on catecholamine turnover in the hypothalamus and on serum LH and FSH. Neuroendocrinology 22:206.

Hadley, M. E. 1984. The Endocrine Hypothalamus. In: Endocrinology. 1st ed., pp. 112-139. Prentice-Hall, Inc., Englewood Cliffs, New Jersey.

Haug, E. and K. M. Gautvik. 1976. Effects of sex steroids on prolactin secreting rat pituitary cells in culture. Endocrinology 99:1482.

Hemken, R. W., Bull, L. S., Boling, J. A., Kane, E., Bush, L. P. and R. C. Buckner. 1979. Summer fescue toxicosis in lactating dairy cows and sheep fed experimental strains of ryegrass-tall fescue hybrids. J. Anim. Sci. 49:641.

Hemken, R. W., Jackson Jr., J. A. and Boling. 1984. Toxic factors in tall fescue. J. Anim. Sci. 58:1011.

Henson, M. C., Piper, E. L., Daniels, L. B. and M. K. Barnett. 1984. Effects of lactating dairy cows fed high alkaloid fescue vs. Bermuda grass. Arkansas Farm Research 33:9.

Henson, M. C., Piper, E. L. and L. B. Daniels. 1987. Effects of induced fescue toxicosis on plasma and tissue catecholamine concentrations in sheep. Domestic Animal Endocrinology 4:7.

Hoffmann, B., Schams, D., Bopp, R., Ender, M. L., Gimenez, T. and H. Karg. 1974. Leuteotrophic factors in the cow: Evidence for LH rather than prolactin. J. Reprod. Fert. 40:77.

Howland, B. E., Sanford, L. M. and W. M. Palmer (Eds.). 1984. Serum concentrations of LH, FSH and prolactin in ovariectomized ewes following treatment with androgens or estrogens. Can. J. Anim. Sci. 64:171.

Hunter, R. H. F. 1980. In: Physiology and Technology of Reproduction in Female Domestic Animals, pp. 17-30. Academic Press Inc. (London) Ltd.

Hurley, W. L., Convey, E. M., Leung, K., Edgerton, L. A. and R. W. Hemken. 1981. Bovine prolactin, TSH, $T_4$ and $T_3$ concentrations as affected by tall fescue summer toxicosis and temperature. J. Anim. Sci. 51:374.

Jackson, J. A., Hemken, R. W., Boling, J. A., Harmon, R. J., Buckner, R. C. and L. P. Bush. 1982. Effect of toxic fescue on prolactin levels in dairy cattle. J. Dairy Sci. 65:172 (Suppl. 1).

Koprowski, J. A. and H. A. Tucker. 1973. Serum prolactin during various physiological states and its relationship to milk production in the bovine. Endocrinology 92:1480.

LeBlanc, H., Lachelin, G. C. L., Abu-Fadil, S. and S. S. C. Yen. 1976. Effects of dopamine infusion on pituitary hormone secretions in humans. J. Clin. Endocrinol. Metab. 43:668.

Lumpkin, M. D., Samson, W. K. and S. M. McCann. 1983. Hypothalamic and pituitary sites of action of oxytocin to alter prolactin in secretion in the rat. Endocrinology 112:1711.

Lyons, P. C., Plattner, R. D. and C. W. Bacon. 1986. Occurrence of peptide and clavine ergot alkaloids in tall fescue grass. Science 232:487.

McLeod, B. J., Haresign, W., Peters, A. R. and G. E. Lamming. 1984. Plasma LH and FSH concentrations in prepubertal beef heifers before and in low response to repeated injections of low doses of GnRH. J. Reprod. Fert. 70:137.

McNeilly, A. S. 1980. Prolactin and the control of gonadotrophin secretion in the female. J. Reprod. Fert. 58:537.

Niswender, G. D., Richert Jr., L. E., Midgley Jr., A. R. and A. V. Nalbandov. 1969. Radioimmunoassay for bovine and ovine luteinizing hormone. Endocrinology 84:1166.

Murphy, B. D. and K. Rajkumar. 1985. Prolactin as a luteotrophin. Can. J. Physiol. Pharmacol. 63:257.

Neill, J. D., Freeman, M. E. and S. A. Tillson. 1971. Control of the proestrus surge of prolactin and luteinizing hormone secretion by estrogens in the rat. Endocrinology 89:1448.

Ojeda, S. R., Advis, J. P. and W. W. Andrews. 1980. Neuroendocrine control of the onset of puberty in the rat. Federation Proc. 39:2365.

Padmanabhan, V. and E. M. Convey. 1979. Estradiol-17β stimulates basal and thyrotropin releasing hormone induced prolactin secretion by the bovine pituitary cells in primary culture. Molecular and Cellular Endocrinology 14:103.

Piper, E. L. and M. C. Henson. Effects of feeding fescue or Bermuda grass hay on lamb weights and peripheral prolactin levels in crossbred ewes. J. Anim. Sci. 59 (Suppl. 1):292.

Ragavan, V. V. and A. G. Frantz. 1981. Opioid regulation of prolactin secretion: Evidence for a specific role of β-endorphin. Endocrinology 109:1769.

Padmanabhan, V. and E. M. Convey. 1979. Estradiol-17β stimulates basal and thyrotropin releasing hormone induced prolactin secretion by the bovine pituitary cells in primary culture. Molecular and Cellular Endocrinology 14:103.

Piper, E. L. and M. C. Henson. 1983. Effects of feeding fescue or Bermuda grass hay on lamb weights and peripheral prolactin levels in crossbred ewes. J. Anim. Sci. 59:292 (Suppl. 1).

Ragavan, V. V. and A. G. Frantz. 1981. Opioid regulation of prolactin secretion: Evidence for a specific role of β-endorphin. Endocrinology 109:1769.

Richards, J. S. and J. J. Williams. 1976. Luteal cell receptor content for prolactin (PRL) and luteinizing hormone (LH): Regulation by LH and PRL. Endocrinology 99:1571.

Robyn, C., Delvoye, P., Nokin, J., Vekemans, M., Badawi, M., Perez-Lopez, F. R. and M. L'Hermite. 1973. Prolactin and human reproduction. In: J. L. Pasteels and C. Robyn (Eds.) Human Prolactin, pp. 167-188.

Rosenzweig, L. J. and Y. S. Kanwar. 1982. Dopamine internalization by and intracellular distribution within prolactin cells and somatotrophs of the rat anterior pituitary as determined by quantitative electron microscopic autoradiography. Endocrinology 111:1817.

Rzepkowski, R. A., Ireland, J. J., Fogwell, R. L., Chapin, L. T. and H. A. Tucker. 1982. Serum luteinizing hormone, follicle stimulating hormone and prolactin response to photoperiod during the estrous cycle of Holstein heifers. J. Anim. Sci. 55:1125.

Samson, W. K., Lumpkin, M. D. and S. M. McCann. 1986. Evidence for a physiological role for oxytocin in the control of prolactin secretion. Endocrinology 119:554.

SAS. 1982a. SAS User's Guide: Basics. Statistical Analysis System Institute, Inc., Raleigh, N.C.

SAS. 1982b. SAS User's Guide: Statistics. Statistical Analysis System Institute, Inc., Raleigh, N.C.

Schams, D. and H. Karg. 1972. The immediated response of the plasma prolactin level to oestrogen infusions in dairy cows. Acta endocr. 69:47.

Schillo, K. K., Dierschke, D. J. and E. R. Hauser. 1983. Estrogen-induced release of luteinizing hormone in prepubertal and postpubertal heifers. Theriogenology 19:7272.

Shaban, M. S. and P. F. Terranova. 1985. Delayed puberty in hyperprolactinemic rats is related to suppressed ovarian and serum progesterone. Proc. Soc. Sci. Res., p. 110.

Shaban, M. S. and P. F. Terranova. 1986. 2-Bromo-α-Ergocryptine Mesylate (CB-154) inhibits prolactin and luteinizing hormone secretion in the prepubertal female rat. Biol. Reprod. 34:788.

Sinha, Y. N. and H. A. Tucker. 1968. Mammary development and pituitary prolactin level of heifers from birth through puberty and during the estrous cycle. J. Dairy Sci. 52:507.

Smith, V. G., Beck, T. W., Convey, E. M. and H. A. Tucker. 1974. Bovine serum prolactin, growth hormone, cortisol and milk yield after ergocryptine. Neuroendocrinology 15:172.

Smith, V. G., Hacker, R. R. and R. G. Brown. 1977. Effect of alterations in ambient temperature on serum prolactin alterations in ambient temperature on serum prolactin concentrations in steers. J. Anim. Sci. 44:645.

Staigmiller, R. B., Short, R. E. and R. A. Bellows. 1979. Induction of LH surges with 17β Estradiol in prepuberal beef heifers: An age dependent response. Theriogenology 11:453.

Swanson, L. V. and H. D. Hafs. 1971. LH and prolactin in blood serum from estrus to ovulation in Holstein heifers. J. Anim. Sci. 33:1038.

Thompson Jr., D. L., Pickett, B. W. and T. M. Nett. 1978. Effect of season and artificial photoperiod on levels of estradiol-17 and estrone in blood serum of stallions. J. Anim. Sci. 47:184.

Varney, D. R., Ndefru, M., Jones, S. L., Newsome, R., Siegel, M. R. and P. M. Zavos. 1987. The effect of feeding endophyte infected tall fescue seed on reproductive performance in female rats. Comp. Biochem. Physiol. 87C:171.

Veldhuis, J. D., Klase, P. A., and J. F. Strauss III. 1980. Divergent effects of prolactin upon steroidogenesis by porcine granulosa cells in vitro: Influence of cytodifferentiation . Endocrinology 107:42.

Vescovi, P. P., Pezzarossa, A., Ceresini, G., Rastelli, G., Valenti, G. and G. Gerra. 1985. Effects of dopamine receptor stimulation on opiate-induced modifications of pituitary-gonadal function. Hormone Res. 21:155.

Wang, C., Hsueh, A. J. W. and G. F. Erickson. 1979. Induction of functional prolactin receptors by follicle-stimulating hormone in rat granulosa cells in vivo and in vitro. J. Biol. Chem. 254:11330.

Wehrenberg, W. B., McNicol, D., Wardlaw, S. L., Frantz, A. G. and M. Ferin. 1981. Dopaminergic and serotonergic involvement on opiate-induced prolactin release in monkeys. Endocrinology 109:544.

Wettemann, R. P. and H. A. Tucker. 1974. Relationship of ambient temperature to serum prolactin in heifers. Proc. Soc. Exper. Biol. Med. 146:908.

Wuttke, W., Cassell, E. and J. Meites. 1971. Effects of ergocornine on serum prolactin and LH, on hypothalamic content of PIF and LRF. Endocrinology 88:737.

Zamir, N., Ben-Ishay, D., Wiedenfeld, J. and R. A. Siegel. 1983. Stress-induced secretion of adrenocorticotropin, corticosterone, and prolactin in experimentally and genetically hypertensive rats. Endocrinology 112:580.

Zavos, P. M., Varney, D. R., Siegel, M. R., Hemkin, J. A., Jackson, J. A. and L. P. Bush. 1987. Effects of feeding endophyte-infected tall fescue seed on the reproductive performance in male and female CD-1 mice by combination crosses. Theriogenology 27:541.

Zeilmaker, G. H. and R. A. Carlsen. 1962. Experimental studies on the effect of ergocornine methanesulphonate on the luteotrophic function of the rat pituitary gland. Acta Endocr. 41:321.

# APPENDICES

BOSTON MASS

# APPENDIX A

## RADIOIODINATION PROCEDURES

LH: Chloramine-T Technique (Brown et al., 1983)

Protein: LER 1072-2

Materials needed:

Plastic 1.5 ml Microcentrifuge vial

0.5 M Phosphate buffer (pH 7.5) (PB)

0.05 M Phosphate buffer (pH 7.5) (PB)

Chloramine-T (1 µg/µl in 0.05 M PB)

Sodium Metabisulfite (2 µg/µl in 0.05 M PB)

Bovine Serum Albumin (BSA)

G 25-M Sephadex column

Proceed in a step-wise addition:

1. 2.5 µg LER 1072-2 in 25 µl of 0.5 M PB (pH 7.5)
2. Add 500 µCi 125-I (Amersham Co.)
3. Add 15 µg of Chloramine-T
4. Mix gently, for 1 minute
5. Add 60 µl of Sodium Metabisulfite
6. Add 100 µl of eluent buffer (0.05 M PB + 0.1% BSA)
7. Load onto a G 25-M Sephadex column for separation
8. Elute with 0.05 M PB + 0.1% BSA

FSH: Iodogen Technique (Bolt and Rollins, 1983)

Protein: USDA bFSH-BP3

Materials needed:

Wheaton 1.5 ml flat bottom serum vial, acid washed*

0.5 M PB (pH 7.4)

0.25 M PB (pH 7.4)

0.50 M PB (pH 7.4)

0.5 M Sodium Diphosphate solution

Sodium Metabisulfite (25 µg/ml in 0.25 M PB)

Potassium Iodide (1 mg/ml in 0.5 M Sodium Diphosphate)

BSA

G 25-M Sephadex column

*Plate the reaction vessel with a solution of iodogen in chloroform at a concentration of 2 µg/50 µl.

1. 5 mg in 5 ml; iodogen/chloroform
2. 40 µl of #1 on 940 µl of chloroform

Proceed in a step-wise addition:

1. 2.5 µ bFSH-BP3 in 35 µl of 0.5 M PB
2. Add 400 µCi 125-I (Amersham Co.)
3. Vortex for 10 minutes (thorough, but gentle)
4. Add 200 µl Sodium Metabisulfite
5. Add 200 µl Potassium Iodide
6. Load on G 25-M Sephadex column for separation
7. Elute with 0.05 M PB + 0.1% BSA

Prolactin: Iodogen Techique (Bolt and Rollins, 1983, modified)

Protein: USDA-bPRL-I-1

Materials needed:

Wheaton 1.5 ml flat bottom serum vial, acid washed*

0.5 M PB (pH 7.4)

0.25 M PB (pH 7.4)

0.05 M PB (pH 7.4)

0.5 M Sodium Diphosphate solution

Sodium Metabisulfite (25 µg/ml in 0.25 M PB)

Potassium Iodide (1 mg/ml in 0.5 M Sodium Diphosphate)

BSA

G 25-M Sephadex column

*Plate the reaction vessel with a solution of iodogen in chloroform at a concentration of 4 µg/50 µl.

1. 50 mg in 5 ml; iodogen/chloroform
2. 80 µl of #1 on 900 µl of chloroform

Proceed in a step-wise addition:

1. 5.0 µg bPRL-I-1 in 35 µl of 0.5 M PB (pH 7.4)
2. Add 400 µCi 125-I (Amersham Co.)
3. Vortex 5 minutes (thorough, but gentle)
4. Add 200 µl Sodium Metabisulfite
5. Add 200 µl Potassium Iodide
6. Load on G 25-M Sephadex column for separation
7. Elute with 0.05 M PB + 0.1% BSA

Column Preparation:

Gel filtration was used to separate LH, FSH and PRL radiolabelled compounds. Sephadex G 25-M gel (Pharmacia Co.) suspended in 0.05 M

sodium phosphate buffer was placed into a 1 x 20 cm glass column. Approximately 10 ml of the elution buffer (0.05 M sodium phosphate buffer + 0.1% BSA) was used to rinse the column just prior to use. Buffer pH for LH was 7.5 and for FSH and PRL was 7.4.

Isolation Technique:

Upon loading iodinated material into the column, isolation of the labelled protein is accomplished through fractionatig the eluted material in 1 ml fractions. Radioactivity is monitored using a portable Geiger counter. Radioactivity is eluted from the column in two peaks; the first peak is the protein fraction and the second peak is the free iodide. The labelled protein is eluted after approximately 11-13 fractions with the free iodide coming off the column at approximately 20-25 fractions.

## TRACER VERIFICATION

Trichloroacetic Acid Test: (TCA precipitates out the protein therefore, the test verifies that what is labelled is the protein hormone).

Materials needed:

Diluted radiolabelled protein (≅ 32,000 cpm)

Four 1.5 ml plastic microcentrifuge tubes

18% Trichloroacetic acid in 0.09 M PB

1% BSA in 0.5 M PB (pH 7.5)

Procedure:

1. Add 25 µl of the diluted tracer to the microcentrifuge tubes

2. Set aside two tubes as Controls
3. To the remaining tubes, add 100 µl of cold (4°C) 1% BSA, then add 200 µl TCA
4. Vortex rapidly for 10 seconds
5. Centrifuge treated tubes at 3,000 rpm for 5 minutes
6. Aspirate supernatant from the treated tubes and dispose of properly
7. Count the Control and Treated tubes on a gamma counter

Calculation of Labelled Hormone:

To calculate % labelled hormone:

avg cpm of TREATED TUBES ÷ avg cpm of CONTROL TUBES

# APPENDIX B

## RADIOIMMUNOASSAY TECHNIQUES

Table B-I. Protocol for LH: Niswender as Modified by D. J. Bolt (1981)

| | Sequence | | | | |
|---|---|---|---|---|---|
| TUBES | 1<br>RIA BUFFER<br>(µl) | 2<br>SAMPLE<br>(µl) | 3<br>STOCK Ab<br>(µl) | 4<br>1st Ab<br>(µl) | 5<br>TRACER<br>(µl) |
| TC | --- | --- | --- | --- | 100 |
| NSB | 700 | --- | --- | --- | 100 |
| Bo | 500 | --- | --- | 200 | 100 |
| Standards | 300 | 200 | --- | 200 | 100 |
| QC | 100 | 400 | --- | 200 | 100 |
| Max Bo | 500 | --- | 200 | --- | 100 |
| BLANK | 500 | --- | --- | 200 | 100 |
| SAMPLE | 100* | 400 | --- | 200 | 100 |

*Sample volume (sample + buffer) must equal 500 µl, therefore, if 400 µl of sample is used then add 100 µl of buffer or if 300 µl of sample is used add 200 µl of buffer.

TC = Total count or the total amount (counts per minute) of tracer added to each tube.

NSB = Nonspecific binding, amount of interference of impurities in the assay tubes, buffer, etc.

Bo = Total binding (%) capacity of the working dilution of tracer and antibody to be used as the basis for determining hormone concentrations.

Standards = Known amounts of hormone used to construct the standard reference curve.

QC = Quality control, a standard unknown plasma sample used in assay to control intra- and interassay variation.

Max Bo = Maximum binding (%) capacity of the tracer and antibody to control the quality of the tracer.

Sample = The unkown amount of the hormone that is to be measured.

To mix Standard: (LH Standard ampule contains 32 ng/800 µl).

Number the Standard tubes: 31, 62, 125, 250, 500, 1,000, 2,000, 4,000.

Place 800 µl of RIA Buffer in each tube then add the contents of the ampule to the tube numbered 4,000. Mix well and perform serial dilutions (i.e., 4,000, 2,000, 1,000, etc.) by placing 800 µl from the previous tube into the next and so on.

Proceed in a step-wise addition:

1. Add sample or standard to numbered assay tubes
2. Add 200 µl of anti-LH antibody (1:40,000) to all tubes except TC and NSB tubes
3. Add 100 µl of LH-$I^{125}$
4. Vortex
5. Incubate for 48 hours at room temperature
6. Add 200 µl EDTA (Sigma Chemical Co.) to all tubes except TC
7. Add 400 µl Normal Rabbit Serum (NRS) (Miles Scientific, Inc.) to all tubes except TC
8. Add 200 µl 2nd Ab (Miles Scientific, Inc.) to all tubes except TC
9. Vortex
10. Add 1 ml PEG (Sigma Chemical Co.) to all tubes except TC
11. Incubate for 3-16 hours at 4°C
12. Centrigue at 3,000 rpm for 20 minutes
13. Decant supernatant and discard properly
14. Count the "pill" on the gamma counter

See Appendix C for RIA buffer, Ab buffer, EDTA, NRS and PEG buffer recipes.

First Ab recipe:

x/300 = total volume/Ab concentration needed = volume of stock Ab required.

Total volume = [# of tubes + 25 (for error)] x 200 µl

Ab concentration = 1:40,000

Stock Ab concentration = 1:300

Then take: Total volume - volume of stock needed = amount of Ab buffer required.

Tracer recipe:

[# of tubes + 30] x 18,000 cpm/tube = total cpm needed

Total cpm - stock cpm/µl (usually 32,000 cpm) = volume of stock tracer needed

Total volume = [# of tubes + 25] x 200 µl

Total volume ÷ 5 = volume of 2nd Ab stock needed

Total volume = 2nd Ab volume = amount of RIA buffer required

Protocol for FSH: Bolt and Rollins, 1983:

The procedure is the same as that for LH except for the standard curve parameters, Ab dilution and tracer cpm. To mix standards: (FSH Standard ampule contains 1,024 ng/800 µl). Number of the Standard tubes: 1, 2, 4, 8, 16, 32, 64, 128 ng. Place 800 µl of RIA Buffer in each tube then add the contents of the ampule to the tube numbered 128. Mix well and perform serial dilutions (i.e., 128, 64, 32, etc.). Working Ab dilution for FSH is 1:10,000. Tracer: 20,000 cpm

Protocol for PRL: Bolt and Rollins, 1983 (modified):

The procedure is the same as that for FSH except for the standard curve parameters and Ab dilution. To mis Standards: (PRL Standard

aliquot: 102.4 ng/800 µl). Number of the Standard tubes: 0.25, 0.50, 0.1, 0.2, 0.4, 0.8, 1.6, 3.2, 6.4, 12.8 ng. Working Ab dilution for PRL is 1:200,000.

The protocol for Estradiol: Thomspon et al., 1978:

Proceed in step-wise addition:

1. Add 2 ml of unknown sample to extraction tube
2. Add 2,000 cpm of $^{3}H\text{-}E_2$ for recovery
3. Add 10 ml of Benzene:Methanol (90:10)
4. Vortex vigorously layer with liquid nitrogen
5. Freeze aqueous layer with liquid nitrogen
6. Decant organic layer onto an LH-20 Sephadex column
7. Collect fraction
8. Evaporate organic layer with Nitrogen gas
9. Add 500 µl of assay buffer
10. Remove 100 µl to count recovery
11. Transfer 200 µl to assay tube in duplicate
12. Add 100 µl diluted antiestradiol antiserum (1:50,000)
13. Add 100 µl tracer ($^{3}H\text{-}E_2$:20,000 cpm)
14. Vortex gently
15. Incubate for 24 hours at 4°C
16. Add 500 µl of Dextran coated charcoal to all but TC tubes
17. Vortex tubes and incubate for 15 minutes
18. Centrifuge for 10 minutes at 3,000 rpm
19. Decant supernatant into scintillation vials (Fisher Co.)
20. Add 10 ml scintillation fluid (Scintiverse II, Fisher Co.)
21. Count vials for 1 minute in a liquid scintillation counter

# APPENDIX C

## BUFFERS AND REAGENTS

### Sodium Phosphate Buffers

1. Dibasic F.W. = 268.07 (hydrous or anhydrous)

   To make up 500 ml of 0.5 M Sodium Phosphate Buffer, dibasic:

   Add 67 gms of Sodium Phosphate (dibasic) to a 500 ml volumetric flask then fill halfway with double distilled water. Stir until dissolved then fill to volume with more double distilled water and stir for an additional 10-15 minutes. Filter the solution into the 0.45 μm filter storage container (a vacuum is required). Store at room temperature. Buffer will keep 4-6 weeks.

2. Monobasic F.W. = 137.99 (hydrous or anhydrous)

   To make up 500 ml of 0.5 M Sodium Phosphate Buffer, monobasic:

   Add 34.49 gms of Sodium Phosphate (monobasic) to a 500 ml volumetric flask then fill halfway with double distilled water. Stir until dissolved then fill to volume with more double distilled water and stir for an additional 10-15 minutes. Filter the solution into the 0.45 μm filter storage container (a vacuum is required). Store at room temperature. Buffer will keep 4-6 weeks.

### Reagents for LH, FSH and PRL RIA

1. Phosphate Buffer (PB): (See phosphate buffer pH chart for mixture). Use 0.5 M Sodium Phosphates and dilute with double distilled water to obtain desired molarity of buffer.

NOTE: To adjust pH use 1 M, 3 M or 10 M NaOH (base) or 5'sulfosalicylic acid (acid).

2. Basic RIA Buffer: Use a volumetric flask. Mix solution using 0.01 M PBS with a pH of 7.5; readjust pH after mixing. Add material to flask before adding liquid; only fill about halfway with PBS and dissolve materials before completing the mixture.

| MATERIALS | LITER | ½ LITER |
|---|---|---|
| 0.1% BSA | 1 gm | 0.5 gm |
| 0.135 M NaCl | 7.89 gm | 3.95 gm |
| 0.005 M $NaHCO_3$ | .420 gm | 0.21 gm |
| 0.01% Thimerosal | 100 mg | 50 mg |

3. EDTA: Use a volumetric flask. Mix a 0.1 M solution using double distilled water and adjust the pH to 7.5 ± 0.5.

| MATERIALS | LITER | ½ LITER |
|---|---|---|
| (Di)NaEDTA | 37.22 gm | 18.6 gm |
| 0.01% Thimerosal | 100 mg | 50 mg |

4. 2% Normal Rabbit Serum (NRS) v/v: Mix using a 0.01 M PBS with a pH of 7.5; readjust pH after mixing solution.

| MATERIALS | LITER | ½ LITER |
|---|---|---|
| Normal Rabbit Serum | 20 ml | 10 ml |
| 0.01% Thimerosal | 100 mg | 50 mg |

5. Polyethylene glycol (PEG) v/v: Mix a 5% solution in 0.01 M PBS with pH of 7.5; readjust after mixing.

| MATERIALS | LITER | ½ LITER |
|---|---|---|
| PEG | 50 gm | 25 gm |
| 0.01% Thimerosal | 100 mg | 50 mg |

6. First Ab Buffer: Use a volumetric flask. Mix using a 0.1 M PBS with a pH of 7.5; readjust the pH after mixing.

| MATERIALS | LITER | ½ LITER |
|---|---|---|
| 0.135 M NaCl | 7.89 gm | 3.95 gm |
| 0.005 M $NaHCO_3$ | 0.420 gm | 0.21 gm |
| 0.05 M (Di) NaEDTA | 18.6 gm | 9.3 gm |
| 1:300 NRS | 3.3 mls | 1.65 mls |
| 0.01% Thimerosal | 100 mg | 50 mg |

Reagents for Estradiol RIA

1. Dextran coated charcoal:

   Mix: 0.625 gm Norit A charcoal (Sigma Chemical Co.)
   0.0625 gm Dextran T-70 (Pharmacia Co.)
   100 ml Assay Buffer*

2. Assay Buffer: (for 2 liters)

   Mix: gm Sodium Phosphate (monobasic)
   32.7 gm Sodium Phosphate (dibasic)
   2 gm Sodium azide
   18 gm Sodium chloride
   2 gm gelatin

# VITA

Pamela Pecon McKenzie, daughter of Brian and Margaret Pecon, was born in Tuscan, Arizona, on March 12, 1960. She graduated from Germantown High School, Germantown, Tennessee, in May 1978. She entered the University of Tennessee, Martin, in September 1978, then went on to Memphis State University where she graduated with a Bachelor of Science degree in Biology in December of 1983. In the fall of 1985, she accepted a teaching assistantship at The University of Tennessee, Knoxville, and began study toward a Master of Science degree in Animal Science with a concentration in Reproductive Physiology. In August of 1987, she married John R. McKenzie. Upon completion of her graduate research in December 1987, she was awarded her degree.

The author will begin work on a Doctor of Philosophy degree in Animal Science at the University of Tennessee, Knoxville.