INFLUENCE OF A TWICE A DAY FEEDING REGIMEN AFTER PHOTOSTIMULATION ON THE REPRODUCTIVE PERFORMANCE OF BROILER BREEDER HENS

by

JESSICA MARIE SPRADLEY

(Under the direction of Adam J. Davis)

ABSTRACT

In a commercial setting broiler breeders are typically provided a restricted amount of feed once a day during the laying period, and this feed is rapidly consumed, leaving the birds to fast for extended periods of time before the next feeding. In the current research, the effects of shortening the daily fasting period on the reproductive performance of broiler breeder hens was investigated by implementing a twice a day (2x) versus once a day (1x) feeding program after photostimulation. The hens fed 2x produced significantly more eggs during the early lay period and had a significantly greater cumulative percent hen day egg production than the hens fed 1x. Cumulative mortality was also significantly higher, however, for the hens fed 2x than the hens fed 1x. The results indicate that providing broiler breeder hens feed 2x compared to 1x after photostimulation can enhance reproductive performance during the early lay period.

KEYWORDS Broiler breeder hen, Twice a day feeding, Egg production

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B.S., The University of Georgia, 2007

A Thesis Submitted to the Graduate Faculty of the University of Georgia in Partial Fulfillment of the Requirements for the Degree

MASTER OF SCIENCE

ATHENS, GEORGIA

2007

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August 2007

DEDICATION

This thesis is dedicated to my friends who have been a tremendous support over the past year.

ACKNOWLEDGEMENTS

I would like to acknowledge Adam Davis for his support over the past four years, for believing in me, and for his assistance in writing this thesis. I thank Michael Lacy, Jeanna Wilson, and the Poultry Science Department for this opportunity. I would like to acknowledge Elizabeth Freeman for her assistance with all of my data and her words of encouragement. I would also like to thank the farm crew, especially George, Carl, and Beverly, for all of their help.

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1. REPRODUCTIVE ENDOCRINOLOGY OF BROILER BREEDER HENS

1.1. Structure of the Hen Ovary

In the domestic hen, only the left ovary develops and this ovary consists of a pool of follicles arranged in a size hierarchy. Small white follicles, large white follicles, and small yellow follicles make up the prehierarchical follicles (increasing in size, respectively), while 5-8 large yellow follicles are the hierarchical follicles. Small white follicles measure less than 2 mm in diameter, large white follicles measure 2-5 mm in diameter, and small yellow follicles measure between 5 and 12 mm in diameter. The individual large yolk-filled preovulatory yellow follicles are 12-40 mm in diameter depending on the stage of their development. These large yellow follicles can be categorized by their size as well as by their time until ovulation. The largest sized follicle is designated as the F1 follicle, and it will typically ovulate within 24 hours, the next largest follicle is the F2 follicle and it will ovulate in about 48 hours, followed by the F3 follicle which will ovulate in 72 hours, and so on for the other follicles. After the F1 follicle ovulates the F2 follicle becomes the new F1 follicle, and succeeding follicles each advance one place in the hierarchy, and an additional follicle is recruited into the hierarchy from the pool of small yellow follicles. Similarly, several large white follicles and small white follicles also grow and advance to the next size category. Follicular recruitment into the hierarchy is a highly selective process. It is estimated that only 5% of the growing prehierarchical follicles will survive to reach a size of 6-8 mm (Gilbert *et al*., 1983a). Thus, the vast majority of follicles undergo follicular atresia with the individual follicular cells dying by apoptosis (Johnson *et al*., 1996a).

The outer structure of the avian ovarian follicle consists of several tissue layers, beginning with the theca layer which is the outermost layer and is followed in subsequent order by a basement membrane, a granulosa layer, an inner perivitelline layer, and a plasma membrane which surrounds the yolk. The theca layer can be further subdivided into two tissue layers, the theca interna and externa, and both of these layers contain vasculature while the granulosa layer does not. Granulosa cells are multilayered in the prehierarchical follicles, but as follicles mature into hierarchical follicles the granulosa cells migrate and form a monolayer.

1.2. LHRH, FSH, and LH

Gonadotrophin releasing hormone (GnRH) is released from the hypothalamic median eminence in response to environmental and physiological cues (Contijoch *et al*., 1992; Advis and Contijoch, 1993). GnRH initiates the reproductive cycle by stimulating the production and release of follicle stimulating hormone (FSH) and luteinizing hormone (LH) from the anterior pituitary. In the domestic hen, adequate photostimulation (greater than 12 hours), attaining a body weight sufficient to support reproduction, and adequate nutrition are key cues that stimulate GnRH production. In avian species, GnRH is specifically referred to as luteinizing hormone releasing hormone (LHRH) because experimentally only LH release from the anterior pituitary is stimulated by GnRH (Krishnan *et al.,* 1993; Proudman *et al*., 2006). Three forms of GnRH (GnRH I, GnRH II, and 1GnRH III) have been identified; however, only GnRH I appears to control LH secretion *in vivo* in avian species (Katz *et al*., 1990; Sharp *et al*., 1990; Wilson *et al*., 1990a, 1990b, 1991; Millam *et al*., 1998).

FSH receptor (FSH-R) expression in the hen ovary is highest in the granulosa cells of the small yellow follicles, and the level of expression decreases with follicular maturation (Calvo and Bahr, 1983; Ritzhaupt and Bahr, 1987; You *et al*., 1996; Woods and Johnson, 2005). Theca cells express far less FSH receptors than granulosa cells and the level of FSH receptor expression in the theca cells does not vary significantly with follicular maturation (Gilbert *et al*., 1985; Etches and Cheng, 1981; You *et al*., 1996). FSH has several functions: it promotes granulosa cell proliferation and maturation (Davis *et al*., 2000a; 2001); helps maintain the follicular hierarchy through prevention of atresia (Palmer and Bahr, 1992; Johnson *et al*., 1996a, 1999); induces LH receptor, steroidogenic acute regulatory protein, and P450 cholesterol side chain cleavage (P450 SCC) enzyme expression in granulosa cells for subsequent steroid production (Li and Johnson, 1993; Johnson *et al*., 2001, 2004); and stimulates progesterone production (Calvo and Bahr, 1983; Robinson *et al*., 1988; Davis *et al*., 1999, 2001; Johnson *et al*., 2004).

LH receptor (LH-R) expression is highest in the granulosa cells of the large follicles, especially the F1-F3 follicles (Calvo *et al*., 1981; Calvo and Bahr, 1983; Gilbert et al., 1983b, 1985; Johnson *et al*., 1996b). LH receptor mRNA expression in the theca tissue varies little with follicular development (Johnson *et al*., 1996b), and LH promotes steroidogenesis by the theca cells of prehierarchical and hierarchical follicles (Robinson and Etches, 1986; Kowalski *et al*., 1991). Decreased expression of the mRNA for the LH receptor in 3-5 mm diameter prehierarchical follicles is associated with atresia (Johnson, 1996b). LH promotes granulosa cell growth (Davis *et al*., 2001) and progesterone production (Davis *et al*., 1999; Johnson *et al*., 2004). Plasma LH concentrations peak 4-6 hours prior to ovulation (Etches, 1990).

1.3. Steroidogenesis of the Hen Ovary

In the avian ovary, steroids are synthesized by two metabolic pathways: the Δ4 and Δ5 pathways. Both pathways begin by converting cholesterol into pregnenolone through the activity of P450 SCC enzyme. From this point pregnenolone can be metabolized into two different compounds depending upon the pathway followed. In the Δ4 pathway, pregnenolone is converted by 3β-hydroxysteroid dehydrogenase (3βHSD) into progesterone, which can be metabolized to 17α-OH-progesterone by the enzyme 17α hydroxylase. The 17α-OH-progesterone can then be converted to androstenedione by 17, 20 lyase. In the Δ5 pathway pregnenolone is converted into 17α-OH-pregnenolone by 17α hydroxylase and the 17α-OH-progesterone is then converted by 17, 20 lyase to dehydroepiandrosterone (DHEA), which can be metabolized to androstenedione by 3βHSD. Androstenedione is the common end product of both the Δ4 and Δ5 steroidogenesis pathways, and it can be metabolized into testosterone which then can be aromatased to estrogen.

The granulosa cells of the prehierarchical follicles are steroidogenically incompetent because they lack P450 SCC activity (Lee and Bahr, 1990; Tilly *et al*., 1991). However, the theca cells of the prehierarchical follicles via the Δ5 steroidogenic pathway produce estrogens, and these cells are the primary source for circulating estradiol (Senior and Furr, 1975; Lee and Bahr, 1989, 1993). Plasma concentrations of estrogen peak 4-6 hours before ovulation. Estrogen stimulates the hypothalamus and pituitary to express progesterone receptors (Wilson and Sharp, 1976). Estrogen also stimulates the liver to produce lipid and vitellogenin for yolk formation (Deeley *et al*., 1975) and aids in calcium metabolism for shell formation and medullary bone deposition (Etches, 1987). Estrogen increases the expression of its own receptor in the oviduct which increases estrogen's effects on oviductal functions (Takahashi *et al*., 2004).

Estrogen also increases expression of progesterone receptors in the ovary and oviduct, which promotes the formation of tubular secretory glands for albumen and shell secretion and the contractile activity of the myometrium (Yoshimura and Bahr, 1991).

Both the granulosa and theca cells of the hierarchical follicles produce steroids. The granulosa cells express very low levels of 17α-hydroxylase and thus utilize the Δ4 pathway to produce progesterone (Etches and Duke, 1984; Lee and Bahr, 1989, 1993; Johnson *et al*., 1996a). The theca cells of the hierarchical follicles also use the Δ4 pathway to produce progesterone which they metabolize to androgens. More importantly, the theca cells of the hierarchical follicles, except those of the F1 follicle, metabolize the progesterone produced by the granulosa cells into androgens (Etches, 1990). In the F1 follicle, the theca cells do not metabolize the progesterone produced by the granulosa cells (Marrone and Hertelendy, 1985), and thus the F1 follicle is the primary source of plasma progesterone. Plasma progesterone produced by the F1 follicle binds to progesterone receptors in the hypothalamus to increase production and release of LHRH I, which increases the release of LH from the anterior pituitary (reviewed by Advis and Contijoch, 1993). The released LH travels through the circulatory system to the ovary and binds to its receptors on the granulosa cells of the F1 follicle to stimulate more progesterone production thus creating a positive feedback loop that leads to a surge in both LH and progesterone production that induces ovulation (Wilson and Sharp, 1976; Robinson and Etches, 1986). Plasma concentrations of progesterone peak 4-6 hours before ovulation. Progesterone and LH bind to their receptors in the cells along the stigma of the F1 follicle, activating the production of enzymes such as collagenase which degrade the tissue along the stigma and allow the rupture of the F1 follicle for ovulation (Isola *et al*., 1987; Yoshimura and Bahr, 1991).

1.4. Activin and Inhibin

Activin and inhibin are two closely related dimeric glycoprotein hormones that share a common protein subunit. Activin is composed of a combination of two distinct but similar β subunit proteins. Activin exists in three different forms: activin A (consisting of two β_A subunits), activin B (two β_B subunits), and activin AB (one β_A subunit and one β_B subunit). Inhibin is composed of one α subunit protein and one β subunit protein and exists as either inhibin A (with one α and one β_A subunit) or inhibin B (with one α subunit and one β_B subunit). Activin and inhibin not only modulate FSH secretion from the pituitary but also affect other reproductive functions in mammalian species (reviewed by Halvorson and DeCherney, 1996, Mather *et al*., 1997, Woodruff, 1998, 2002, Welt *et al*., 2002, and Phillips and Woodruff, 2004).

Follistatin, a soluble binding protein, binds activin and inhibin through their common β-subunit (Nakamura *et al*., 1990). However, follistatin binds inhibin with less affinity than it does with activin (Shimonaka *et al*., 1991; Krummen *et al*., 1993). Many of the biological actions of activin seem to be neutralized by the binding of activin with follistatin (reviewed by Michel *et al*., 1993), but binding of activin by follistatin may not neutralize all biological activity of activin (Mather *et al*., 1993). Changes in the bioactivity of inhibin once bound to follistatin have not been characterized.

The mRNA expression and protein expression of the inhibin and activin subunits as well as follistatin are well characterized in the laying hen ovary (Davis and Johnson, 1998; Lovell *et al*., 1998, 2003; Johnson *et al*., 2005). The granulosa cells of the largest hierarchical follicles of the ovary produce inhibin A while the small follicles produce inhibin B (Lovell *et al*., 1998, 2003; Johnson *et al*., 2005). In particular, the F1 follicle serves as the primary source for inhibin A (Lovell *et al*., 1998). Activin A production is greater in the theca cells than the granulosa cells

(Lovell *et al*., 1998). Activin A production gradually increases from the small prehierarchical follicles to the F4 follicle, but then rapidly declines after the F4 stage (Lovell *et al*., 2003). Follistatin is produced by both the theca and the granulosa cells, and its production by the granulosa cells is highest in the prehierarchical follicles (Lovell *et al*., 2003).

In avian species, active immunization against the inhibin α-subunit resulted in accelerated puberty and enhanced hen day egg production in Japanese quail (Moreau *et al*., 1998), increased testicular weight in cockerels (Lovell *et al*., 2000), and increased numbers of preovulatory follicles in turkey hens (Ahn *et al*., 2001). In addition, broiler hens which naturally produce fewer eggs than laying hens have higher follicular mRNA expression of the inhibin α-subunit than laying hens (Safi *et al*., 1998; Slappey and Davis, 2003). Furthermore, laying hens that have short sequence lengths compared to those with long sequence lengths before a pause day have a greater granulosa cell expression of the mRNA for the inhibin α-subunit in the F1 and F4 follicles (Wang and Johnson, 1993). Although an over expression of the inhibin α-subunit appears to be correlated with decreased reproductive capability, the exact roles that activin and inhibin play in follicular development are just starting to be discerned. In general, the addition of activin A to granulosa cell cultures induces the mRNA expression of LH (Johnson *et al*., 2004; Johnson *et al*., 2006) and FSH (Davis *et al*., 2001; Johnson *et al*., 2004; Woods and Johnson, 2005; Johnson *et al*., 2006) receptors. Activin A also inhibits granulosa cell proliferation in granulosa cell cultures from hierarchical follicles (Davis *et al*., 2001; Johnson *et al*., 2006) but not in granulosa cell cultures from prehierarchical follicles (Johnson *et al*., 2006). In contrast, the addition of inhibin A to cultured granulosa cells did not affect the expression of LH or FSH receptors and had no effect on granulosa cell proliferation (Johnson *et al*., 2006).

1.5. Metabolic Hormones and Ovarian Development

1.5.1. Leptin

Leptin, a protein hormone synthesized and secreted by adipose tissue in mammals, regulates food intake and energy expenditure (reviewed by Friedman and Halaas, 1998, and Houseknecht and Portocarrero, 1998), and influences the onset of reproductive puberty and gonad steroidogenesis (reviewed by Smith *et al*., 2002, Ebling, 2005, Zieba *et al*., 2005, and Budak *et al*., 2006). In avian species, leptin is produced in the liver in response to feeding (Taouis *et al*., 1998; Ashwell *et al*., 1999; Kochan *et al*., 2006). The biology of the chicken leptin receptor has also been fairly well characterized (Horev *et al*., 2000; Ohkubo *et al*., 2000). Leptin's role in avian reproduction is not well investigated; however, based on preliminary reports, it may provide an endocrine mechanism tieing nutritional status to reproduction. Paczoska-Eliasiewicz *et al*. (2003) reported that hens injected with leptin twice a day during a five day fast had a delay in the cessation of egg laying, less hierarchical follicle regression, and lower rates of fasting-induced follicular apoptosis than non injected fasted birds. The leptin receptor is expressed in the hen ovary (Paczoska-Eliasiewicz *et al*., 2003; Ohkubo *et al*., 2000). Cassy *et al*. (2004) reported that the mRNA for the leptin receptor is detected in both granulosa and theca cells of the four largest preovulatory follicles of broiler hens and that the expression of the leptin receptor is increased in the granulosa cells of broiler breeders fed *ad libitum* versus feed restricted.

1.5.2. Ghrelin

In mammals, the hormone ghrelin is produced by the stomach. Ghrelin production is increased when there is a negative energy balance and many of its physiological actions involve

stimulating feed intake and influencing metabolism (reviewed by Korbonits *et al*., 2004, Van der Lely *et al*., 2004, and Ueno *et al*., 2005). Ghrelin has been found to suppress LH secretion in both intact and ovariectomized mammals and may be a mechanism by which insufficient caloric intake depresses reproduction in females (Furuta *et al*., 2001; Fernandez-Fernandez *et al*., 2004; Vulliemoz *et al*., 2004; Tena-Sempere, 2005). In chickens, ghrelin mRNA expression is highest in the proventriculus (Kaiya *et al*., 2002; Richards *et al*., 2006). Plasma ghrelin levels increase when chicks are fasted and after refeeding return to baseline levels (Kaiya *et al*., 2007). The mRNA for the ghrelin receptor has been detected in follicular fragments that contain both theca and granulosa cells (Sirotkin *et al*., 2006).

1.5.3. Thyroid Hormone

In mammalian species, thyroid hormones (T3 and T4) are well established as regulators of metabolism, but there is emerging evidence that they may be involved in regulating reproduction as well. Elevated levels of thyroid hormone can delay sexual maturity, alter gonadotropin release, and increase sex hormone binding globulin production such that steroid hormone activity is altered (Fitko and Szelezyngier, 1994; Doufas and Mastorakos, 2000). Low levels of thyroid hormones are also associated with decreased androgen production (Doufas and Mastorakos, 2000). In avian species, thyroid hormones help regulate body temperature (Danforth and Burger, 1984) and growth and maturation (Bouvet *et al*., 1987). The role of thyroid hormones in regulating reproduction in avian species has not been examined extensively. Exogenous thyroid hormone will stimulate testicular growth in quail (Follett and Nicholls, 1985; Yoshimura *et al*., 2003). In addition, T4 concentrations are elevated and T3 concentrations are depressed during molting (Brake *et al*., 1979; Lien and Siopes, 1989; Davis *et al*., 2000b). The

elevated T4 levels during molting are interesting given that it has been shown feed restricted broiler breeder hens (Bruggeman *et al*., 1997), cockerel chicks food deprived for about one day (Buyse *et al*., 2000), and male quail deprived of food for three days (Kobayashi and Ishii, 2002) all have reduced plasma concentrations of T3. More research is needed to determine whether thyroid hormones play a significant role in avian reproduction.

1.6. Summary

The developmental stage of the large yolk-filled preovulatory follicles on the hen ovary is visually apparent based on the well defined size hierarchy of the follicles. Although the development of the hierarchical follicles is tightly regulated with an interval of 24-26 hours between each consecutive ovulation, the endocrinology involved in maintaining the follicular hierarchy on the ovary is not completely understood. The steroid and gonadotropin profiles associated with follicular growth in the hen are well described. Very little is known, however, about the role of ovarian produced hormones such as activin and inhibin in the development and maintenance of the highly ordered follicular hierarchy. Furthermore, the involvement of hormones such as leptin and ghrelin, which may regulate follicular development based on the hen's nutritional status, is just starting to be explored.

2. FEEDING REGIMENS AND THEIR EFFECTS ON BROILER BREEDER HENS

2.1. The Necessity of Feed Restriction

Through genetic selection and better bird management, today's broilers reach a market weight of 2 to 2.5 kilograms in 6 weeks or less. This rapid growth rate in broilers is facilitated by a nearly insatiable appetite. These voracious appetites and rapid growth rates, however, are counter productive to optimal reproductive performance in the genetically similar parent stocks of broilers. Optimum reproductive performance in broiler breeders is dependent in large part on attaining an ideal body weight to support reproduction, consuming a nutritionally adequate diet, and being properly photostimulated. In the US, broiler breeder pullets are reared on predominantly corn/soybean meal diets which are the same type of diets used to feed commercial broilers. Interestingly, the ideal body weight for reproduction is very similar to 6 week market size, but optimum sensitivity to photostimulation for the initiation of reproduction in broiler breeders does not occur until about 20 weeks of age.

Ovarian development and egg production are very sensitive to stimulation by increasing photoperiod length. A photoperiod of 12 to 14 hours in laying hens is sufficient to stimulate maximum plasma LH concentrations and egg production (reviewed by Sharp, 1993, and Etches, 1996). However, chicks are born photorefractory and acquire maximum sensitivity to light only after they have been exposed to short day lengths (Etches, 1996). The duration of the exposure to short days before photosensitivity is acquired is not well determined, but it appears to be 8-12 weeks (reviewed by Etches, 1996). Hens that are not photostimulated will still reach sexual maturity and produce eggs. Domestic fowl reared on a daily, continuous lighting schedule that

provides either 6 hours or 22 hours of light will reach sexual maturity at about 21 weeks of age (Johnson, 2000). Similarly, Wilson and Cunningham (1980) reported that hens provided 8 hours of light daily started to reach sexual maturity at 19 weeks of age; however, plasma LH concentrations and egg production were significantly lower compared to hens that were photostimulated with 16 hours of light at 17 weeks of age.

Given that maximum egg production at minimal cost is the goal for broiler breeder farmers, broiler breeder pullets must be managed so that ideal body weight for the onset of reproduction is achieved at about 20 to 21 weeks of age and matches the acquisition of photosensitivity for reproduction. To prevent broiler breeder pullets from growing too quickly and becoming obese prior to the photosensitivity-based sexual maturity that occurs at 20 to 21 weeks of age, current pullet management protocols call for dietary intake to be severely restricted. Typically, feed allocations are 60-80 percent less during the rearing period and 25-50 percent less during the laying period than what the breeder pullets/hens would consume if fed *ad libitum*.

There are two basic approaches to feed restricting broiler breeders, a severe quantitative restriction of the typical energy dense corn and soybean meal based diet or a qualitative restriction in which the nutrient density of the diet is diluted so that more feed can be fed at a given time. Typically for the quantitative restriction feeding programs, the diet utilized is so nutrient dense that the amount of feed available on daily basis during the rearing period is not enough to be distributed to all pullets or breeder hens equitably. Therefore, the pullets are typically fed on a skip a day basis so that the feed allotment for two days can be combined. By combining two days worth of feed, the amount of feed that is fed is large enough that it can be distributed to all the available feeder space, which reduces the competition among the birds and

helps ensure that the less aggressive birds also get their portion of feed. The reduction in competition for food results in improved flock body weight uniformity (Bartov *et al*. 1988). Improved uniformity leads to better overall flock performance since the nutrient and management requirements of uniform birds are similar and feeding regimes can be tailored to the average bird size. Flock body weight uniformity is also important later in the production cycle because better uniformity has been linked with an earlier average age at first egg (Petitte *et al*., 1982) and greater total egg production during the lay period (Hudson *et al*., 2001). During the early egg production period the amount of feed needed to support egg production allows the total volume of feed for the flock to be sufficient so that the hens can be placed on an everyday feeding schedule.

In an effort to lessen the severity of the feed restriction associated with quantitative feed restriction programs, researchers have developed and tested qualitative feed restriction programs. The goal of the qualitative feed restriction programs is to increase the amount of feed available to be fed through the use of feed diluents or by utilizing less nutrient dense feed ingredients. Zuidhof *et al*. (1995) fed broiler breeder hens standard rearing and laying diets from 0 to 56 weeks of age or standard diets diluted with either 15% or 30% with ground oat hulls. The birds fed diluted diets had better flock body weight uniformity and decreased stress levels than birds fed standard undiluted diets. The decreased stress levels and better flock uniformity values probably resulted from the increased availability of feed. In fact, during the lay period the average time until all the feed was consumed was 264, 349, and 489 minutes for the standard, and the 15% oat hull and the 30% oat hull diet, respectively. Interestingly, hens fed the 15% oat hull diet had the highest egg production and chick production of all the treatments through 56 weeks of age. Egg production for the birds fed the 30% oat hull diet was equivalent to the

control birds. Tolkamp *et al*. (2005) also utilized oat hulls (40% of diet) as a dietary diluent, and combined this diet with Ca proprionate, an appetite suppressant. Tolkamp *et al*. fed this diet *ad libitum* to broiler breeder pullets during the rearing phase. Even though the pullets were fed *ad libitum* and consumed 6.54 kg/bird more feed than the pullets fed a control diet lacking the oat hulls and Ca proprionate, they gained body weight at an equivalent rate to the control birds and had equal subsequent reproductive performance.

Hogsette *et al*. (1976) tested the effects of qualitatively restricting broiler breeder hens by using an inert dietary diluent. They fed hens a typical corn/soybean meal laying diet or this diet mixed with 50 g of sand/kg of diet in 3 separate experiments (with hens at 66 weeks of age, 33 weeks of age, and 32 weeks of age). They noted no differences in egg production, egg weight, fertility, or hatchability between the two dietary treatments in any of their experiments. However, they did report that birds on the qualitatively restricted diet gained less body weight, resulting in energy and nutrient savings and less energy spent to produce an egg. Thus, a diet with 5% sand reduced the cost of feed by 3% while not compromising the reproductive performance of the hens.

Lordelo *et al*. (2004) utilized a different approach to qualitative feed restriction. Instead of diluting the diet with "filler" such as sand or oat hulls, broiler breeder pullets were reared from 2 to 18 weeks of age with either a standard corn/soybean meal diet or a diet in which the soybean meal was replaced with cottonseed meal. Cottonseed meal has a lower nutrient density than soybean meal. The birds fed the cottonseed meal diet had to be fed a greater amount of feed in order to achieve the same body weight as those fed the diet containing soybean meal. In particular, the birds could be fed more of the corn/cottonseed meal diet without increasing body weight gain compared to the birds fed the corn/soybean meal diet because of the very low levels

of total and available lysine in cottonseed meal relative to soybean meal. As a result of being provided access to more feed than the birds fed the diet containing soybean meal, the birds fed the corn/cottonseed meal diet had significantly better flock body weight uniformity.

2.2. Advantages of Feed Restriction

2.2.1. Body Weights

Obviously, feed restriction during rearing reduces the body weights of broiler breeders when compared to *ad libitum* fed birds (Robbins *et al*., 1986; Bruggeman *et al*.1999, 2005; Richards *et al*., 2003; Onagbesan *et al*., 2006). Bruggeman *et al.* (1999) reported that at the end of 15 weeks, birds fed *ad libitum* were twice the weight of birds that were quantitatively feed restricted beginning at 7 weeks of age. Onagbesan *et al*. (2006) determined that the body weights of the birds fed *ad libitum* were significantly heavier at all ages (up through 36 weeks) compared to birds that were restricted by 55% of what they would consume *ad libitum*. Renema *et al.* (1999a) examined body weight gain between photostimulation and sexual maturity in broiler breeder pullets fed *ad libitum* or feed restricted. The pullets fed *ad libitum* gained significantly more weight than the feed restricted pullets and the increased body weight of the *ad libitum* fed birds was distributed across fat, muscle, and organ tissue.

Although much more research needs to be completed there are some indications that the excessive food intake and weight gain associated with *ad libitum* feeding permanently alters normal metabolic and endocrine functions. Cassy *et al*. (2004) grew broiler breeders with *ad libitum* or restricted access to feed during a 20 week rearing period. Subsequently all the hens were allowed *ad libitum* feeding during the laying period. When sampled at 32 weeks of age the

birds that had been fed *ad libitum* during the rearing period had higher hepatic leptin mRNA expression but equivalent plasma leptin levels to the birds that had been feed restricted during rearing. In addition, the levels of leptin receptor mRNA expression in the F1, F3, and F4 follicles were significantly higher in the birds that had been fed *ad libitum* versus those that had been feed restricted during rearing, indicating that feeding programs utilized in rearing may permanently alter subsequent ovarian endocrinology. In contrast, even though fasting plasma glucose concentrations were significantly lower in birds fed *ad libitum* compared to feed restricted birds, when measured at 32 weeks of age the protein expression of insulin signaling pathway proteins in small white follicles was not different between the two groups of birds (Metayer *et al*., 2006). Finally, Chen *et al*. (2006) conducted short term studies with 35 week old broiler breeder hens. One group of hens was fed the breeder recommended feed amount of 145 g/day while another group of hens was fed 290 g/day. At the end of the 10 day experimental period the hens fed the higher amount of feed had lower egg production, heavier body weights, larger abdominal fat pad weights, and higher plasma concentrations of insulin, glucose, leptin, triacylglycerol, and nonesterified fatty acids. The hens fed the larger amount of feed also exhibited hierarchical follicular atresia. Although limited in number and scope, these research reports clearly indicate the potential linkage between nutrient intake and follicular development and the need for additional research in this area.

2.2.2. Sexual Maturity

Sexual maturity occurs at an earlier age in broiler breeders reared on an *ad libitum* feeding regimen as opposed to a restricted feeding regimen (Heck *et al*., 2004; Hocking, 2004; Renema and Robinson, 2004; Bruggeman *et al*., 2005). Heck *et al*. (2004) reported that the

onset of egg production occurred at 20 weeks for broiler breeders reared on an *ad libitum* feeding regimen, but occurred at 25.4 weeks for broiler breeders reared on a restricted feeding regimen, even though the hens from both treatments were exposed to the same lighting schedule. Depending on the genetic strain of the broiler breeder pullets, Bruggeman *et al.* (2005) reported a delay in sexual maturity of only 2 or 3 weeks in birds feed restricted during rearing compared to birds fed *ad libitum*. Melnychuk *et al*. (2004) also reported that broiler breeder pullets that had been fed *ad libitum* after photostimulation at 21 weeks of age attained sexual maturity about 2 weeks earlier than pullets that were maintained on a feed restriction feeding program. However, if photostimulation was delayed until 24 weeks of age there was no difference in the age of sexual maturity between the feed restricted and *ad libitum* fed pullets. Melnychuck *et al.* (2004) reasoned that by 24 weeks of age all the pullets had reached a threshold body weight regardless of the feeding program and thus responded equally to photostimulation. Subsequently, Hocking (2004) determined that the onset of egg production was linearly related to body weight in broiler breeders fed a relatively constant quantity of feed after photostimulation.

The earlier onset of sexual maturity in broiler breeder hens fed *ad libitum* versus those fed on a restricted basis is reflected in their reproductive hormone profiles. The increase in the LHRH I content of the median eminence of broiler breeder hens typical prior to sexual maturity was significantly delayed and then sharply increased just before egg production in broiler breeder pullets feed restricted from 2 to 24 weeks of age compared to birds fed *ad libitum* (Bruggeman *et al*., 1998a). Bruggeman *et al*. (1998b) reported 16 week old broiler breeder pullets that were fed *ad libitum* during rearing had significantly higher plasma FSH and estradiol concentrations compared to pullets that had been feed restricted during rearing. Similarly, Onagbesan *et al*. (2006) reported plasma estradiol levels were higher and peaked at an earlier age

in broiler breeder hens fed *ad libitum* compared to hens feed restricted beginning at 2 weeks of age. However, once the restricted-fed hens reached peak plasma estrogen levels, they maintained higher levels of plasma estradiol than their *ad libitum*-fed counterparts. Even when an *ad libitum* feeding regimen is not implemented in broiler breeder hens until the time of photostimulation, differences in plasma estrogen concentrations are apparent. Renema *et al.* (1999b) determined plasma estrogen concentrations in broiler breeder hens either fed *ad libitum* or feed restricted at the time of photostimulation at 21 weeks of age. Each hen within the two feeding regimes was also assigned to a body weight category of high, standard, or low. At sexual maturity all the hens from the 3 body weight categories that were fed *ad libitum* had higher plasma estradiol concentrations than their feed restricted counterparts. In addition, the timing of peak estradiol production was delayed in the feed restricted hens compared to hens fed *ad libitum.*

2.2.3. Egg Production

Although sexual maturity is reached earlier in broiler breeder pullets fed *ad libitum* than in feed restricted-fed pullets, *ad libitum* fed breeders produce fewer total and settable eggs in a production cycle than hens that were feed restricted during rearing and then given feed *ad libitum* at sexual maturity (Heck *et al*., 2004; Bruggeman *et al.*, 2005; Onagbesan *et al*., 2006). A further gain in total egg production can be achieved when broiler breeder hens are feed restricted in rearing as well as during the production cycle (Yu *et al.*, 1992a). The reason for the poor egg production in broiler breeder hens fed *ad libitum* compared to feed restricted broiler breeder hens is due to poorer livability and propensity for abnormal follicular development. Broiler breeder hens fed *ad libitum* during rearing have higher ovary weights than feed restricted

hens during rearing (Yu *et al*., 1992a). The increase in ovarian weight is associated with the development of more large yellow preovulatory follicles (Hocking *et al*. 1987). The increased number of large yellow follicles is often manifested as a double hierarchy with pairs of follicles of similar weights (Whitehead and Hocking, 1998) which is linked to an increased incidence of double-yolked eggs, erratic ovulations and atresia of large yellow follicles (Hocking *et al*. 1989). Restricted feeding of broiler breeder hens during the rearing and laying periods reduces the number of large yellow follicles in the ovary of broiler breeder hens (Hocking *et al*. 1987, 1989; Heck *et al*., 2004; Hocking and Robertson, 2005) and thus reduces the incidence of multiple ovulations in a single day and the number of abnormal eggs laid (Fattori *et al*. 1991; Yu *et al*., 1992a; Heck *et al*., 2004) as well as decreasing the number of atretic follicles on the ovary (Hocking and Robertson, 2005). Furthermore, broiler breeder hens that have been feed restricted during rearing and production lay longer sequences (Robinson *et al*., 1991a) and persist in lay longer (Fattori *et al*., 1991) compared to full-fed broiler breeder hens.

2.2.4. Welfare Issues

Ad libitum feeding of broiler breeder hens is thought to be detrimental to the welfare of the birds because it encourages obesity and all of the related negative effects including higher mortality (Katanbaf *et al*., 1989; Bruggeman *et al*., 2005), leg and joint problems (reviewed by Renema and Robinson, 2004), and other metabolic disorders. However, in some respects *ad libitum* feeding may be better for the welfare of the birds because it reduces stress (Kubikova *et al*., 2001). Increased stress in broiler breeder hens that are feed restricted is observable by increased abnormal behaviors, including more time spent pecking at the feeders, less time sitting, more time pacing, and less time preening (Kubikova *et al*., 2001). Plasma corticosterone levels

may also signal the amount of stress a bird is experiencing as higher levels of corticosterone are indicative of higher stress levels in avian species. Broiler breeder hens that are feed restricted according to the breeder's guidelines have higher corticosterone levels than birds fed *ad libitum*, fed twice the amount of the restricted birds, or fed a qualitatively restricted diet diluted with 30% hardwood dust (Kubikova *et al*., 2001). Thus, feed restricted birds may be stressed because they are hungry, bored, or challenged by the increased competition amongst their flock mates when feed is given to them.

Qualitatively restricted diets may be better for animal welfare compared to quantitatively restricted diets. Sandilands *et al.* (2005) noted lower cortisol levels, less object picking (less signs of boredom), and less feed motivation in qualitatively restricted birds compared to birds quantitatively restricted throughout their lives. Zuidhof *et al*. (1995) also reported that broiler breeders fed a qualitatively restricted diet implemented during the rearing and lay periods spend less time at the water source and that their heterophil:lymphocyte ratios suggest a lower stress level at 12 weeks of age compared to a standard quantitatively restricted diet implemented during the rearing and lay periods. Kubikova *et al*. (2001) also reported that hens which were qualitatively feed restricted had lower corticosterone levels than hens that were quantitatively feed restricted, but they had higher corticosterone levels than hens fed *ad libitum.*

2.3. The Timing of Feed Restriction Programs for Optimum Egg Production

Even though restricting the amount of feed fed to broiler breeder hens may increase their stress, the egg production benefits associated with feed restricting broiler breeder hens is

believed to outweigh feeding them *ad libitum* in a commercial setting. However, while the necessity of feed restricting broiler breeders is widely recognized by poultry scientists and producers, the degree of feed restriction as well as the timing and duration of feed restriction during a broiler breeder's life cycle is not agreed upon. An *ad libitum* feeding regimen may be successfully used at certain times of the broiler breeders' lives. Bruggeman *et al*. (1999) indicated that *ad libitum* feeding from 1-7 weeks of age followed by feed restriction from 7-15 weeks of age followed by *ad libitum* feeding to first egg, resulted in improved reproductive performance compared to any other combination of *ad libitum* or restricted feeding during the rearing period. Pym and Dillon (1974) reported it was best to have severe restriction during the rearing period beginning at 10 weeks of age followed by *ad libitum* feeding during the laying period for optimum egg production. Total egg production was also better through 68 weeks of age in female broilers which were restricted during rearing (through 24 weeks of age) and then fed *ad libitum* than birds fed *ad libitum* throughout their lives (Robbins *et al*., 1986). In addition, Robbins *et al*. (1988) reported that egg production was better in broiler breeder hens fed *ad libitum* during weeks 24-31 and 24-64 compared to hens feed restricted their entire lives (Robbins *et al*., 1988). In complete contrast, Robinson *et al*. (1991a) reported that *ad libitum* feeding during the breeding period resulted in lower egg production. McDaniel *et al*. (1981) and Yu *et al*. (1992a, b) suggested that feed restriction should occur in both the rearing and breeding periods for optimum reproductive performance, and most broiler breeder hens in commercial production are feed restricted in both the rearing and breeding periods.

One common industry method of feed restriction is to utilize the previously mentioned skip a day feeding regimen from around 2 weeks of age until sexual maturity followed by feeding restricted feed amounts on an every day basis during the breeding/egg production period

(Bell and Weaver, 2002). The skip a day feeding method is used during the rearing period because, as previously mentioned, it allows for better flock body weight uniformity and because it decreases body weight gains, delays sexual maturity, and increases the number of settable eggs produced by broiler breeder hens compared to an every day feeding program during rearing (Wilson *et al*., 1989).

Often in commercial settings the skip a day feeding program was continued until the broiler breeder flock reached 5 percent egg production. This was done to control flock body weight uniformity and to help control body weight gain since even a very slight excess of body weight prior to peak production results in a significant decrease in total egg production (reviewed by Robinson *et al*., 1991b). Recently, Gibson (2006) reported that initiating an everyday feeding regimen after photostimulating broiler breeder hens for reproduction increased total egg production by about 19 eggs per bird by the end of 65 weeks of age, compared to continuing the skip a day feeding regime until 5 percent egg production was reached. Gibson also reported that plasma estrogen levels were increased and plasma progesterone levels were decreased for the entire breeding period in the hens fed on a skip a day basis until 5 percent egg production compared to the hens that were fed everyday.

2.4. Summary

Feed restriction methods fall into two categories, qualitative and quantitative. In quantitative feed restriction programs small amounts of a typical diet are fed, while in qualitative restriction programs the nutrient content of typical diet is diluted so that more of it can be fed.

Restricting feed intake has several advantages over *ad libitum* feeding in broiler breeder hens, including lower and more uniform body weights, lower mortality, delayed sexual maturity, and better egg production. Although the best timing and the degree to which feed needs to be restricted is not completely defined for optimum egg production and livability, in most commercial settings the broiler breeders are restricted during both the rearing and breeding periods. Typically some form of a quantitative skip a day feed restriction program is used during the rearing phase followed by a quantitative every day feed restriction program during the breeding/egg production period.

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3. STATEMENT OF PURPOSE

Broiler breeder pullets are typically reared using a quantitative feed restriction program to prevent them from gaining too much body weight before reaching sexual maturity. Broiler breeder pullets that are not feed restricted during rearing will not only become over weight, but will also have less uniform flock body weights, reach puberty at an earlier age, and lay fewer total eggs, than pullets that are feed restricted during rearing.

After photostimulation for reproduction, broiler pullets are still feed restricted and this restriction continues throughout their production cycle to prevent excessive body fat accumulation once the birds have reached a mature body size. By preventing obesity the birds will produce more eggs and these eggs will have a higher fertility rate. Because sexually maturing broiler breeders should not be fed *ad libitum* in commercial settings they are almost exclusively fed their total allotment of fed once a day and in some cases during the early lay period once every two days. The birds consume the available feed within a couple of hours and thus have a substantial fasting period between feedings. Previous research from our laboratory indicated that the extended fasting period associated with continuing a skip a day feeding program after photostimulation until 5 percent egg production was very detrimental to total egg production. Broiler breeder pullets that were placed on a once a day feeding program after photostimulation produced 19 more total eggs per bird than those that were given the same amount of total feed but were maintained on a skip a day feeding program until 5 percent egg production was reached at which point they were switched to the once a day feeding program. Interestingly, the average gain of 19 eggs per bird resulted from a cumulative gain in egg

production throughout the production cycle which ended when the birds were 65 weeks of age. Thus, the longer fasting periods during the early production period in the skip a day birds permanently altered their reproductive performance capabilities. Therefore, the goal of the current research is to determine if reproductive performance of broiler breeder hens can be improved by splitting the daily feed allotment offered during the entire breeding period into two feeding periods separated by 8.5 hours.

CHAPTER 4

INFLUENCE OF A TWICE A DAY FEEDING REGIMEN AFTER PHOTOSTIMULATION ON THE REPRODUCTIVE PERFORMANCE OF BROILER BREEDER HENS[1]

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To be submitted to *Poultry Science*

ABSTRACT In a commercial setting broiler breeders are typically provided a restricted amount of feed once a day, and this feed is rapidly consumed leaving the birds to fast for extended periods of time before their next feeding. In the current research the effects on reproductive performance of implementing a twice a day (2x) versus a once a day (1x) feeding program after photostimulation were investigated. Pullets and cockerels were reared using a skip a day feeding program. All pullets were weighed at 20 wk of age and then distributed into 30 laying pens such that each pen had a similar body weight distribution. Each individual laying pen consisted of 35 hens and 4 roosters. At 21 wk of age the birds were photostimulated for reproduction and 15 of the laying pens were placed on a 1x feeding schedule while the other 15 pens were placed on a 2x feeding schedule. The total amount of feed provided per day to all the laying pens was the same but the birds fed 1x received all of their feed at 6:30 *a.m.*, while the birds fed 2x received 60% of their total feed allotment at 0630 h and the other 40% at 1500 h. Even though both treatment groups began egg production at the end of week 23, the birds fed 2x laid significantly ($P \leq 0.05$) more eggs through 42 wk of age than those fed 1x. Additionally, the average egg weight for the entire production period until the birds were 60 wk of age was greater for the hens fed 2x versus 1x. Overall body weight uniformity for the entire laying period was significantly better for hens fed 2x versus 1x. Cumulative mortality was significantly higher, however, for hens fed 2x than those fed 1x. The results indicate that providing broiler breeder hens feed 2x after photostimulation can enhance reproductive performance during the early lay period.

KEYWORDS: broiler breeder hen, twice a day feeding, egg production

INTRODUCTION

The typical poultry industry practice of feed restricting broiler breeder hens decreases body weight gain (Robbins *et al*., 1986; Katanbaf *et al*., 1989; Bruggeman *et al*.1999, 2005; Onagbesan *et al*., 2006), delays the onset of sexual maturity (Robbins *et al*., 1986; Yu *et al*., 1992; Heck *et al*., 2004; Bruggeman *et al*., 2005; Hocking and Robertson, 2005; Onagbesan *et al*., 2006) and decreases mortality (Robbins *et al*., 1986; Katanbaf *et al*., 1989; Heck *et al*., 2004 Bruggeman *et al*., 2005). In addition, feed restriction during the rearing and the laying period reduces the number of large follicles on the ovary of broiler breeder hens (Hocking *et al*., 1987; 1989; Heck *et al*., 2004; Hocking and Robertson, 2005). But more importantly, broiler breeder hens which have been feed restricted produce more eggs (Yu *et al*. 1992; Heck *et al*., 2004; Bruggeman *et al*., 2005; Onagbesan *et al*., 2006) lay longer sequences (Robinson *et al*., 1991), persist in lay longer (Fattori *et al*., 1991), lay fewer abnormal eggs and have fewer multiple ovulations in a single day (Fattori *et al*., 1991; Yu *et al*., 1992; Heck *et al*., 2004) compared to full-fed broiler breeder hens.

Despite the improvement in egg production of broiler breeder hens resulting from feed restriction programs, these hens still have inferior total egg production compared to commercial layer strain hens. Interestingly, total egg production in broiler breeder hens may be depressed as a result of poultry industry feed restriction practices. Broiler breeder hens are typically provided feed once a d during reproduction. This feed is quickly consumed by the hens and thus they will fast for a significant portion of each d. Morris and Nalbandov (1961) suggested that the lack of gonadotropin secretion from the pituitary was responsible for the loss of egg production in fasted birds. Subsequently Scanes *et al*. (1976) reported that plasma LH concentrations were

significantly depressed in 6 wk old male chicks fasted for 12 h compared to control fed cockerels. In addition, fasted laying hens have lower plasma concentrations of LH after 48 h of fasting and lower estradiol and progesterone concentrations after 24 h of fasting compared to *ad libitum* fed control hens (Tanabe *et al*., 1981). Based on these previous reports, the reproductive capability of chickens may be compromised by even short term fasting. Therefore, the goal of the current research is to determine if reproductive performance of broiler breeder hens can be improved by splitting the feed allotment offered each d during the entire breeding period into two feeding periods separated by 8.5 h instead of offering the feed allotment once a d as is typically done in a commercial setting.

MATERIALS AND METHODS

At one d of age 1,300 Cobb 500 slow-feathering pullets were randomly divided among 4 rooms and 500 Cobb cockerels were divided among 2 rooms. The rooms measured 7.32 m x 9.14 m, and had pine shavings for litter. The rooms were environmentally controlled, with a temperature maintained at 32.2° C for the first wk, and then the temperature was decreased by about 2.8° C every wk thereafter until a target temperature of 21° C was reached. From 1-3 d of age the chicks were given 24 h of light per d and then from 4-14 d of age the amount of light was decreased from 24 to 8 h per d. The 8 h per d lighting schedule was then maintained until the birds reached 21 wk of age. All birds were fed a standard corn/soy diet (Table 1) *ad libitum* from 0-2 wk of age, and then fed a developer diet (Table 1) from 2-23 wk of age. From 2 to 21 wk of age the birds were feed restricted and fed on a skip a day (every other day) basis. Feed was distributed by automatic chain feeders, and the birds were given *ad libitum* access to water

from nipple drinkers. A random selection of 10% of the birds from each room was weighed every wk to adjust feed allocations so that BWG of the pullets and cockerels matched the recommended guidelines of the primary breeder. All pullets were wing-banded for identification purposes. All animal procedures were approved by the Animal Care and Use Committee of the University of Georgia.

At 20 wk of age all the pullets were weighed. Pullets were selected and assigned to 30 laying pens to assure the weight profile in each pen was similar. Each laying pen contained 35 pullets and 4 roosters. Each pen measured 3.65 m x 2.75 m and consisted of 2/3 litter and 1/3 elevated slats, and had one six-hole nest box located in the slat area. Each pen contained 3 hen feeder pans which were fitted with rooster exclusion grills. The feeding system provided 9.14 cm of feeder space per hen. Males were given their own feeder pan which was elevated in height to prevent females from consuming their feed. The male to female ratio was kept between 10-11% throughout the experiment by replacing male mortality from a pool of extra males. Per standard industry practice, when the hens were 50 wk of age 26 wk old males from another flock were added to the pens to stimulate mating and assure continued fertility. Subsequent male mortality replacements came from this younger male flock.

Photostimulation occurred at 21 wk of age by providing 14 h of light, and this length of light was continued each d through 60 wk of age. At 21 wk of age the birds in half of the 30 pens were switched from the skip a day feeding to 1x feeding, while the remaining pullets in the other 15 pens were switched to 2x feeding. The birds for the 1x feeding treatment received all of their feed every day at 0630 h, while the birds for the 2x treatment received 60% of their total daily feed allotment at 0630 h and 40% at 1500 h. The birds in both treatments received the same total amount of daily feed. Roosters were fed their daily allotment of feed at 0630 h. At 23

wk of age the birds were switched to a breeder layer diet (Table 1). Before the layer diet was pelleted, 100 g of rice hulls were added per kg of diet to increase feed volume to ensure all the birds fed 2x had access to feed at the 1500 h feeding. The rice hulls were considered not to add any significant nutrient value to the diet for purposes of calculating daily nutrient intake.

All mortality was recorded and necropsies were performed on all hen mortality from 23-60 wk of age. Birds were weighed weekly before the morning feeding from 21-40 wk of age and every other wk from 42-60 wk of age. A rotating sample of 5 of the 15 pens per treatment was weighed during each weigh period which allowed for individual pens to be weighed every 3 weigh periods. All birds were weighed at 21, 32, 40, 52, and 59 wk to accurately determine whole flock body weight uniformity. Eggs were manually collected 3-4 times per d. Hen housed and hen day egg production were calculated weekly from daily egg counts and the number of hatchable, abnormal, cracked, double yolked, dirty, and total eggs was recorded daily for each pen.

Ninety hatching eggs (eggs not abnormal in shape, cracked, double yolked, dirty, or surrounded by only shell membranes and no shell) from each pen were collected and stored at 18.3-19.9º C for no more than 7 d every other week when the hens were between 26 and 41 wk of age and then every 4 wk thereafter. Eggs were incubated at 37.8º C with 53% relative humidity d 0-18, and then at 37.2º C with 70% relative humidity d 19-21. Eggs were candled on d 12 of incubation and transferred for hatching on d 19. During candling, transfer, and after hatching, eggs were characterized as being infertile, cracked, contaminated, containing early dead embryos (less than 7 d), mid dead embryos (7-14 d), or late dead embryos (15-21 d). Eggs cracked during transfer to the hatcher were removed from the data set as lost eggs. After hatching the number of live and dead pips and live and dead chicks were determined.

Beginning when the birds were 26 wk of age, all hatching eggs from two d of production were weighed every other week. Specific gravities were determined using the saline flotation method (Phillips and Williams, 1943) on 20 eggs per pen from one production day every other week when the hens were 30-40 wk of age, and then once every 4 wk thereafter. Also, when the hens were 46 wk of age, all hatching eggs produced by the hens of each pen over a 2 d period were individually weighed and then the weight of the individual components (shell, albumen, and yolk) of each egg were determined.

Statistical Analyses

Data were subjected to ANOVA according to the General Linear Model procedure in order to detect significant differences between the 1x and 2x feeding treatments. All statistical procedures were done with the Minitab Statistical Software package (Release 13, State College, PA). Differences were considered significant when *P*-values were < 0.05.

RESULTS

Weekly differences in BW between the birds fed 1x and 2x were not significantly different during most of the experiment (Table 2). For the few weeks in which the differences in BW were significant, body weights were higher in hens fed 2x than the hens fed 1x during the first half of the experimental period. However, for the weeks in which the differences in BW were significant during the second half of the experimental period, it was the hens fed 2x that weighed more than the hens fed 1x. For the entire experimental period the difference in BW was only 13 g (mean ± SEM values were 3,555 ± 57 and 3,568 ± 59 g/hen for the birds fed 1x and

2x, respectively), but this small difference was statistically significant ($P = 0.016$). After wk 22 of age the weekly CV of BW was significantly lower for the hens fed 2x versus those fed 1x in wks 27, 29, 30, 32, 33, 38, and 40 (Table 2). The weekly trend in the CV for BW resulted in a highly significant ($P = 0.0001$) overall improvement in BW uniformity for the hens fed 2x versus 1x. The mean ± SEM CV for BW for the entire experimental period was 9.22 ± 0.08 and 8.49 ± 0.09 for the birds fed 1x and 2x, respectively.

The hens fed 1x and 2x began producing eggs during wk 23 of age, reached 25% egg production during wk 25 of age and reached 50% production during wk 26 of age (Table 3). For the hens fed 2x, hen day egg production peaked at 78.64% at 29 wk of age while the hens fed 1x peaked at 74.84% at wk 30 of age. Hen day egg production was generally significantly higher in the hens fed 2x compared to the hens fed 1x in the first half of the experimental period (Table 3); however, this trend did not continue during the last half of the experimental period. Nonetheless, the overall percent hen day egg production was significantly greater (58 versus 56 percent) for the hens fed 2x compared to the hens fed 1x. Through 41 wk of age total hen day egg production was also significantly ($P < 0.01$) higher (83 versus 78 eggs/bird) for the hens fed 2x compared to those fed 1x. At the end of the experiment the hens fed 2x produced 149 eggs/hen compared to 145 eggs/hen for the hens fed 1x. This difference was not significant.

Cumulative mortality at the end of the experiment was significantly higher in the birds fed 2x compared to those fed 1x (Table 4). Necropsy records indicated that 75% of the hens that died in the 1x treatment group were either out of lay (regressed ovary) or going out of lay (multiple regressing hierarchical follicles). In contrast, of the total number of hens that died in the 2x treatment, 63% of them were in active lay (normal ovarian hierarchy and/or egg in oviduct) while 37% were out of lay or going out of lay. Given the higher mortality of the birds in

the 2x treatment, it is not surprising that hen housed egg production was sometimes significantly higher for the hens fed 1x versus 2x for some of the individual wk of production during the last half of the experiment (Table 3). However, since hen housed egg production was significantly higher for some of the wk in the first part of the experimental period for the hens fed 2x, total hen housed egg production was not significantly different through 41 wk of age (78 versus 79 eggs per hen on the 1x and 2x treatments, respectively) or through 59 wk of age (140 versus 137 eggs per hen on the 1x and 2x treatments, respectively).

The percent of hatching eggs out of the total number of eggs produced each wk by the hens fed 2x was at times significantly greater than the percent of hatching eggs produced by the hens fed 1x (Table 5). For the entire production period, the hens fed 2x produced a significantly ($P < 0.001$) greater (92.45 ± 0.28 versus 91.13 ± 0.31) percentage of hatching eggs of total eggs produced than the hens fed 1x. The increased production of hatching eggs by the hens fed 2x compared to those fed 1x was due to the difference in the production of dirty eggs by the hens in the two feeding treatments. Typically, each wk the hens fed 1x tended to produce more dirty eggs than the hens fed 2x (Table 5). For the entire egg production period of the experiment, the hens fed 1x had a significantly ($P < 0.001$) greater (5.07 ± 0.21 versus 3.94 ± 0.24) percentage of dirty eggs of the total eggs produced than the hens fed 2x. The weekly proportion of cracked eggs, membrane eggs, and double yolked eggs produced by the hens in both feeding treatments were generally the same (Table 6), and for the entire production period there were no differences in these egg types between the two feeding treatments (data not shown).

Biweekly egg weights were significantly greater for the hens fed 2x compared to those fed 1x except on wk 28, 56 and 58 (Table 7). For the entire experimental period the mean ± SEM egg weight was 67.87 ± 0.33 and 68.84 ± 0.34 g for the hens fed 1x and 2x, respectively.

This overall difference in egg weight between the two treatments was significant ($P \leq 0.001$). Eggs produced by the hens from the two feeding treatments when they were 46 wk of age did not differ significantly in their percentage of albumen, yolk and shell (Table 9). The specific gravities of the eggs produced by the hens from the two feeding treatments also did not differ significantly (Table 8).

Fertility and hatchability of eggs produced by the hens fed either 1x or 2x were not significantly different (Table 10). The cumulative difference in hatchability and fertility between the two feeding treatments was also not significantly different (data not shown). The only significant difference in the hatchability of fertile eggs between the two treatments occurred at wk 60 (Table 10), but the overall hatchability of fertile eggs did not differ between the two treatments (data not shown). The incidence of early dead, mid dead and late dead embryos as well as the incidence of live and dead pips in the eggs incubated during the experiment, was also similar for the two feeding treatments with only two exceptions (Table 11).

DISCUSSION

The present study indicates that feeding broiler breeder hens 2x rather than 1x improves egg production from the initiation of lay through peak egg production. Cave (1981) reported that feeding broiler breeder hens 3 times a day increased the percent hen day egg production in the first 10 wk period of the production cycle when compared to hens fed once or twice a day. Hens fed once, twice, or 3 times a day did not vary in percent hen day egg production. Similarly, de Avila *et al*. (2003a, b) did not observe a difference in total egg production through 66 wk of age between broiler breeder hens fed 1x and 2x. De Avila *et al*. (2003a) did report that the time to

5% egg production was shorter for the flock fed 2x, but the age at first egg and the age to reach 50% production did not vary between the hens fed 1x and 2x (de Avila *et al.*, 2003b).

An even greater difference in overall egg production between the hens fed 1x and 2x in the present research may have been achieved if mortality had not been different between the two treatments. Mortality was high for both treatments based on breeder guidelines (Cobb, 2005), but comparable to the 17.1 % mortality reported for commercial practice in the US during the breeding period (Agristats, 2007) and actually lower than what has been experienced in previous experiments with slow feathering breeder strains (Hudson *et al*., 2004). However, by the end of the experiment the birds fed 2x had experienced a significantly higher level mortality than those fed 1x. More importantly 67% of the hens that died in the 2x treatment were still producing eggs prior to their deaths compared to only 25% of the hens still producing eggs in the 1x feeding treatment. Possibly the resources needed to produce more eggs when the hens were fed 2x resulted in the greater loss of egg producing hens in the 2x treatment, but the loss of these hens was very detrimental to overall egg production. Further research is needed to determine if feeding hens 2x is detrimental to livability or if this finding was a peculiarity to the specific strain of hens utilized for this experiment. Cave (1981) and de Avila (2003b) using different strains of broiler breeder hens than were used in the present research did not report an increase in mortality when feeding broiler breeder hens 2x.

The decision to split the daily feed allotment into a 60:40 ratio was based on assuring there was a sufficient volume of feed at both feeding times to minimize competition amongst the birds. Furthermore since the start of the experiment was occurring in the summer, a slightly lower proportion of the feed was provided for the afternoon feeding (1500 h) than the morning feeding (0630 h) in order to have slightly less heat generated in the hottest part of the day

associated with increased feeding and metabolic activity. For their research de Avila *et al*. (2003a, b) split the daily feed allotment equally and fed half of the feed allotment at 0630 h and the other half at 1530 h. Cave (1981) took a different approach and fed 34 percent of the daily feed allotment at 0500 h and the remainder of the feed allotment was fed 4 h before the lights went out. Surprisingly, despite concern in the present experiment that flock body weight uniformity might be difficult to control by splitting the daily feed allotment into 2 feeding periods compared to one feeding, the birds fed 2x actually had better body weight uniformity than the birds fed 1x. Neither Cave (1981) nor de Avila *et al*. (2003a, b) reported findings on body weight uniformity.

The increase in egg size of about one g for hens fed 2x compared to 1x observed in the current research is consistent with the earlier report by Cave (1981). The increase in egg size may be related to providing feed later in the day rather than feeding 2x. Broiler breeder hens provided feed 1x in the afternoon produce larger eggs than those provided feed 1x in the morning (Farmer *et al.,* 1983; Brake and Peebles, 1986). In the current research, the increase in egg size resulted from an increase in all the major individual components of the egg. Egg shell quality (based on specific gravity measurements) was not compromised in the larger sized eggs produced by the hens fed 2x.

Feeding the hens 2x significantly reduced the production of dirty eggs. Possibly the increased physical activity associated with receiving feed 2x promoted the production of less floor eggs which are also typically more prone to becoming dirty. Both the female feeder pans and nest boxes were located in the slat area of our pens and thus the hens on the 2x feeding treatment may have spent more time on the slats than the hens on the 1x feeding treatment. Providing feed in the afternoon did not appear to interfere with mating behavior since egg

fertility was not different between the hens fed 1x and 2x. Finally, the proportion of total eggs collected before and after 1500 h was equivalent for both treatments (data not shown), so the additional afternoon feeding did not shift the timing of oviposition.

In summary, feeding broiler breeder hens 2x improved the total number of eggs produced per hen through 42 wk of age and the overall percent hen day egg production through 59 wk of age. However, these gains in egg production were lost on a hen housed basis due to the significantly higher level of mortality associated with feeding broiler breeder hens 2x after photostimulation for reproduction through 59 wks of age. Feeding broiler breeder hens twice a day increased egg weight without compromising shell quality, decreased the production of dirty eggs, and had no effect on fertility. Further research with different strains of broiler breeder hens is needed before a definitive recommendation on the utility of feeding broiler breeder hens 2x can be made.

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TABLE 1. Composition of the experimental diets.

Ingredient	Diet		
	Starter[1]	Developer[2]	Layer[3]
	----------% of diet----------		
Corn	62.95	65.88	70.80
Soybean meal, 48% CP	22.24	15.00	18.11
Poultry Fat	0.00	0.00	0.91
Wheat middlings	10.53	14.83	0.00
Limestone	1.16	1.28	7.33
Dicalcium phosphate	1.75	1.57	1.49
Sodium chloride	0.54	0.60	0.51
Vitamin premix[4]	0.50	0.50	0.50
DL-Methionine	0.15	0.13	0.15
L-Lysine HCl	0.10	0.13	0.12
Trace mineral premix[5]	0.08	0.08	0.08
Calculated analysis[6]			
M. E. (kcal/kg)	2,865.00	2,920.00	2,920.00
Crude protein (%)	18.00	15.00	15.00
Lysine (%)	1.00	0.83	0.83
Calcium (%)	0.91	0.92	3.22
Methionine and cystine (%)	0.73	0.64	0.64
Available phosphorus (%)	0.45	0.42	0.38

[1]Starter diet was fed from 0 to 2 wk of age

[2]Developer diet was fed from 2 to 23 wk of age

[3]Layer diet was fed from 23 to 60 wk of age. To add more bulk to the layer diet 100 g of rice hulls were added per kg of layer diet before pelleting.

[4]Vitamin premix provided the following per kg of diet: vitamin A, 5,510 IU; vitamin D_3, 1,100 IU; vitamin E, 11 IU; vitamin B_{12}, 0.01 mg; riboflavin, 4.4 mg; niacin, 44.1 mg; d-panthotenic acid, 11.2 mg; choline, 191.3 mg; menadione sodium bisulfate, 3.3 mg; folic acid, 5.5 mg; pyridoxine HCl, 4.7 mg; thiamin, 2.2 mg; d-biotin, 0.11 mg; and ethoxyquin, 125 mg.

[5]Trace mineral premix provided the followng in mg per kg of diet: Mn, 60; Zn, 50; Fe, 30; I, 1.5; and Se 0.5.

[6]Calculated analyis was based on Dale (2001).

TABLE 2. Body weight (BW) and coefficient of variation (CV) of BW of broiler breeder hens fed either once a day or twice a day after photostimulation for reproduction[1].

		Feeding schedule			
		Once a day		Twice a day	
Age (week)	n	BW[2] (g)	CV[2]	BW[2] (g)	CV[2]
20	15	2,111 ± 0	9.44 ± 0.02	2,111 ± 0	9.45 ± 0.02
21	15	2,157 ± 3	9.78 ± 1.17	2,144 ± 3*	9.80 ± 0.16
22	5	2,260 ± 5	9.31 ± 0.32	2,264 ± 7	9.04 ± 0.15
23	5	2,400 ± 7	8.97 ± 0.18	2,403 ± 7	8.82 ± 0.27
24	5	2,585 ± 8	9.63 ± 0.55	2,585 ± 16	9.05 ± 0.38
25	5	2,712 ± 11	9.09 ± 0.48	2,720 ± 4	9.56 ± 0.37
26	5	2,920 ± 15	8.31 ± 0.38	2,944 ± 21	8.03 ± 0.41
27	5	3,070 ± 16	8.55 ± 0.16	3,092 ± 8	7.16 ± 0.48*
28	5	3,137 ± 9	8.52 ± 0.62	3,118 ± 11	8.19 ± 0.51
29	5	3,251 ± 12	8.48 ± 0.42	3,263 ± 18	7.16 ± 0.35*
30	5	3,418 ± 38	8.52 ± 0.57	3,383 ± 14	6.86 ± 0.33*
31	5	3,504 ± 7	8.54 ± 0.60	3,449 ± 21*	7.68 ± 0.61
32	15	3,586 ± 12	8.33 ± 0.31	3,585 ± 13	7.33 ± 0.23*
33	5	3,649 ± 15	8.62 ± 0.57	3,661 ± 19	6.79 ± 0.30*
34	5	3,692 ± 24	8.73 ± 0.46	3,668 ± 19	7.93 ± 0.51
35	5	3,769 ± 20	8.10 ± 0.31	3,744 ± 13	7.93 ± 0.51
36	5	3,825 ± 21	8.61 ± 0.40	3,822 ± 15	7.78 ± 0.36

37	5	3,885 ± 30	8.88 ± 0.59	3,877 ± 25	7.36 ± 0.41
38	5	3,932 ± 13	10.19 ± 0.71	3,862 ± 5*	8.00 ± 0.37*
39	5	3,966 ± 24	8.79 ± 0.54	3,966 ± 9	8.02 ± 0.51
40	15	4,003 ± 15	9.20 ± 0.37	3,962 ± 11*	7.95 ± 0.27*
42	5	4,076 ± 27	9.65 ± 0.92	4,088 ± 20	7.93 ± 0.88
44	5	4,117 ± 31	10.05 ± 0.05	4,082 ± 16	8.60 ± 0.46
46	5	4,200 ± 20	9.16 ± 0.49	4,192 ± 22	8.99 ± 0.36
48	5	4,299 ± 19	9.06 ± 0.34	4,341 ± 15	8.80 ± 0.78
50	5	4,303 ± 36	9.73 ± 0.56	4,311 ± 28	8.65 ± 0.38
52	15	4,429 ± 16	9.25 ± 0.25	4,501 ± 18*	8.73 ± 0.29
54	4	4,501 ± 29	9.07 ± 0.27	4,546 ± 23	8.49 ± 0.62
56	4	4,472 ± 65	11.09 ± 0.69	4,535 ± 45	9.73 ± 0.20
58	4	4,637 ± 24	10.42 ± 0.43	4,776 ± 33*	9.51 ± 0.48
60	15	4,676 ± 24	10.06 ± 0.35	4,822 ± 25*	9.29 ± 0.36

*Significantly different from the corresponding value for the once a day fed hens, ($P \leq 0.05$).

[1]Pullets were moved to laying pens at 20 wk of age and photostimulated for reproduction at 21 wk of age.

[2]Values are means ± SEM, n = 4, 5, or 15 replicate pens of 35 hens each for both feeding treatments. BW values are calculated on a per bird basis. Due to the significant labor involved in weighing all the birds, only one-third of the replicate pens were weighed on a rotating basis for most weeks.

TABLE 3. Hen day egg production (HDEP) and hen housed egg production (HHEP) through 59 weeks of age for broiler breeder hens fed either once a day or twice a day after photostimulation for reproduction.

	Feeding schedule			
	Once a day		Twice a day	
Age	HDEP[1]	HHEP[1]	HDEP[1]	HHEP[1]
week	----------%----------			
23	0.25 ± 0.12	0.25 ± 0.12	0.57 ± 0.16	0.57 ± 0.16
24	3.73 ± 0.50	3.73 ± 0.50	6.32 ± 0.54*	6.29 ± 0.55*
25	22.51 ± 1.03	22.45 ± 1.02	29.40 ± 1.48*	29.14 ± 1.51*
26	49.42 ± 1.20	49.22 ± 1.24	57.65 ± 1.25*	56.82 ± 1.17*
27	66.06 ± 0.99	65.69 ± 1.02	70.59 ± 0.78*	69.25 ± 0.81*
28	70.79 ± 1.21	70.37 ± 1.30	75.43 ± 1.11*	73.69 ± 1.24
29	73.22 ± 1.18	72.52 ± 1.26	78.64 ± 1.17*	76.38 ± 1.35*
30	74.84 ± 1.17	73.82 ± 1.30	77.33 ± 1.00	74.18 ± 1.13
31	73.42 ± 1.34	72.00 ± 1.44	75.86 ± 1.21	72.54 ± 1.36
32	71.84 ± 0.98	70.37 ± 1.22	75.68 ± 1.02*	72.00 ± 1.03
33	72.53 ± 1.11	70.78 ± 1.31	75.70 ± 1.10*	71.73 ± 1.27
34	71.57 ± 1.28	69.28 ± 1.50	74.51 ± 0.98	70.23 ± 1.10
35	69.27 ± 0.92	66.83 ± 1.07	72.32 ± 0.89*	68.00 ± 0.85
36	69.83 ± 1.14	67.32 ± 1.27	71.80 ± 1.26	67.35 ± 1.04
37	66.44 ± 0.91	63.95 ± 1.05	69.54 ± 1.30	64.90 ± 1.07
38	66.53 ± 0.85	64.00 ± 1.04	67.64 ± 1.10	62.94 ± 0.97

39	66.05 ± 0.83	63.29 ± 1.11	68.80 ± 1.04*	63.73 ± 0.90
40	65.45 ± 0.99	62.59 ± 1.12	66.40 ± 1.01	61.14 ± 1.00
41	64.45 ± 1.04	61.66 ± 1.28	67.78 ± 0.98*	62.01 ± 1.18
42	64.26 ± 1.26	61.16 ± 1.38	62.79 ± 1.21	57.28 ± 1.18*
43	61.33 ± 0.99	58.20 ± 1.16	62.12 ± 1.43	56.44 ± 1.20
44	62.51 ± 1.13	59.21 ± 1.33	61.30 ± 1.25	55.76 ± 1.23
45	60.58 ± 1.15	57.25 ± 1.23	59.32 ± 1.31	53.93 + 1.18
46	57.78 ± 1.28	54.53 ± 1.42	58.44 ± 1.22	52.68 ± 1.18
47	56.07 ± 1.18	52.73 ± 1.25	56.06 ± 1.64	50.12 ± 1.31
48	56.44 ± 1.67	52.60 ± 1.64	55.31 ± 0.80	49.06 ± 0.63*
49	55.62 ± 1.46	51.59 ± 1.51	55.39 ± 1.34	47.16 ± 1.20*
50	54.76 ± 1.04	50.67 ± 1.21	52.82 ± 1.01	46.40 ± 0.68*
51	52.08 ± 1.28	47.95 ± 1.36	51.10 ± 1.27	44.68 ± 0.83*
52	51.54 ± 1.22	47.32 ± 1.23	50.75 ± 1.20	44.27 ± 0.76*
53	50.68 ± 1.31	46.34 ± 1.35	48.85 ± 1.16	42.42 ± 0.88*
54	48.73 ± 1.23	44.41 ± 1.33	48.34 ± 0.99	41.85 ± 0.97
55	46.87 ± 1.30	42.50 ± 1.38	46.67 ± 1.35	40.08 ± 1.05
56	47.73 ± 1.45	43.02 ± 1.39	45.56 ± 1.43	38.97 ± 0.88*
57	44.66 ± 1.64	39.92 ± 1.55	45.91 ± 1.24	39.07 ± 1.41
58	44.35 ± 1.66	39.07 ± 1.41	43.49 ± 1.30	36.63 ± 1.30
59	42.77 ± 1.68	37.55 ± 1.41	44.16 ± 1.90	36.65 ± 1.50

*Significantly different from the corresponding value for the once a day fed hens, ($P \leq 0.05$).

[1]Values are means ± SEM, n = 15 replicate pens of 35 hens for both feeding schedules. HDEP equals the percent of hens in lay corrected for mortality while HHEP equals the percent of hens in lay based on the original number of hens placed in each replicate pen.

TABLE 4. Cumulative mortality of broiler breeder hens from 23 through 59 weeks of age fed either once a day or twice a day after photostimulation at 21 weeks of age[1].

	Feeding Schedule	
Age	Once a day	Twice a day
Week	----------%----------	
23	0.00 ± 0.00	0.20 ± 0.20
24	0.19 ± 0.19	0.77 ± 0.34
25	0.38 ± 0.26	0.77 ± 0.34
26	0.57 ± 0.31	1.53 ± 0.55
27	0.57 ± 0.31	2.10 ± 0.71
28	0.95 ± 0.46	2.29 ± 0.70
29	1.14 ± 0.47	3.44 ± 0.85*
30	1.52 ± 0.47	4.01 ± 0.88*
31	2.10 ± 0.65	4.39 ± 0.78*
32	2.10 ± 0.65	4.96 ± 0.90*
33	3.05 ± 0.65	5.54 ± 0.94*
34	3.43 ± 0.75	5.73 ± 0.93
35	3.62 ± 0.76	5.73 + 0.93
36	3.62 ± 0.76	6.11 ± 0.83*
37	3.81 ± 0.87	6.68 ± 0.91*
38	4.19 ± 0.88	6.87 ± 0.87*
39	4.38 ± 0.88	7.25 ± 0.88*
40	4.38 ± 0.88	8.02 ± 0.98*

41	4.76 ± 0.77	8.40 ± 1.06*
42	4.95 ± 0.76	8.78 ± 1.23*
43	5.33 ± 0.73	8.78 ± 1.23*
44	5.52 ± 0.76	8.78 ± 1.23*
45	5.52 ± 0.76	8.97 ± 1.25*
46	5.91 ± 0.90	9.93 ± 1.50*
47	6.48 ± 0.81	10.50 ± 1.62*
48	7.24 ± 0.88	11.26 ± 1.56*
49	7.43 ± 0.87	11.45 ± 1.63*
50	8.00 ± 0.85	12.03 ± 1.59*
51	8.00 ± 0.85	12.03 ± 1.59*
52	8.38 ± 0.94	12.61 ± 1.60*
53	8.95 ± 1.00	12.80 ± 1.61*
54	9.14 ± 1.09	13.56 ± 1.64*
55	9.71 ± 1.14	13.75 ± 1.71
56	10.48 ± 1.24	13.94 ± 1.65
57	10.86 ± 1.16	14.32 ± 1.83
58	12.00 ± 1.09	16.04 ± 1.87
59	12.00 ± 1.09	17.56 ± 1.95*

* Significantly different from the corresponding value for the once a day fed hens, ($P \leq 0.05$).

[1]Prior to week 23, pullets that died were replaced with spare pullets to maintain all breeder pens at 35 pullets per pen. Values are means ± SEM, n = 15 replicate pens of 35 hens for each feeding schedule.

TABLE 5. Percent hatching and dirty egg production per week through 59 weeks of age for broiler breeder hens fed either once a day or twice a day after photostimulation for reproduction.

	Feeding schedule			
	Once a day		Twice a day	
Age	Hatching[1]	Dirty[1]	Hatching[1]	Dirty[1]
week	----------%----------			
23	66.70 ± 23.60	25.0 ± 16.00	80.60 ± 11.60	13.90 ± 11.10
24	74.19 ± 5.35	10.95 ± 3.92	78.89 ± 3.64	13.45 ± 3.09
25	78.20 ± 1.98	14.58 ± 1.69	81.91 ± 1.56	11.18 ± 1.50
26	86.90 ± 0.81	4.86 ± 0.72	88.54 ± 0.89	3.79 ± 0.43
27	88.92 ± 0.88	5.61 ± 0.88	90.13 ± 0.65	3.44 ± 0.54*
28	91.53 ± 0.64	4.47 ± 0.48	92.31 ± 0.70	2.45 ± 0.40*
29	89.62 ± 0.90	5.62 ± 0.86	91.78 ± 0.77	3.24 ± 0.50*
30	90.52 ± 0.62	4.34 ± 0.46	91.85 ± 0.58	3.06 ± 0.36*
31	91.30 ± 0.81	4.79 ± 0.46	93.57 ± 0.70*	2.87 ± 0.45*
32	92.50 ± 0.73	3.67 ± 0.34	93.68 ± 0.60	2.86 ± 0.36
33	92.93 ± 0.66	3.45 ± 0.53	94.37 ± 0.40	2.51 ± 0.23
34	91.32 ± 0.74	5.05 ± 0.49	93.46 ± 0.70*	3.32 ± 0.52*
35	92.95 ± 0.79	3.80 ± 0.48	94.36 ± 0.44	2.69 + 0.33
36	91.67 ± 0.91	4.36 ± 0.71	93.47 ± 0.35	3.30 ± 0.33
37	93.80 ± 0.54	3.20 ± 0.48	93.37 ± 0.83	3.56 ± 0.58
38	92.75 ± 0.56	4.00 ± 0.38	95.26 ± 0.48*	2.23 ± 0.39*

39	93.47 ± 0.42	3.82 ± 0.40	94.92 ± 0.43	2.36 + 0.33*
40	94.27 ± 0.99	1.85 ± 0.42	95.38 ± 0.41	1.82 + 0.38
41	95.12 ± 0.60	3.00 ± 0.42	95.80 ± 0.40	1.97 ± 0.34
42	94.68 ± 0.56	2.64 ± 0.35	95.75 ± 0.38	2.20 ± 0.28
43	93.48 ± 0.49	4.09 ± 0.47	94.61 ± 0.62	3.02 ± 0.46
44	92.65 ± 0.55	4.20 ± 0.50	93.04 ± 0.60	4.25 ± 0.46
45	93.29 ± 0.35	4.82 ± 0.33	94.17 ± 0.62	3.71 ± 0.51
46	93.18 ± 0.78	4.19 ± 0.55	95.17 ± 0.81	3.30 ± 0.66
47	92.40 ± 0.50	5.15 ± 0.36	92.96 ± 0.59	4.15 ± 0.41
48	90.15 ± 0.84	7.52 ± 0.63	91.84 ± 0.95	5.12 ± 0.62*
49	92.04 + 0.56	4.63 ± 0.55	93.28 ± 0.81	3.72 ± 0.64
50	90.37 ± 1.07	6.23 ± 0.60	92.45 ± 0.71	4.97 ± 0.50
51	94.40 ± 0.91	3.54 ± 0.63	94.53 ± 0.55	2.83 ± 0.35
52	92.47 ± 0.68	5.24 ± 0.70	93.35 ± 0.99	3.10 ± 0.42*
53	94.23 ± 0.66	3.13 ± 0.57	96.20 ± 0.78	1.98 ± 0.52
54	92.53 ± 0.78	5.08 ± 0.57	93.94 ± 0.87	2.73 ± 0.60
55	92.39 ± 0.63	4.00 ± 0.58	93.67 ± 0.88	3.23 ± 0.49
56	87.93 ± 0.94	7.65 ± 0.94	89.60 ± 1.37	5.76 ± 0.91
57	92.63 ± 0.86	4.82 ± 0.72	91.75 ± 0.85	4.20 ± 0.46
58	92.13 ± 0.80	4.37 ± 0.60	93.45 ± 0.60	4.10 ± 0.62
59	93.26 ± 0.70	4.75 ± 0.69	93.96 ± 0.66	3.43 ± 0.52

*Significantly different from the corresponding value for the once a day fed hens, ($P \leq 0.05$).

[1]Values are means ± SEM, n = 15 replicate pens of 35 hens for each feeding schedule. Values are the percent of hatching and dirty eggs out of the total number of eggs produced per pen each week. Hatching eggs exclude eggs that were abnormal in shape, cracked, double yolked, dirty, or were surrounded by only shell membranes and no shell. Dirty eggs were contaminated with feces and/or blood.

TABLE 6. Abnormal (A), membrane (M), double-yolked (DY), and cracked (C) egg production through 59 weeks of age for broiler breeder hens fed either once a day or twice a day after photostimulation for reproduction.

	Feeding schedule							
	Once a day				Twice a day			
Age	A[1]	M[1]	DY[1]	C[1]	A[1]	M[1]	DY[1]	C[1]
Week	----------%----------							
23	0.00 ± 0.00	0.00 ± 0.00	0.00 + 0.00	8.33 ± 8.33	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	5.56 ± 3.67
24	0.00 ± 0.00	0.83 ± 0.83	6.74 ± 2.45	7.28 ± 2.54	0.48 ± 0.48	0.00 ± 0.00	1.70 ± 1.00	5.49 ± 1.78
25	1.07 ± 0.50	0.00 ± 0.00	1.66 ± 0.35	4.49 ± 0.74	1.09 ± 0.35	0.08 ± 0.08	2.61 ± 0.69	3.13 ± 0.46
26	2.07 ± 0.38	0.07 ± 0.07	2.62 ± 0.42	3.49 ± 0.44	1.95 ± 0.37	0.10 ± 0.07	2.56 ± 0.50	3.06 ± 0.48
27	1.49 ± 0.29	0.04 ± 0.04	2.42 ± 0.26	1.52 ± 0.28	1.93 ± 0.35	0.16 ± 0.07	2.68 ± 0.37	1.65 ± 0.26
28	0.78 ± 0.15	0.23 ± 0.11	1.17 ± 0.21	1.83 ± 0.30	1.21 ± 0.20	0.08 ± 0.05	2.29 ± 0.27*	1.67 ± 0.34
29	1.04 ± 0.15	0.11 ± 0.08	1.29 ± 0.21	2.32 ± 0.28	1.29 ± 0.23	0.04 ± 0.04	1.73 ± 0.19	1.93 ± 0.20
30	1.66 ± 0.28	0.00 ± 0.00	1.34 ± 0.22	2.15 ± 0.34	2.13 ± 0.40	0.07 ± 0.05	1.25 ± 0.22	1.64 ± 0.20
31	0.39 ± 0.11	0.04 ± 0.04	1.28 ± 0.35	2.21 ± 0.30	0.94 ± 0.23*	0.00 ± 0.00	0.86 ± 0.25	1.76 ± 0.30
32	0.77 ± 0.14	0.08 ± 0.05	0.90 ± 0.23	2.09 ± 0.39	0.87 ± 0.24	0.00 ± 0.00	0.65 ± 0.15	1.94 ± 0.24

33	0.94 ± 0.21	0.04 ± 0.04	0.70 ± 0.15	1.93 ± 0.32	0.86 ± 0.15	0.04 ± 0.04	0.46 ± 0.13	1.75 ± 0.24
34	0.97 ± 0.21	0.13 ± 0.10	0.41 + 0.13	2.12 ± 0.26	0.94 ± 0.24	0.00 ± 0.00	0.47 ± 0.18	1.81 ± 0.26
35	0.73 ± 0.16	0.04 ± 0.04	0.33 ± 0.12	2.16 ± 0.54	0.84 ± 0.14	0.12 ± 0.12	0.21 ± 0.10	1.79 ± 0.19
36	0.96 ± 0.36	0.00 ± 0.00	0.54 ± 0.15	2.47 ± 0.40	0.97 ± 0.20	0.08 ± 0.08	0.49 ± 0.14	1.90 ± 0.26
37	0.85 ± 0.16	0.00 ± 0.00	0.42 ± 0.12	1.74 ± 0.30	0.59 ± 0.18	0.04 ± 0.04	0.30 ± 0.11	2.14 ± 0.27
38	0.38 ± 0.13	0.09 ± 0.06	0.40 ± 0.13	2.44 ± 0.46	0.34 ± 0.12	0.00 ± 0.00	0.26 ± 0.08	1.91 ± 0.29
39	0.43 ± 0.17	0.00 ± 0.00	0.09 ± 0.06	1.69 ± 0.20	0.90 ± 0.14*	0.04 ± 0.04	0.17 ± 0.09	1.61 ± 0.21
40	0.66 ± 0.15	0.00 ± 0.00	0.17 ± 0.08	3.04 ± 0.66	0.49 ± 0.10	0.09 ± 0.09	0.18 ± 0.08	2.04 ± 0.26
41	0.17 ± 0.07	0.00 ± 0.00	0.14 ± 0.07	1.61 ± 0.34	0.21 ± 0.10	0.09 ± 0.06	0.04 ± 0.04	1.56 ± 0.31
42	0.65 ± 0.19	0.00 ± 0.00	0.09 ± 0.06	1.93 ± 0.26	0.63 ± 0.19	0.00 ± 0.00	0.00 ± 0.00	1.42 ± 0.23
43	0.32 ± 0.12	0.00 ± 0.00	0.13 ± 0.07	1.88 ± 0.27	0.46 ± 0.19	0.05 ± 0.05	0.05 ± 0.05	1.80 ± 0.29
44	1.03 ± 0.23	0.00 ± 0.00	0.17 ± 0.13	1.96 ± 0.37	0.81 ± 0.19	0.05 ± 0.05	0.05 ± 0.05	1.08 ± 0.35
45	0.58 ± 0.18	0.00 ± 0.00	0.23 ± 0.11	1.07 ± 0.26	0.40 ± 0.14	0.05 ± 0.05	0.22 ± 0.10	1.45 ± 0.20
46	0.53 ± 0.18	0.00 ± 0.00	0.26 ± 0.13	1.85 ± 0.24	0.35 ± 0.12	0.00 ± 0.00	0.00 ± 0.00*	1.19 ± 0.50
47	0.50 ± 0.19	0.00 ± 0.00	0.10 ± 0.07	1.86 ± 0.29	0.65 ± 0.18	0.06 ± 0.06	0.00 ± 0.00	2.18 ± 0.40
48	0.68 ± 0.20	0.00 ± 0.00	0.11 ± 0.07	1.55 ± 0.30	0.72 ± 0.16	0.00 ± 0.00	0.06 ± 0.06	2.26 ± 0.48

49	0.37 ± 0.13	0.00 ± 0.00	0.10 ± 0.07	2.86 ± 0.37	0.70 ± 0.21	0.00 ± 0.00	0.05 ± 0.05	2.25 ± 0.40
50	1.16 ± 0.28	0.00 ± 0.00	0.05 ± 0.05	2.18 ± 0.53	0.41 ± 0.15*	0.05 ± 0.05	0.00 ± 0.00	2.13 ± 0.51
51	0.51 ± 0.18	0.00 ± 0.00	0.00 ± 0.00	1.55 ± 0.38	0.67 ± 0.17	0.00 ± 0.00	0.00 ± 0.00	2.00 ± 0.38
52	0.39 ± 0.20	0.00 ± 0.00	0.06 ± 0.06	1.84 ± 0.46	0.18 ± 0.10	0.00 ± 0.00	0.06 ± 0.06	3.30 ± 0.72
53	0.30 ± 0.17	0.00 ± 0.00	0.06 ± 0.06	1.28 ± 0.31	0.39 ± 0.19	0.00 ± 0.00	0.07 ± 0.07	1.36 ± 0.39
54	0.43 ± 0.17	0.06 ± 0.06	0.13 ± 0.09	1.77 ± 0.38	0.79 ± 0.17	0.00 ± 0.00	0.19 ± 0.10	2.35 ± 0.38
55	0.52 ± 0.16	0.00 ± 0.00	0.19 ± 0.10	2.94 ± 0.61	0.26 ± 0.17	0.00 ± 0.00	0.07 ± 0.07	2.78 ± 0.52
56	1.57 ± 0.31	0.00 ± 0.00	0.60 ± 0.26	2.26 ± 0.45	1.21 ± 0.42	0.00 ± 0.00	0..06 ± 0.06	3.36 ± 0.60
57	0.70 ± 0.20	0.00 ± 0.00	0.06 ± 0.06	1.78 ± 0.34	0.77 ± 0.26	0.00 ± 0.00	0.08 ± 0.08	3.20 ± 0.65
58	0.63 ± 0.23	0.00 ± 0.00	0.30 ± 0.13	2.51 ± 0.40	0.37 ± 0.18	0.07 ± 0.07	0.14 ± 0.10	2.07 ± 0.38
59	0.29 ± 0.18	0.00 ± 0.00	0.00 ± 0.00	1.71 ± 0.33	0.32 ± 0.21	0.00 ± 0.00	0.08 ± 0.08	2.22 ± 0.47

*Significantly different from the corresponding value for the once a day fed hens, ($P \leq 0.05$).

[1]Values are means ± SEM, n = 15 replicate pens for each feeding program. Values are the percent of abnormal, membrane, double-yolked or cracked eggs out of the total number of eggs produced per pen per wk. Abnormal eggs were misshaped eggs.

TABLE 7. Biweekly egg weights for eggs produced by broiler breeder hens fed either once a day or twice a day after photostimulation for reproduction.[1]

	Feeding schedule	
Age	Once a day	Twice a day
Week	----------g----------	
26	54.17 ± 0.29	54.98 ± 0.17*
28	58.20 ± 0.20	58.74 ± 0.20
30	61.22 ± 0.15	62.10 ± 0.20*
32	62.95 ± 0.19	63.91 ± 0.19*
34	64.98 ± 0.27	65.87 ± 0.21*
36	66.89 ± 0.21	67.50 ± 0.24
38	68.26 ± 0.20	69.09 ± 0.27*
40	68.91 ± 0.31	69.74 ± 0.27*
42	69.52 ± 0.19	70.49 ± 0.31*
44	70.46 ± 0.15	71.47 ± 0.22*
46	71.10 ± 0.19	72.48 ± 0.22*
48	71.23 ± 0.24	72.40 ± 0.29*
50	71.85 ± 0.21	73.11 ± 0.24*
52	72.10 ± 0.32	73.01 ± 0.24*
54	72.43 ± 0.27	73.51 ± 0.30*
56	72.99 ± 0.22	73.77 ± 0.35
58	72.72 ± 0.28	73.27 ± 0.39
60	75.56 ± 0.25	74.28 ± 0.30*

*Significantly different from the corresponding value for the once a day fed hens, ($P \leq 0.05$)

[1] Values are means ± SEM, n = 15 replicate pens of 35 hens each for both feeding treatments. All the hatching eggs produced over a 2 d period for every pen were weighed for a given age period.

TABLE 8. Percent of total egg weight for individual egg component in eggs produced by broiler breeder hens fed either once a day or twice a day after photostimulation for reproduction.[1]

	Feeding schedule			
	Once a day		Twice a day	
Egg component	n	-------%------	n	------%------
Albumen	520	55.29 ± 0.038	488	55.45 ± 0.040
Yolk	521	32.29 ± 0.128	493	32.22 ± 0.120
Shell	546	12.42 ± 0.102	508	12.33 ± 0.095

[1]Values are means ± SEM. All hatching eggs produced over a 2 d period were collected from each feeding treatment when the hens were 46 wk of age. Not all egg components have the same n due to the loss of some components during the separation and weighing process.

TABLE 9. Specific gravities of eggs produced by broiler breeder hens fed either once a day or twice a day after photostimulation for reproduction.[1]

	Feeding schedule	
Age (week)	Once a day	Twice a day
30	1.0874 ± 0.0003	1.0874 ± 0.0002
32	1.0886 ± 0.0003	1.0884 ± 0.0004
34	1.0824 ± 0.0004	1.0815 ± 0.0003
36	1.0854 ± 0.0004	1.0860 + 0.0003
38	1.0845 ± 0.0003	1.0844 + 0.0002
42	1.0829 ± 0.0003	1.0830 ± 0.0003
46	1.0828 ± 0.0003	1.0828 ± 0.0004
50	1.0841 ± 0.0003	1.0846 ± 0.0003
54	1.0820 ± 0.0003	1.0815 ± 0.0004
58	1.0830 ± 0.0005	1.0834 ± 0.0005

[1]Values are means ± SEM, n = 15 replicate pens of 35 hens each for both feeding treatments. Specific gravities were determined for all hatching eggs collected over a one day period for each pen at given age period.

TABLE 10. Fertility, hatchability, and hatchability of fertile eggs produced by broiler breeder hens fed either once a day or twice a day after photostimulation for reproduction.

	Feeding schedule					
	Once a day			Twice a day		
Age	Fertility[1]	Hatchability[1]	Hatch of fertile[1]	Fertility[1]	Hatchability[1]	Hatch of fertile[1]
Week	----------%----------					
26	94.44 ± 1.40	84.26 ± 1.95	88.86 ± 1.33	95.89 ± 0.90	84.88 ± 1.49	87.64 ± 1.16
28	97.69 ± 0.44	89.48 ± 0.86	91.13 ± 0.96	98.14 ± 0.26	89.14 ± 0.95	90.62 ± 0.92
30	97.09 ± 0.82	85.26 ± 0.90	87.45 ± 0.93	97.62 ± 0.64	87.62 ± 1.12	89.07 ± 0.99
32	97.09 ± 1.21	86.96 ± 1.32	89.22 ± 0.77	97.98 ± 0.55	87.31 ± 1.02	88.59 ± 1.06
34	95.15 ± 1.35	83.89 ± 1.86	87.61 ± 1.50	96.80 ± 0.72	87.79 ± 1.22	90.09 ± 0.77
36	91.15 ± 2.34	82.52 ± 2.79	89.78 ± 1.08	92.30 ± 1.88	83.29 ± 1.99	89.64 ± 0.83
39	88.70 ± 3.60	80.62 ± 3.68	89.79 ± 1.01	94.04 ± 1.71	85.00 ± 1.93	89.58 ± 1.07
41	89.28 ± 2.53	79.22 ± 2.51	89.09 + 1.02	93.69 ± 1.74	84.28 ± 1.51	90.04 ± 0.96
44	93.05 ± 1.79	83.26 ± 1.60	88.92 ± 1.09	92.18 ± 2.03	80.69 ± 2.26	87.30 ± 1.50
48	93.73 ± 0.99	80.86 ± 1.56	85.76 ± 1.21	92.34 ± 1.02	77.27 ± 1.82	83.62 ± 1.21

52	89.52 ± 2.20	87.94 ± 0.85	82.89 ± 1.42	90.50 ± 1.89	84.94 ± 1.11	83.24 ± 1.43
56	90.46 ± 2.08	75.71 ± 2.03	84.50 ± 1.46	90.04 ± 2.13	76.39 ± 2.31	82.80 ± 1.18
60	90.10 ± 2.55	78.66 ± 2.72	85.43 ± 1.11	90.58 ± 1.63	74.99 ± 1.91	81.91 ± 1.24*

*Significantly different from the corresponding value for the once a day fed hens, ($P \leq 0.05$)

[1] Values are means ± SEM, n = 15 replicate pens of 35 hens for each feeding schedule. Ninety eggs from each replicate pen were incubated for each age period.

TABLE 11. The incidence of early dead (ED), mid dead (MD) and late dead (LD) embryo mortality as well as the incidence of pips (P) in eggs incubated from broiler breeder hens fed either once a day or twice a day after photostimulation for reproduction.

	Feeding schedule							
	Once a day				Twice a day			
Age	ED[1]	MD[1]	LD[1]	P[1]	ED[1]	MD[1]	LD[1]	P[1]
Week	----------%----------							
26	6.00 ± 0.53	0.08 ± 0.08	2.83 ± 0.64	0.87 ± 0.31	7.02 ± 0.98	0.00 ± 0.00	2.87 ± 0.57	0.83 ± 0.32
28	4.48 ± 0.67	0.08 ± 0.08	2.84 ± 0.49	0.45 ± 0.15	5.13 ± 0.65	0.15 ± 0.10	2.31 + 0.49	0.89 ± 0.27
30	4.24 ± 0.58	0.07 ± 0.07	4.32 ± 0.46	1.93 ± 0.43	3.50 ± 0.54	0.00 ± 0.00	3.66 ± 0.70	1.72 ± 0.37
32	4.24 ± 0.57	0.00 ± 0.00	3.51 ± 0.62	1.72 ± 0.36	4.49 ± 0.63	0.00 ± 0.00	3.66 ± 0.54	1.79 ± 0.40
34	5.29 ± 0.86	0.00 ± 0.00	3.21 ± 0.33	1.79 ± 0.40	3.36 ± 0.47	0.00 ± 0.00	3.35 ± 0.56	1.78 ± 0.43
36	4.24 ± 0.58	0.00 ± 0.00	2.31 ± 0.44	1.19 ± 0.30	4.92 ± 0.53	0.00 ± 0.00	2.38 ± 0.55	0.97 ± 0.21
39	3.62 ± 0.59	0.00 ± 0.00	2.33 ± 0.39	0.75 ± 0.24	3.47 ± 0.44	0.00 ± 0.00	2.70 ± 0.47	1.29 ± 0.27
41	4.02 ± 0.61	0.00 ± 0.00	3.29 ± 0.57	0.74 ± 0.26	3.94 ± 0.73	0.00 ± 0.00	2.31 ± 0.48	1.64 ± 0.26*
44	3.51 ± 0.54	0.00 ± 0.00	3.59 + 0.62	1.12 ± 0.33	3.95 ± 0.57	0.00 ± 0.00	3.58 ± 0.76	2.98 ± 0.92

48	4.27 ± 0.72	0.00 ± 0.00	5.53 ± 0.72	1.72 ± 0.42	5.75 ± 0.62	0.00 ± 0.00	4.56 ± 0.78	1.12 ± 0.22
52	4.55 ± 0.60	0.00 ± 0.00	5.74 ± 0.80	2.60 ± 0.83	5.65 ± 0.58	0.00 ± 0.00	4.96 ± 0.67	1.92 ± 0.39
56	6.50 ± 0.81	0.15 ± 0.10	4.66 ± 0.51	1.91 ± 0.38	5.43 ± 0.70	0.00 ± 0.00	4.48 ± 0.56	1.95 ± 0.47
60	5.05 ± 0.58	0.00 ± 0.00	3.46 ± 0.59	1.28 ± 0.31	5.83 ± 0.83	0.08 ± 0.08	5.29 ± 0.50*	2.14 ± 0.36

*Significantly different from the corresponding value for the once a day fed hens, ($P \leq 0.05$).

[1]Values are means ± SEM, n = 15 replicate pens of 35 hens each for both feeding treatments. Ninety eggs from each replicate pen were incubated for each age period. Embryo mortality was classified as early dead (less than 7 d) mid dead (7-14 d) or late dead (15-21 d of incubation). Pips included both live and dead pips at the time of hatch.

5. CONCLUSIONS

Feed restriction in broiler breeder hens is necessary during both the rearing and laying periods due to their tendency for excessive weight gain and a resultant decrease in reproductive capabilities when they are fed *ad libitum*. Under typical commercial feed restriction guidelines the amount of feed that can be fed is so small that it necessitates feeding the hens on a skip a day feeding regimen during the rearing period. By combining two days worth of feed there is enough feed to distribute in the automatic feeders so all the birds have access to feed. Later when the birds reach reproductive maturity the amount of the daily feed allotment increases to support egg laying. The increased amount of feed allows the birds to be placed on an every day feeding regimen during the laying period. Even when the hens are placed on an everyday feeding schedule, they receive only one meal a day and thus spend much of each day in a fasting state. The results of the current research indicate that shortening the fasting period by splitting the daily feed allotment into two feeding periods may improve the reproductive performance of broiler breeder hens.

Broiler breeder hens that were fed twice a day after photostimulation and throughout the laying period had significantly greater hen day egg production during the early lay period as well as significantly greater cumulative hen day percent egg production compared to hens fed only once a day. Even though cumulative hen day egg production per bird was not significantly different, the increase of 4 eggs more per bird in the hens fed twice a day would have an economic impact. With 13 million broiler breeders in lay in Georgia per year, a 4 egg per bird increase in production would result in an annual increase of about 52 million eggs.

However, at this point we would not recommend to broiler breeder farm managers to feed their birds twice a day since the increase in egg production was off-set by a significant increase in hen mortality in the hens fed twice a day. The current research was conducted with Cobb slow feathering broiler breeder hens, which are known to have a high mortality rate that is enhanced even further when the hens have high production rates. Interestingly a majority of the mortality that occurred in the birds fed twice a day was of hens in active lay. Further research is needed utilizing different strains of broiler breeder hens before a final recommendation on feeding twice a day can be made.