5-1-2009

The effects of feeding a ground raw soybean diet on reproductive performance and carcass quality of prepubertal gilts

Daniel Jason Sykes

THE EFFECTS OF FEEDING A GROUND RAW SOYBEAN DIET ON REPRODUCTIVE PERFORMANCE AND CARCASS QUALITY OF PREPUBERTAL GILTS

By

Daniel Jason Sykes

A Thesis
Submitted to the Faculty of
Mississippi State Universtiy
in Partial Fulfillment of the Requirements
for the Degree of Masters of Science
in Animal Physiology
in the Department of Animal and Dairy Sciences

Mississippi State, Mississippi

May 2009

THE EFFECTS OF FEEDING A GROUND RAW SOYBEAN DIET ON REPRODUCTIVE PERFORMANCE AND CARCASS QUALITY OF PREPUBERTAL GILTS

By

Daniel Jason Sykes

Approved:

Peter L. Ryan Associate Professor Animal & Dairy Sciences Graduate Coordinator (Director of Thesis)	Mark A. Crenshaw Extension Swine Specialist Animal & Dairy Sciences (Committee Member)
Brian J. Rude Associate Professor Animal & Dairy Sciences (Committee Member)	Fred L. Cunningham Clinical Instructor CVM Pathobiology & Population Medicine (Committee Member)

Melissa Mixon
Interim Dean
College of Agriculture and
Life Sciences

Name: Daniel Jason Sykes

Date of Degree: May 2, 2009

Institution: Mississippi State University

Major Field: Animal Physiology

Major Professor: Dr. Peter Ryan

Title of Study: THE EFFECTS OF FEEDING A GROUND RAW SOYBEAN DIET ON REPRODUCTIVE PERFORMANCE AND CARCASS QUALITY OF PREPUBERTAL GILTS

Pages in Study: 77

Candidate for Degree of Masters of Science

The administration of raw soybeans to the diet of prepubertal gilts had no effect ($p>0.05$) on age to puberty, number of corpora lutea, or embryos present. Also, the number of pigs farrowed live and 28d litter weights did not differ between gilts consuming the raw soybean diet and gilts consuming the control diet. There was a reduction ($p<0.05$) in the number of pigs weaned in the group consuming the raw soybeans but no differences ($p>0.05$) in weaning weights. Gilts fed the raw soybean diet had a reduction ($p=0.05$) of hot carcass weights compared to gilts consuming the control diet but exhibited no differences for other carcass parameters measured. Thermal imaging was able to differentiate gilts in estrus versus diestrus. Gilts in estrus had greater ($p<0.05$) surface area temperatures than gilts in diestrus.

DEDICATION

This thesis is dedicated to my grandfather John C. Davis who passed away this past year.

ACKNOWLEDGEMENTS

I first would like to thank my parents Mike and Nancy Sykes, my grandmother Ruth Davis and my sister Laura for all of their support and love. Thank you for believing in me and helping me achieve my goals even if they did change along the way. I would also like to thank my friends who have been there with me through graduate school. You will never know how much you mean to me and I would not have made it through without you. I also would like to thank my major professor Dr. Ryan for his support and believing in me. Thank you for giving me this opportunity and sticking with me even when I was being very difficult. To Erin Schenck, you have been a life saver and thank you for all of you help and the listening ear. Also I would like to express my gratitude to the Animal and Dairy Sciences department and all of the staff and faculty. Lastly I would like to thank you, Thurston. You have been here supporting me the entire time and have been with me through the good and bad. You will never know what you mean to me and thank you for putting up with me and still supporting me.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER I

INTRODUCTION

Soybean meal is commonly used in swine diets as a source of supplemental protein. Although soybeans provide an abundant source of protein, there are some attributes of raw soybeans that may be problematic for animal growth. Soybeans are known to contain an anti-trypsin factor that has been shown to retard the growth of growing swine. However more recently, attention has been directed toward other compounds present in raw soybeans that might affect reproductive performance. These compounds, referred to as phytoestrogens, are biologically active and have estrogen-like properties. Phytoestrogens can competitively bind with estrogen receptors and mimic the actions of endogenous estrogens. The processing of raw soybeans through heating and alcohol extraction degrades the antitrypsin factors present along with reducing the phytoestrogen content.

The ability of phytoestrogens to mimic endogenous estrogens has led researchers to evaluate the potential effects of consuming diets with increased phytoestrogen content. Estrogens play an important role in the development of reproductive tissues and organs. Exposure to environmental estrogens at critical developmental periods could alter the development of these tissues and organs and affect reproductive performance. Therefore, the following hypothesis was tested; the consumption of a diet containing raw soybeans

at 24% of the total diet can alter the reproductive performance of prepubertal gilts. As mentioned previously, soybeans contain protease (trypsin) inhibitors that can retard the growth of young swine and potentially impact carcass quality. These protease inhibitors block enterokinase activity on trypsin activation which reduces the amount of protein digested and absorbed. The degradation of proteins to amino acids is essential for muscle growth and a lack of protein absorption could adversely affect carcass quality. Therefore, the carcass quality of gilts fed a diet containing raw soybeans was evaluated and compared to gilts fed a diet containing soybean meal.

As a means of assessing onset of puberty and estrus, both exposure to a mature boar and observation of reproductive behavior and digital infrared thermal imaging was employed. Digital infrared thermal imaging is a non-invasive technique that can be used to detect symmetrical and asymmetrical temperature gradients of surface areas of animals. The surface of the skin acts as a cooling system emitting heat. Changes in vascularization and blood flow during events such as stress and estrus can alter the surface area temperature of the skin. The thermal gradients given off by the skin can be measured through thermal imaging. During estrus there is increased vascularization and blood flow to the vulva. In swine the vulva is prominent and unobstructed for easy assessment. Therefore, the third hypothesis of this thesis was tested; thermal imaging of the vulva can differentiate between the state of estrus and diestrus of the gilt.

CHAPTER II

LITERATURE REVIEW

Swine Industry

With the decrease in the number of farms, farmers have relied heavily on advancements made in technology to improve production efficiency. With the decline in small farms the number of animals on larger farms has continued to increase (NAHMS, 2006, Part IV). In a report conducted by the USDA National Animal Health Monitoring System (NAHMS) in 2006, the total number of hogs and pigs has been gradually increasing since the year 2000 in the U. S. Although the numbers of swine operations have decreased almost 25% since 2000, the numbers of pigs are increasing and are currently over 60 million according to the same report. Of the more than 60 million pigs, only about 9.7% make up the breeding population compared to 12.6% in 1990 (NAHMS, 2006, Part IV). Therefore, much emphasis is placed on the 9.7% to efficiently produce over 8 million more pigs now than in 1990.

Litter size is an important component for the measure of reproductive efficiency in the swine industry (Johnson *et al.*, 1999). In 2006, approximately 6.09 million swine were a part of the national breeding herd with the average litter size of 10.8 pigs born alive and 9.38 pigs weaned per litter. Through genetic selection and meticulous culling practices, the average litter size of pigs born alive has increased by over a full pig since

1995. Reproductive failure accounts for over 26% of the breeding age females culled, with performance (small litter size, increased preweaning mortality or decreased birth weight) accounting for approximately 13% of the females culled (NAHMS, 2006, Part I). Litter size is greatly influenced by ovulation rate, uterine capacity and embryo survival (Marsteller *et al.*, 1997). Although the number of pigs born alive has increased, so have the number of stillborns and mummified fetuses (NAHMS, 2006, Part IV). The incidence of stillborns and mummies have been associated with many factors including nutrition, disease, mating frequency, lactation length, intrauterine crowding and hormone concentrations (Baysinger, *et al.,* 1997; Marsteller *et al.,* 1997; Xue *et al.*, 1998; Vallet *et al.*, 2002; Vinscky *et al*., 2006). Most of the factors listed can be altered through managerial techniques and therefore do not merit much discussion. However the ability to influence pig survival through intrauterine crowding and hormone concentrations are more complex.

Uterine capacity is the number of fully formed fetuses that can be maintained in the uterus until farrowing. Uterine capacity is influenced by uterine blood flow and uterine protein secretion. Uterine blood flow is said to reach its maximum at 5 fetuses per horn (Vallet *et al*., 2006). Giesert and others (1990) suggested that placental estrogens regulate uterine blood flow (UBF). Uterine blood flow increases with the increase of estrogen during the follicular phase of the estrous cycle. This course of action is also seen during pregnancy. Estrogen receptors present in the endothelial and vascular smooth muscle of the uterine artery appear to be mediators of UBF (Chang and Zhang, 2009). Although the mechanisms behind UBF are still poorly understood, it appears that the

concentrations of estrogen present in the circulatory system could alter UBF and uterine capacity.

Progesterone is essential for the uterus to maintain pregnancy. Maternal recognition of pregnancy in the pig occurs at approximately day 11 after the onset of estrus. Estrogens secreted by the embryo redirect the secretion of prostaglandin $F_{2\alpha}$ into the uterine lumen away from the vascular drainage to prevent the lyses of the corpus luteum (CL) (Bazer, 1989). This allows the CL's to maintain their viability and continue to secrete progesterone. It has been suggested that progesterone secreted before day 11 of pregnancy can have a detrimental effect on the uterine capacity and embryo survival (Vallet and Christenson, 2004). Estrogen secretion is also important during days 14 to 18 and day 25 and beyond of gestation to maintain luteal function (Geisert *et al*., 1990).

Estrogen

Endogenous estrogens 17β-estradiol (E_2), estrone (E_1) and estriol (E_3) are steroid hormones that are derivatives of cholesterol (Gruber *et al.,* 2002). Cholesterol is transported via the bloodstream to the ovary where it binds with specific receptors before being transported to the mitochondria and converted to testosterone through a series of enzymatic reactions (Gruber *et al.*, 2002). As part of the "two-cell, two-gonadotropin" model described by Gruber, androgens (i.e. testosterone) are secreted by thecal cells in response to luteinizing hormone (LH). Gruber also stated that granulosa cells express androgen receptors during antral development. Androgens are then converted to estrogen through follicle stimulating hormone (FSH) induced expression of cytochrome P450

(Hillier, 2001) in the smooth endoplasmic reticulum of follicular granulosa cells (Gruber *et al.,* 2002). Granulosa cells of the ovary are the primary sources of estradiol.

Estrogens are transported via the bloodstream bound to sex-hormone-binding globulin and albumin to their target cells. The actions of estrogens are determined by structure of the hormone and the estrogen receptor involved. There are two known subtypes of estrogen receptors (ER), estrogen receptor alpha (ERα) and estrogen receptor beta (ERβ) (Gruber *et al*., 2002). Although both receptors are expressed in the ovary and uterus (Kuiper *et al.,* 1997), ERα is expressed in greater quantities in reproductive tissues, while ERβ is predominantly expressed in the prostate, lung, bladder, brain and granulose cells (Kuiper *et al*., 1997; Nynca and Ciereszko, 2006).

Estrogens play an important role in the development and growth of reproductive tissues and the onset of puberty. Estrogens stimulate proliferation of the epithelial and stromal layers of the uterus and vagina, water retention, vascularization and growth of sexual organs (Gruber *et al*., 2002). The development of reproductive organs to sustain pregnancy is one criterion for the onset of puberty. Other criteria for the onset of puberty include the age at first estrus and the age at first ovulation. In the prepubertal animal, the hypothalamic gonadotropin-releasing hormone (GnRH) neurons cannot release enough GnRH to stimulate FSH and LH secretion. The secretion of FSH and LH is essential for folliculogenesis. Without the development of follicles, estradiol concentrations remain decreased and unable to generate activation of GnRH neurons in the surge center of the hypothalamus (Senger, 1999a).

The life cycle of the preovulatory follicle can be divided into three phases; FSH-dependent progression, LH-responsive maturation along with estrogen secretion and ovulation (Hillier, 2001). As described by Hillier (2001), during the FSH-dependent stage and LH-responsive stage, pituitary secreted FSH and LH stimulates the recruitment of ovarian follicles. Hillier also stated that theca cells within the ovary contain LH receptors that produce testosterone when LH binds to the receptors. Testosterone is then diffused into the Granulosa cells which contain FSH receptors. The binding of FSH to its receptors initiates the synthesis of enzymes that convert testosterone to estradiol (Hillier, 2001). Once estradiol concentrations reach its threshold, an LH surge occurs initiating a cascade of events leading to ovulation (Senger, 1999b). At the time of ovulation, estradiol concentrations are increased and elicit behavioral responses. In females, lordosis, crouching and increased activity are signs of estrus that are associated with increased concentrations of estradiol and progesterone (Senger, 1999c).

Soybeans

According to the 2008 USDA Economic Research Service (ERS), soybean production in the U.S. peaked at almost 78 million acres in 2008. Although the number of farms raising soybeans has decreased to about 317 thousand farms, acreage per farm along with crop yields per acre have increased according to the USDA ERS report. Nearly all of the soybeans produced in the U.S. are crushed to extract the oil. Soybean meal accounts for 50 to 75% of the value of soybeans and is the most important protein feed (USDA, ERS, 2008). Soybean meal is commonly used in livestock diets due to the

high protein content. Livestock diets account for approximately 98% of the total consumption of soybean meal (USDA, ERS, 2008). Traditionally, soybean meal has been used as a cheaper source of protein for cattle and swine diets. Raw soybeans however, are known to contain a number of compounds that include allergenic proteins, goitrogens, lectins, phytates, phytoestrogens, protease (trypsin) inhibitors and saponins (Liener, 1994; Cheeke, 1998). The processing of soybeans to soybean meal reduces exposure of the animal to these compounds. The most common methods of soybean processing are heating and alcohol extraction. Heating soybeans denatures and inactivates proteins which reduces the trypsin inhibitor content. Alcohol extraction removes compounds soluble in alcohol including saponins and phytoestrogens (Anderson and Wolf, 1995).

Phytoestrogens

Classification and Sources

Phytoestrogens are naturally occurring biological compounds in legumes and grains (Dusza *et al*., 2006). Phytoestrogens are structurally and functionally similar to endogenous estrogens and can mimic the actions of endogenous estrogens (Kaldas and Hughes, 1989). Compared to endogenous estrogens, phytoestrogens have weaker binding affinities (10^{-2} to 10^{-3} fold; Benassayag *et al.*, 2002). However, increased concentrations of phytoestrogens present in the circulation could account for their ability to competitively bind with estrogen receptors and mimic endogenous estrogens. The influence of phytoestrogens is dependent on the dose of phytoestrogens consumed,

presence of endogenous estrogens, receptor type and location and the class in which they belong (Benassayag *et al.*, 2002).

Phytoestrogens can be classified into three main classes based on their chemical structures: isoflavones, coumestans and lignans (Kurzer and Xu, 1997). These compounds are diphenolic and are structurally similar to natural estrogens (Kurzer and Xu, 1997). The isoflavones (mainly genistein and daidzein) and the coumestans are more extensively studied because of their abundance in plants (Dusza *et al.,* 2006). Soybeans contain the greatest dietary source of isoflavones (Dixon, 2004). According to Rozman *et al*. (2006), the isoflavone content of raw soybeans ranges from 1.35 mg/g to 1.86 mg per 100g of dry weight. The processing of raw soybeans by alcohol extraction significantly decreases the isoflavone content and therefore processed soybean meal found in typical swine diets has reduced concentrations of isoflavones than diets formulated using raw soybeans (Rozman *et al*., 2006).

Metabolism and Bioavailability

Most isoflavones (i.e. genistein and daidzein) are present in plants as water soluble glycosides. The glycosides are hydrolyzed by bacteria present within the large intestine to allow for absorption of genistein and daidzein in a more active form (Dixon, 2004; Murkies *et al*., 1998; Vaya & Tamir, 2004). Of these compounds, daidzein may be further metabolized into equol which has the highest estrogenic action of the isoflavonoids (Vaya & Tamir, 2004). These compounds can then enter circulation or undergo further metabolism and be excreted in bile and urine (Murkies *et al*., 1998).

Although phytoestrogens have a lower binding affinity for estrogen receptors, they are able to compete with endogenous estrogens for receptors. Endogenous estrogens are bound to serum proteins in the circulation leaving less than 5% free (Gruber *et al.,* 2002). Phytoestrogens are less likely to be bound to serum proteins therefore there are greater concentrations of unbound phytoestrogens in the circulation available for estrogen receptor binding (Brown & Setchell, 2001; Gruber *et al*., 2002). The half life of plasma phytoestrogens is estimated to be between 5 and 8 hrs (Mazur, 2000).

Estrogen Receptor Binding

Phytoestrogens have the ability to exhibit both estrogenic and antiestrogenic actions (Murkies *et al*., 1998). These effects begin with the ability of phytoestrogens to bind with estrogen receptors (Whitten & Patisaul, 2001). There are two subtypes of estrogen receptors present in mammals, estrogen receptor alpha (ERα) and estrogen receptor beta (ERβ). Phytoestrogens have been shown to bind to both receptors; however, there are some compounds that have greater binding affinities for specific receptors. For example, genistein, an isoflavone, has shown to have a greater affinity for ERβ. Estrogen receptor alpha is found to be more prevalent in reproductive tissues (i.e. uterus, ovary, testis) and has a more direct influence on reproductive organ development. Estrogen receptor β has been found not only in the uterus but also in the prostate, lung, bladder, brain and in porcine granulose cells (Kuiper *et al*., 1997; Nynca and Ciereszko, 2006). The ability of phytoestrogens to bind to both receptors provides the opportunity for phytoestrogens to influence functions other than just reproduction.

Reproductive Tract Development

Development of the reproductive tract is largely dependent on the availability of estrogens during the prepubertal period (Ford *et al*., 2006). Estradiol plays a crucial role in the development of the female reproductive organs (Griffin and Ojeda, 2000). Estrogens stimulate proliferation of the epithelial and stromal layers of the uterus and vagina, water retention, vascularization and growth of sexual organs (Gruber *et al*., 2002). In those animals that have decreased concentrations of estrogen, the administration of phytoestrogens can promote the development of reproductive tissues (Ford *et al*., 2006). Phytoestrogens have been shown to increase wet tissue weight of the uterus and cervix in the mouse and porcine (Whitten *et al*., 1995; Ford *et al*., 2006; Santell *et al*., 1996). Ford *et al.* (2006) administered genistein to ovariectomized gilts and found that development of the reproductive tissues was similar to that of endogenous estrogen. Ford's research provides definitive evidence of the sensitivity of reproductive tissues in the gilt to exogenous estrogens such as phytoestrogens.

Ovarian Cyclicity

The ability of phytoestrogens to mimic endogenous estrogens enables them to influence ovarian cycles. Phytoestrogens have been shown to inhibit and stimulate serum concentrations of progesterone (P_4) and estradiol-17 β (E_2) (Dusza *et al*., 2006; Nynca and Ciereszko, 2006; Dixon, 2004.). However it appears that the effects of phytoestrogens on serum P_4 and E_2 concentrations are species and dose dependent. In the rabbit, bovine and rat, phytoestrogens have been shown to stimulate P_4 and have no affect on E_2 production of ovarian follicles (Kaldas and Hughes, 1989; Makarevich *et al*.,

1997). Nynca and Ciereszko (2006) reported that genistein inhibited P_4 production from granulose cells but did not affect P_4 secretion from whole porcine follicles or E_2 production. In contrast, Makarevich *et al.* (1997) found that genistein stimulated E_2 secretion but had no affect on P_4 in the porcine granulose cells. Either the stimulation or inhibition of E_2 or P_4 can greatly alter the estrous cycle. Murrill and others (1996) evaluated the ovaries of mice treated with genistein. They found no differences in the number of follicles or CL present on the ovaries of mice treated with genistein compared to the controls. However, a study conducted by Jefferson and others (2005) reported that mice receiving an elevated dose of genistein (50 mg/kg/d) had fewer CL than mice receiving a low dose of genistein (0.5 or 5.0 mg/kg) and control mice. There were no differences in the number of CL present between the controls and low dose treatment groups.

The ability of phytoestrogens to influence ovarian activity appears to be both species and dose dependant. Estrogen is known to have proliferative effects on granulosa cells. Granulosa cells predominantly express ERβ which has a greater binding affinity for phytoestrogens. However from the previous studies mentioned, the role of phytoestrogens as estrogen mimics, agonists or antagonists in ovarian activity is still unclear.

Estrus Behavior

Phytoestrogens also have the ability to influence estrus behaviors. Studies with mice have reported that prepubertal mice receiving dietary phytoestrogens reach puberty at earlier ages than those receiving a phytoestrogen-free diet (Khan *et al*., 2008; Murrill *et al*., 1996; Whitten *et al*., 1995). Khan and others (2008) also reported an increase in

sexual behaviors from those mice consuming a diet containing phytoestrogens. However, in a study conducted by Teague (1955), gilts fed alfalfa showed no differences in estrus behavior compared to those fed a legume-free diet. Jefferson and others (2005) reported that mice treated with doses of 0.5 or 5.0 mg/kg/d of genistein showed no differences in age at puberty compared to control mice. Jefferson also reported that mice administered increased doses of genistein (50 mg/kg/d) exhibited delays in reaching puberty. Although there were no statistical differences reported with age to puberty, there were irregularities in estrous cycles of mice treated with genistein. Along with Jefferson, other researchers have noted that mice treated with phytoestrogens have irregular estrous cycles resulting in prolonged periods of estrus or diestrus (Murrill *et al*., 1996; Whitten *et al*., 1995; Whitten *et al.,* 2001).

Estrus behavior is directly influenced by the ovarian cycle. The actions of phytoestrogens on the ovarian cycle are immensely variable. The ability of phytoestrogens to influence estrus behavior has been reported (Murrill *et al*., 1996; Whitten *et al*., 1995; Whitten *et al.*, 2001) however there are still many uncertainties as to the mechanisms, dose and species dependencies.

Pregnancy

Pregnancy rate can be affected by the exposure of dams to phytoestrogens. Jefferson and others (2005) reported a dose dependent decrease in pregnancy rates of mice treated with genistein with the low dose (0.5 mg/kg) having the greatest pregnancy rates and the high dose (50 mg/kg) having the lowest pregnancy rates. Although there were differences in pregnancy rates, the number of live pups delivered did not differ

between the treatment groups. Crenshaw and Danielson (1985a) reported similar findings in swine. Gilts fed a raw soybean diet had no differences in the number of pigs farrowed live, number of pigs weaned or pig weaning weight. However, researchers did observe an increase in pig birth weight from sows fed the raw soybean diet.

Raw Soybeans and Carcass Quality

Growth performance and carcass value are important aspects to the profitability of the swine industry. Pork carcasses are graded based on quality and yield. Quality is assessed by the characteristics of the lean and fat at the 10th rib. The yield grade is based on the carcass yield from the ham, loin, picnic shoulder and Boston butt. Backfat thickness and degree of muscling are also taken into consideration when determining yield grade (USDA, AMS, 1985). Carcass quality is greatly affected by genetic selection, management strategies and nutrition.

Researchers have shown that soybeans contain protease (trypsin) inhibitors that can retard the growth of growing swine (Combs *et al*., 1967; Crenshaw and Danielson, 1985b; Jimenez *et al*., 1963; Pontif *et al*., 1987). Trypsin inhibitors bind with trypsin preventing the breakdown of proteins in the small intestine (Cheek, 1998). The lack of protein breakdown limits the ability of proteins to be absorbed and utilized within the body potentially affecting carcass quality. In a study conducted by Felton *et al* (2004), a decrease of hot carcass weight and longissimus muscle area of yearling steers fed a whole raw soybean diet was reported. Pontif *et al.* (1987) also noted a reduction of longissimus and carcass weight of finishing swine fed a raw soybean diet. However, it has been suggested that maturity of the animal may affect its ability to utilize raw soybeans

(Saxena *et al*, 1963; Combs and Wallace, 1969; Crenshaw and Danielson, 1985b) and therefore potentially reduce the negative effects on carcass quality.

Thermal Imaging

Digital infrared thermal imaging (DITI) is a non-invasive technique that can be used to detect symmetrical and asymmetrical temperature gradients of surface areas of animals. The surface of the skin acts as a cooling system emitting heat (Purohit *et al*., 1985), allowing for accurate measure of temperature gradients through thermal imaging. Changes in blood flow and vascularization of tissues can alter the surface temperature of the skin (Winsor, 1971). The normal body maintains a constant temperature and changes in body surface temperature are easily recognized using thermal imaging and may provide a means for the discovery of abnormalities (Purohit *et al*., 1985). The ability to detect changes in thermal gradients of the surface area has led to the utilization of thermal imaging for detection of lameness and injury of cattle and horses (Purohit, 1980; Turner, 1998; Cockcroft *et al*., 2000; Schmidt *et al*., 2003;), as a tool for measuring fertility of bulls and rams (Kastelic *et al*., 1995, 1996; Lunstra and Coulter, 1997; Gabor *et al*., 1998) and evaluating heat stress of dairy cattle (Coppola *et al*., 2002; White *et al*., 2006). More recently researchers have focused on the use of thermal imaging for the detection of pregnancy in both zoological and domestic species (Hilsberg *et al*., 2002; Bowers *et al*., 2004; Jones *et al*., 2005; Durrant *et al*., 2006).

Although there continues to be research evaluating the use of thermal imaging for reproduction purposes, there has been little work in the area of thermal imaging for detection of estrus. Anatomically, the vulva provides a prominent area to measure

thermal gradients. During estrus in many species, the vulva will become swollen (Bearden *et al*., 2004) due to increased blood flow (Frandson *et al*., 2003). Studies evaluating the use of thermal imaging for estrus detection in bovine have met with mixed results. Osawa *et al.* (2004) observed an increase in vulva surface area temperature of cows in estrus versus diestrus. Jones *et al*. (2005) were able to discriminate first estrus from diestrus of dairy cows, but not between subsequent cycles. These studies have provided a foundation for the investigation to more accurately detecting estrus through the use of thermal imaging.

References Cited

Anderson, R. L. and W. J. Wolf. 1995. Compositional changes in trypsin inhibitors, phytic acid, saponins and isoflavones related to soybean processing. Journal of Nutrition. 125(3):581S-588S.

Baysinger, A. K., C. E. Dewy, B. E. Straw, M. C. Brumm, J. Schmitz, A. Doster and C. Kelling. 1997. The effect of PRRSV on reproductive parameters in swine herds. Swine Health and Production. 5(5):173-176.

Bazer, F. W. 1989. Establishment of pregnancy in sheep and pigs. Reproduction, Fertility and Development. 1(3):237-242.

Bearden, H. J., J. W. Fuquay and S. T. Willard. 2004. Applied animal reproduction 6th ed. Pearson Prentice Hall, Upper Saddle River, NJ.

Benassayag, C., M. Perrot-Applanat and F. Ferre. 2002. Phytoestrogens as modulators of steroid action in target cells. Journal of Chromatography B. 777:233-248.

Bowers, S., S. Gandy, B. Anderson, P. Ryan and S. Willard. 2004. Assessment of pregnancy in the mare using digital infrared thermography. ASAS/ADSA National Meeting, St. Louis, Missouri.

Brown, N. M. and K. D. R. Setchell. 2001. Animal models impacted by phytoestrogens in commercial chow: implications for pathways influenced by hormones. Laboratory Investigation. 81(5):735-747.

Chang, K and L Zhang. 2009. Steroid hormones and uterine vascular adaptation to pregnancy. Reproductive Sciences. 15(4):336-348.

Cheeke, P. R. 1998. Natural toxicants in feeds, forages, and poisonous plants. 2nd ed. Interstate Publishers, Inc., Danville, IL.

Cockcroft, P. D., F. D. Henson and C. Parker. 2000. Thermography of a septic metatarsophalangeal joint in a heifer. Veterinary Research. 146:258-260.

Combs, G. E., R. G. Conness, T. H. Berry and H. D. Wallace. 1967. Effect of raw and heated soybeans on gain, nutrient digestibility, plasma amino acids and other blood constituents of growing swine. Journal of Animal Science. 26:1067-1071.

Combs, G. E. and H. D. Wallace. 1969. Raw and heated soybeans for young, growing and finishing swine. Rep. of 14th Annual Swine Filed Day, University of Florida.

Coppola, C. L., R. J. Collier and R. M. Enns. 2002. Evaluation of thermal status of cattle using infrared technology. Proceedings of the Western Section American Society of Animal Science, Fort Collins, Colorado.

Crenshaw, M. A. and D. M. Danielson. 1985a. Raw soybeans for gestating swine. Journal of Animal Science. 60:163-170.

Crenshaw, M. A. and D. M. Danielson. 1985b. Raw soybeans for growing-finishing pigs. Journal of Animal Science. 60:725-730.

Dixon, R. A. 2004. Phytoestrogens. Annual Review of Plant Biology. 55:225-261.

Dusza, L., R. Ciereszko, D. J. Skarzynski, L. Nogowski, M. Opalka, B. Kaminska, A. Nynca, O. Kraszewska, M. Slomczynska, Il Woclawek-Potocka, A. Korzekwa, E. Pruszynska-Oszmalek, and K. Szkudelska. 2006. Mechanism of phytoestrogens action in reproductive processes of mammals and birds. Reproductive Biology. 6 (Suppl. 1):151-174.

Durrant, B. S., N. Ravida, T. Spady and A. Cheng. 2006. New technologies for the study of carnivore reproduction. Theriogenology. 66:1729-1736.

Felton, E. E. and M. S. Kerley. 2004. Performance and carcass quality of steers fed whole raw soybeans at increasing inclusion levels. Journal of Animal Science. 82(3):725-732.

Ford, J. A. Jr., S. G. Clark, E. M. Walters, M. B. Wheeler and W. L. Hurley. 2006. Estrogenic effects of genistein on reproductive tissues of ovariectomized gilts. Journal of Animal Science. 84:834-842.

Frandson, R. D., W. L. Wilke and A. D. Fails. 2003. Anatomy of the Female Reproductive System. In: Anatomy and Physiology of Farm Animals, Troy D (Ed.), Lippincott Williams and Wilkins.

Gabor, G. R., G. Sasser, J. P. Kastelic, G. H. Coulter, D. O. Everson, G. Falkay, M. Mezes, S. Bozo, R. B. Cook and J. V. Csik. 1998. Endocrine and thermal responded to GnRH treatment and prediction of sperm output and viability in Holstein-Friesian breeding bulls. Theriogenology. 50:177-183.

Geisert, R. D., M. T. Zavy, R. J. Moffatt, R. M. Blair and T. Yellin. 1990. Embryonic steroids and the establishment of pregnancy in pigs. Journal of Reproduction and Fertility. Supplement 40:293-305.

Griffin, J. E. and S. R. Ojeda. 2000. Female reproductive function. Pages 202 -242 in Textbook of Endocrine Physiology. Oxford University Press, New York, NY.

Gruber, C. J., W. Tschugguel, C. Schneeberger and J. C. Huber. 2002. Production and actions of estrogens. The New England Journal of Medicine. 346(5):340-352.

Hillier, S. G. 2001. Gonadotropic control of ovarian follicular growth and development. Molecular and Cellular Endocrinology. 179:39-46.

Hilsberg, S., K. Eulenberger and F. Zahorszki. 2002. Application of infrared thermography in pregnancy diagnosis in giraffes. Leipzig. 14-15.

Jefferson, W. N., E. Padilla-Banks and R. R. Newbold. 2005. Adverse effects on female development and reproduction in CD-1 mice following neonatal exposure to the phytoestrogen genistein at environmentally relevant doses. Biology of Reproduction. 73:798-806.

Jimenez, A. A., T. W. Perry, R. A. Pickett and W. M. Beeson. 1963. Raw and heat-treated soybeans for growing finishing swine, and their effect on fat firmness. Journal of Animal Science. 22:471-475.

Johnson, R. K., M. K. Nielson and D. S. Casey. 1999. Responses in ovulation rate, embryonal survival, and litter traits in swine to 14 generations of selection to increase litter size. Journal of Animal Science. 77:541-557.

Jones, M., A. Denson, E. Williams, K. Graves, A. Dos Santos, A. Kouba and S. Willard. 2005. Assessing pregnancy status using digital infrared thermal imaging in Holstein dairy heifers. ASAS/ADSA National Meeting, Cincinnati, Ohio.

Kaldas, R. S. and C. L. Hughes, Jr. 1989. Reproductive and general metabolic effects of phytoestrogens in mammals. Reproductive Toxicology Review. 3:81-89.

Kastelic, J. P., G. H. Coulter and R. B. Cook. 1995. Scrotal surface, subcutaneous, intratesticular and intraepididymal temperatures in bulls. Theriogenology. 44:147-152.

Kastelic, J. P., R. B. Cook, G. H. Coulter and R. G. Saake. 1996. Ejaculation increases scrotal surface temperature in bulls with intact epididymides. Theriogenology. 46:889-892.

Khan A., N. Bellefontaine and D. deCatanzaro. 2008. Onset of sexual maturation in female mice as measured in behavior and fertility: Interactions of exposure to males, phytoestrogen content of diet, and ano-genital distance. Physiology and Behavior. 93:588-594.

Kuiper, G. J. M., B. Carlsson, K. Grandien, E. Enmark, J. Haggblad, S. Nilsson and J. A. Gustafsson. 1997. Comparison of the ligand binding specificity and transcript tissue distribution of estrogen receptors α and β. Endocrinology. 138:863-870.

Kurzer, M. S. and X. Xu. 1997. Dietary phytoestrogens. Annual Review of Nutrition. 17:353-381.

Liener, I. E., 1994. Implication of antinutritional components in soybean foods. Critical Review of Food Science and Nutrition. 34:31-67.

Lunstra, D. D. and G. H. Coulter. 1997. Relationship between scrotal infrared temperature patterns and natural-mating fertility in beef bulls. Journal of Animal Science. 75(3):767-74.

Makarevich, A., A. Sirotkin, T. Taradajnik and P. Chrenek. 1997. Effects of genistein and lavendustin on reproductive processes in domestic animals in vitro. Journal of Steroid Biochemistry and Molecular Biology. 63(4-6):329-337.

Marsteller, T. A., G. A. Armbruster, D. B. Anderson, A. J. Wuethrich, J. L. Taylor and J. T. Symanowski. 1997. Effect of lactation length on ovulation rate and embryo survival in swine. Swine Health and Production. 5(2):49-56.

Mazur, W. Phytoestrogens: Occurrence in foods, and metabolism of lignans in man and pigs. University of Helsinki, 2000, p. 29.

Murkies, A. L., G. Wilcox and S. R. Davis. 1998. Phytoestrogens. Journal of Clinical Endocrinology and Metabolism. 83(2):297-303.

Murrill, W. B., N. M. Brown, J Zhang, P. A. Manzolillo, S. Barnes and C. A. Lamartiniere. 1996. Prepubertal genistein exposure suppresses mammary cancer and enhances gland differentiation in rats. Carcinogenesis. 17(7):1451-1457.

National Animal Health Monitoring System, 2008. Swine 2006, Part I: Reference of swine health and management practices in the United States, 1990-2006.

National Animal Health Monitoring System, 2008. Swine 2006, Part IV: Changes in the pork industry, 1990-2006.

Nynca, A. and R. E. Cierszko. 2006. Effect of genistein on steroidogenic response of granulose cell populations from porcine preovulatory follicles. Reproductive Biology. 6(1):31-49.

Osawa, T., M. Tanaka, M. Morimatsu, K. Hashizume and B. Syuto. 2004. Use of infrared thermography to detect the change in the body surface temperature with estrus in the cow. Proceedings from the Society for Theriogenology Annual Conference, Lexington, Kentucky.

Pontif, J. E., L. L. Southern, D. F. Coombs, K. W. McMillin, T. D. Binder and K. L. Watkins. 1987. Gain, feed efficiency and carcass quality of finishing swine fed raw soybeans. Journal of Animal Science. 64:177-181.

Purohit, R. C. 1980. The diagnostic value of thermography in equine medicine. Proceedings from the Annual Convention of the American Association of Equine Practitioners. 26:317-326.

Purohit, R. C., R. S. Hudson, M. G. Riddell, R. L. Carson, D. F. Wolfe and D. F. Walker. 1985. Thermography of the bovine scrotum. American Journal of Veterinary Research. 46:2388-2392.

Rozman, K. K., J. Bhatia, A. M. Calafat, C. Chambers, M. Culty, R. A. Etzel, J. A. Flaws, D. K. Hansen, P. B., Hoyer, E. H. Jeffery, J. S. Kesner, S. Marty, J. A. Thomas and D. Umbach. 2006. NTP-CERHR expert panel report on the reproductive and developmental toxicity of genistein. Birth Defects Research Part B: Developmental and Reproductive Toxicology. 77:485-638.

Santel, R. C., Y. C. Chang, M. G. Nair and W. G. Helferich. 1996. Dietary genistein exerts estrogenic effects upon the uterus, mammary gland and the hypothalamic/pituitary axis in rats. Journal of Nutrition. 127:263-269.

Saxena, H. C., L. S. Jensen and J. McGinnis. 1963. Influence of age on utilization of raw soybean meal by chickens. Journal of Nutrition. 80:391-396.

Senger, P. L. 1999a. The onset of puberty. In *Pathways to Pregnancy and Parturition*. 1st Revised Edition. 100-114. Ephrata, PA. The Mack Printing Group-Science Press.

Senger, P. L. 1999b. The follicular phase of the estrous cycle. In *Pathways to Pregnancy and Parturition*. 1st Revised Edition. 130-146. Ephrata, PA. The Mack Printing Group-Science Press.

Senger, P. L. 1999c. Reproductive behavior. In *Pathways to Pregnancy and Parturition*. 1st Revised Edition. 188-204. Ephrata, PA. The Mack Printing Group-Science Press.

Schmidt, S., S. Bowers, K. Graves, R. Carroll, J. White and S. Willard. 2003. Use of infrared thermography to assess thermal temperature gradients and pathologies of the bovine claw. ASAS/ADSA National Meeting, Phoenix, Arizona.

Teague, H. S. 1955. The influence of alfalfa on ovulation rate and other reproductive phenomena in gilts. Journal of Animal Science. 14:621-627.

Turner, A. T. 1998. Thermography as an aid in the localization of upper hind limb lameness. Equine Athletics. 11:38-42.

USDA, Agriculture Marketing Service. 1985. United States standards for grades of pork carcasses. http://www.ams.usda.gov/AMSv1.0/getfile?dDocName=STELDEV3060403 (Accessed March 16, 2009)

USDA, Economic Research Service. 2008. Soybeans and oil crops: Background. http://www.ers.usda.gov/Briefing/SoybeansOilcrops/background.htm (Accessed March 3, 2009.)

Vallet, J. L., K. A. Leymaster and R. K. Christenson. 2002. The influence of uterine function on embryonic and fetal survival. Journal of Animal Science. 80:E115-E125.

Vallet, J. L. and R. K. Christenson. 2004. Effect of progesterone, mifepristone, and estrogen treatment during early pregnancy on conceptus development and uterine capacity in swine. Biology of Reproduction. 70:92-98.

Vallet, J. L., B. A. Freking, J. R. Miles, J. A. Nienaber and T. M. Brown-Brandl. 2006. Factors affecting litter size in pigs. Proceedings of the National Swine Improvement Federation Conference and Annual Meeting, Nashville, TN. 31:15-21.

Vaya, J. and S. Tamir. 2004. The relation between the chemical structure of flavonoids and their estrogen-like activities. Current Medicinal Chemistry. 11:1333-1343.

Vinsky, M. D., S. Novak, W. T. Dixon, M. K. Dyck and G. R. Foxcroft. 2006. Nutritional restriction in primiparous sows selectively affects female embryo survival and overall litter development. Reproduction, Fertility and Development. 18:347-355.

White, M. B., M. Jones, S. Schmidt and S. Willard. 2006. Use of digital infrared thermal imaging (DITI) for monitoring environmental physiology in dairy cattle. Journal of Animal Science. 84(1):212.

Whitten, P. L., C. Lewis, E. Russell and F. Naftolin. 1995. Potential adverse effects of phytoestrogens. Journal of Nutrition. 125(3S):771S-776S.

Whitten, P. L. and H. B. Patisaul. 2001. Cross-species and interassay comparisons of phytoestrogen action. Environmental Health Perspectives. 109(1):5-20.

Winsor, T. 1971. Vascular aspects of thermography. Journal of Cardiovascular Surgery. 12:379-388.

Xue, J., T. Lucia, Y. Koketsu, G. D. Dial and W. E. Marsh. 1998. Effect of mating frequency and weaning-to-mating interval on sow reproductive performance. Swine Health and Production. 6(4):157-162.

CHAPTER III

EFFECTS OF A GROUND RAW SOYBEAN DIET ON REPRODUCTIVE EFFICIENCY IN THE GILT

Abstract

Two experiments were conducted evaluating the effects of a ground raw soybean based diet on reproductive efficiency in pre-pubertal gilts. In Experiment 1, gilts were assigned to one of two diets using either soybean meal (SBM) or ground raw soybeans (RSB) as the protein source. Diets were formulated to be iso-nitrogenous (14% CP) and iso-caloric for metabolizable energy. Gilts were monitored daily for estrus starting at 160 days of age and bred by AI on the third standing estrus. After breeding, gilts were maintained on their respective dietary treatments to approximately day 40 of gestation and harvested for analysis of reproductive tracts. There were no differences ($P > 0.10$) for age at first estrus, age at breeding or weights at breeding between the two treatments. Reproductive tracts were evaluated for number of CLs present on ovaries and number of embryos present. No differences were observed between the two treatments for either parameter. In Experiment 2, the same parameters were used as for experiment 1 with the exception that gilts were allowed to farrow and were observed through weaning. No differences ($P > 0.10$) were observed in age at first estrus, age at breeding or weight at breeding between the two treatments. There were also no differences observed for number of pigs farrowed and birth weights of pigs between treatments. However, sows

fed the RSB supplemented diet weaned fewer ($P < 0.05$) pigs than sows supplemented with SBM. Average weaning weights of pigs did not differ ($P > 0.10$) between treatment groups. The sow's return to estrus interval from time of weaning did not differ ($P > 0.10$) between treatments. Further research is needed to confirm whether or not diet was responsible for the reduction in number of pigs weaned.

Introduction

Soybean meal is commonly used in swine diets due to the high content of protein and favorable amino acid profile. However recent evidence suggests that biological compounds known as phytoestrogens present in soybeans might have an effect on reproductive function. It has been reported that phytoestrogens can mimic the actions of endogenous estradiol (Kaldas *et al*., 1989; Adams, 1995). These phytoestrogens can be classified into three classes: isoflavones, coumestans, and lignans, with the isoflavones being the most studied (Dusza *et al*., 2006). The three most common forms of isoflavones found in raw soybeans are genistein, diadzein, and glycitein. Of these compounds genistein is present in the highest concentrations (Wang, 1994). However, the processing of raw soybeans by alcohol extraction reduces the phytoestrogen content (Rozman *et al*., 2006).

Previous research found that phytoestrogens can cause deleterious effects on reproductive function in sheep (Adams, 1988) and cattle (Adams, 1995). However, in a study conducted by Crenshaw and Danielson (1985), gestating swine fed diets composed of raw soybeans showed no adverse effects on number of pigs farrowed or weaned. The differences between these studies give rise to the question as to the role phytoestrogens

have in different species or stages of reproductive development and function differently. In pre-pubertal rats, estrous cycles were initiated when fed diets containing low amounts (100 µg/g) of coumestrol, a type of phytoestrogens (Whiten *et al*., 1995). Drane and others (1981) reported pre-pubertal gilts exhibited an increase in vulva size when fed a diet of 20% soybean meal over those fed a non-soybean meal diet. In a study conducted by Ford *et al*. (2006), ovariectomized gilts were administered doses of genistein ranging from 100 mg/d to 400 mg/d. Gilts administered genistein in the amount of 400 mg/d for 10 days exhibited increased development of the entire reproductive tract.

These findings lead to the question of whether the consumption of phytoestrogens during the appropriate stage of development can have a positive influence on reproductive parameters of gilts. The objective of this study was to evaluate the effects of feeding a ground raw soybean diet on the reproductive performance of pre-pubertal gilts.

Materials and Methods

This study followed the FASS (1999) *Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching* and was approved by the Mississippi State University Institutional Animal Care and Use Committee (Protocol No. 04-072).

Experiment I

Twenty pre-pubertal Yorkshire x Landrace crossbred gilts of Monsanto genetics (BW 79.4 ± 1.4 kg; approx. 144 d of age), were purchased from Prestage Farms (West Point, MS) and housed at the Physiology Research Unit at the Mississippi Agriculture and Forestry Experiment Station (Starkville, MS). Gilts were matched by weight and

assigned to one of two dietary treatments (n = 10/treatment) and initially allowed to acclimate to their environment on a pre-treatment standard gestation corn soybean meal based diet for eleven days. Gilts were vaccinated for Leptospirosis (6 way), Parvovirus and Erysipelothrix (Farrow-Sure Plus B, Pfizer Inc, New York, NY), de-wormed and re-weighed before introducing the experimental diets. Experimental diets consisted of either soybean meal (SBM, control diet) or raw soybeans (RSB, experimental diet) as the primary source of dietary protein. Diets were formulated to have 14% crude protein and to be iso-nitrogenous and iso-caloric for metabolizable energy (ME). Ground Raw Soybeans accounted for about 75% of the dietary protein for the first four weeks (phase I) of the study and then increased to 100% of the dietary protein for the remainder of the study (phase II). The control diet (SBM) was supplemented with poultry fat to equal the ME content of the RSB diet (Table 3.1). Gilts were placed on dietary treatments at approximately 155 days of age with an average body weight of 84.9 ± 1.5 kg and housed by treatment group in outdoor covered pens with *ad libitum* access to food and water. Gilts were monitored daily (0600-0700 hrs) for signs of estrus beginning at 160 days of age with a mature boar and bred on the third detected standing estrus by artificial insemination (AI) using pooled boar semen (Prestage Farms, West Point, MS). Gilts received two services, twelve hours apart, with the first service occurring twelve hours post observation of third standing estrus. Following AI, gilts were individually penned and placed on a ration of 2.3 kg/d of their respective diets until they were slaughtered between 35 and 45 d of gestation for recovery of the reproductive tract. Reproductive tracts were evaluated for CL's present on ovaries and embryo development.

Table 3.1

SWINE DIETS USING SOYBEAN MEAL (SBM) OR RAW SOYBEANS (RSB) AS DIETARY PROTEIN SOURCE

	Phase I - 75% RSB		Phase II - 100% RSB	
Ingredients, %	SBM	RSB	SBM	RSB
Corn	75.04	71.46	74.62	69.72
Soybean meal, 48%	17.86	4.68	17.93	
Raw soybean, ground		17.86		24.28
Poultry fat	1.10		1.45	
Sow 120 Premix*	6.00	6.00	6.00	6.00
Total	100	100	100	100
Calculated Analysis				
Protein	14.00	14.00	14.00	14.00
Calcium	1.44	1.45	1.44	1.46
Phosphorous	0.96	0.95	0.96	0.94
Lysine	0.71	0.70	0.71	0.70
ME, kCal/kg	3262	3261	3280	3280
Laboratory Analysis				
Protein	14.10	13.90	14.80	13.80
Calcium	1.28	1.44	1.50	1.60
Phosphorous	0.95	0.95	1.10	1.10

*Sow 120 premix (Multi-Kare, Inc., Tifton, GA) guaranteed analysis: Ca (19% – 22.8%), P (10%), NaCL (7%-8.4%), SE (0.0005%), biotin (8.38 mg/kg), vitamin A (261,242, USP units/kg), vitamin D (40,123 USP units/kg), vitamin E (750.0 INT units/kg)

Experiment II

Twenty pre-pubertal Yorkshire x Landrace crossbred gilts of Monsanto genetics (BW 73.5 ± 1.1 kg; approx. 140 d of age) were purchased from Prestage Farms (West Point, MS) and housed at the Physiology Unit at the Mississippi Agriculture and Forestry Experiment Station (Starkville, MS). Gilts were matched by weight and assigned to one of two dietary treatments (either RSB or SBM; n = 10/treatment) and initially allowed to acclimate to their environment on a standard gestation corn-soybean meal based diet for

eleven days. Gilts were placed on dietary treatments at approximately 151 days of age with an average body weight of 85.1 ± 1.3 kg and housed by treatment group in outdoor covered pens with *ad libitum* access to food and water. Gilts followed the same experimental plan as explained in Experiment 1 with the following exceptions: 1.) blood was collected by jugular veinapuncture at standing estrus and ten days post standing estrus for analysis of serum progesterone (P_4) and estradiol (E_2) concentrations, and 2.) gilts were allowed to farrow and pigs weaned at 28 d post-partum. Gilts were weighed and placed in farrowing crates at d 111 of gestation and maintained on respective diets until farrowing. Post farrowing, gilts were allowed *ad libitum* access to a standard corn-soybean meal based lactation diet. Litter size, pig birth weights and placenta weights were all collected at farrowing. At 28 d post-partum, pigs were weaned and number of pigs weaned along with pig and sow weights were recorded. Sows were returned to outdoor covered pens post-weaning and evaluated daily for return to estrus. At first estrus post-weaning, sows were terminated from the study.

Radioimmunoassay of P_4 and E_2

Progesterone (P_4) and estradiol (E_2) analysis of serum were conducted using radioimmunoassay specific of P_4 and E_2 (Diagnostic Systems Laboratories, Webster, TX; DSL-3900, DSL-4800). Assay sensitivity for P_4 was 0.02 ng/ml and intra- and inter-assay coefficients of variation were 6.13% and 7.40%, respectively. The intra- and inter-assay coefficients of variation for E_2 were 5.24% and 5.60%, respectively and assay sensitivity was 2.2 pg/ml.

Statistical Analysis

The experiment was analyzed as a complete randomized design with two treatments (RSB and SBM diets). Statistical analyses were performed using GLM procedures of SAS v9.1.3 (SAS Institute, Cary, NC) and least square means (least square mean ± SE) were calculated for both treatments. Data with a P value less than 0.1 but greater than 0.05 were considered as tendencies and data with a P value less than 0.05 were considered to be significant.

Results

Experiment I

Age at Puberty and Breeding

RSB gilts were the first to exhibit signs of estrus and by the second week of estrus detection, 70% of RSB gilts had exhibited signs of estrus with only 40% of the SBM gilts exhibiting estrus. However, despite the fact that the RSB gilts began to exhibit estrus earlier than the SBM gilts, there were no differences ($P > 0.05$) in mean age of gilts at puberty or breeding between the two groups (Table 3.2). There were no differences ($P > 0.05$) for body weights at breeding between the two treatment groups.

Table 3.2

BODY WEIGHTS AND AGES OF GILTS FED A SOYBEAN MEAL OR RAW SOYBEAN BASED DIET

Dietary Treatments*	SBM[1]	RSB[1]	SBM	RSB	P value[1]
	Mean body weights (kg)		Age (d)		
Phase I - 75% RSB					
Initial	84.7 ± 2.3	85.2 ± 2.1	155	155	0.874
Final	110.3 ± 3.0	112.3 ± 2.7	183	183	0.630
Phase II - 100% RSB					
Puberty			193.0 ± 7.3	184.3 ± 3.9	0.776
Breeding	147.8 ± 6.4	136.3 ± 3.8	232.9 ± 7.3	225.6 ± 3.6	0.396
Final	163.2 ± 2.8	157.6 ± 3.0	271.8 ± 5.8	267.6 ± 4.0	0.159

* Dietary Treatments - SBM, Soybean Meal; RSB, Raw Soybeans

[1] P Value for body weight only; ages were not significant

Embryos and Corpora Lutea

Reproductive tracts were collected at time of slaughter and evaluated for the number of CL present on the ovaries and the number of embryos in each horn. One gilt from each treatment was not included in the analysis due to not conceiving (SBM) and the absence of the left uterine horn (RSB). The number of CL's and embryos present did not differ ($P > 0.05$) between the two treatments with the RSB group having 16.9 ± 0.9 total CL's and 13.2 ± 0.6 total embryos and the SBM group 18.3 ± 1.0 total CL's and 14.9 ± 1.6 total embryos, respectively.

Experiment II

Age at Puberty and Breeding

Unlike Experiment 1, SBM gilts were the first to exhibit signs of estrus. However, there were no differences ($P > 0.05$) in age at puberty between the two treatments. There were also no differences ($P > 0.05$) in age at time of first breeding or weights at breeding (Table 3.2).

Progesterone and Estradiol Concentrations

Progesterone and estradiol concentrations for each treatment are shown in Table 3.3. Serum progesterone concentrations were decreased ($P < 0.05$) at estrus than diestrus for both treatments, as expected. However, RSB gilts had reduced serum P_4 concentrations during diestrus than SBM gilts (10.3 ± 0.7 pg/ml and 12.4 ± 0.7 pg/ml, respectively). There were no differences ($P > 0.1$) for P_4 concentrations between the treatment groups during estrus. Estradiol concentrations were increased ($P = 0.05$) during estrus than diestrus and had a tendency to be increased ($P = 0.09$) in RSB than SBM gilts at estrus (Table 3.3).

Litter Size

The number of pigs farrowed, stillborns and mummified fetuses were recorded along with placental and baby pig body weights. The total number of pigs farrowed did not differ ($P > 0.05$) between the two treatments (Table 3.4). The number of stillborns and mummified fetuses were not different between the two treatments as well. Number of

male and female pigs per litter, mean pig birth weight and placenta weight also did not differ ($P > 0.05$) between treatment groups (Table 3.4).

Table 3.3

LSMEAN (± SE) SERUM P_4 AND E_2 CONCENTRATIONS OF BLOOD SERUM COLLECTED FROM GILTS DURING ESTRUS AND DIESTRUS

	SBM	RSB	P Value
P_4 (pg/ml)			
Estrus	1.2 ± 0.5	1.4 ± 0.6	0.769
Diestrus	12.4 ± 0.7	10.3 ± 0.7	0.039
E_2 (ng/ml)			
Estrus	7.9 ± 0.5	9.2 ± 0.6	0.089
Diestrus	7.2 ± 0.7	7.4 ± 0.7	0.836

Means within row are different ($P < 0.05$)

Pigs were weaned at 28 d post farrowing and the number of pigs weaned along with pig body weights was recorded. Raw soybean treated sows weaned fewer ($P < 0.05$) pigs than SBM-treated sows (8.9 ± 0.8 vs. 11.8 ± 0.6, respectively). To help gain a better understanding as to why sows on the RSB diet weaned fewer pigs, the pigs were divided into three weight divisions, light birth weight (<0.9 kg), medium birth weight (0.91 to 1.6 kg) and heavy birth weight (>1.6 kg). There were fewer ($P < 0.01$) pigs from sows fed the RSB diet in the heavy birth weight group, while there was a tendency ($P = 0.07$) to have more numbers pigs from sows fed the RSB diet in the light birth weight group (Table 3.5).

Table 3.4

MEAN NUMBER (± SE) OF PIGS FARROWED, MEAN BIRTH WEIGHTS AND MEAN PLACENTA WEIGHTS FROM SOWS CONSUMING A RAW SOYBEAN BASED DIET OR SOYBEAN MEAL BASED DIET

	SBM	RSB	P Value
Total Pigs Farrowed/Sow	13.8 ± 0.6	13.2 ± 1.3	0.677
Alive	13.0 ± 0.6	11.6 ± 1.2	0.328
Stillborns	0.6 ± 0.3	1.3 ± 0.5	0.245
Mummified Fetus	0.2 ± 0.1	0.3 ± 0.2	0.696
Males	6.2 ± 0.3	5.9 ± 0.8	0.727
Females	7.4 ± 0.6	7.0 ± 0.9	0.715
Mean Pig Birth Weight (kg)	1.4 ± 0.1	1.3 ± 0.1	0.494
Mean Placenta Weight (kg)	3.7 ± 0.2	3.3 ± 0.4	0.408

No differences were observed in parameters measured between treatment groups

Table 3.5

BIRTH WEIGHT CLASSES OF PIGS BASED ON DIETARY TREATMENT

	SBM	RSB	P Value
Light Birth Weight (<0.09 kg)	11	21	0.077
Medium Birth Weight (0.91 to 1.6 kg)	83	87	0.759
Heavy Birth Weight (> 1.6 kg)	42	21	0.008

Number of pigs per weight division was analyzed using chi square of SAS 9.1

Although there were differences in the number of pigs weaned per treatment groups, average weaning weights per pig did not differ (P > 0.05) between treatments; RSB, 7.9 ± 0.5 kg; SBM, 7.5 ± 0.3 kg, respectively.

Sow Weight Loss and Feed Intake During Lactation

Sows were weighed prior to farrowing and no differences ($P > 0.05$) were observed between the two treatments. During lactation, feed intake was measured and sows were weighed at weaning to calculate weight loss during lactation. Body weights and feed consumption are shown in Table 3.6. The RSB sows tended to have a decreased ($P < 0.1$) mean daily feed intake along with total feed consumption during lactation. Sows fed the RSB diet also tended to have reduced ($P < 0.1$) weight loss and feed consumption than SBM sows during lactation.

Table 3.6

MEAN SOW BODY WEIGHTS (± SE) AT FARROWING AND WEANING WITH SOW'S FEED INTAKE AND WEIGHT LOSS DURING LACTATION

	SBM	**RSB**	**P Value**
Pre-Farrow Weight (kg)	208.7 ± 6.0	202.6 ± 7.2	0.35
Weaning Weight (kg)	172.1 ± 7.0	175.9 ± 6.6	0.77
Weight Loss During Lactation (kg)	36.6 ± 3.9	26.8 ± 5.8	0.09
Total Feed Consumption	147.6 ± 7.2	130.1 ± 5.7	0.08
Mean Daily Feed Intake	5.3 ± 0.3	4.7 ± 0.2	0.08

Return to Estrus

Post weaning, sows were removed from farrowing crates and placed in outdoor covered pens with their respective treatment groups to monitor return to estrus. Sows were returned to their original group pens in the months of June through September.

There were no differences (P > 0.05) for days to return to estrus between treatments (RSB, 15.9 ± 4.5 d; SBM, 10.9 ± 4.1 d, respectively); however, sows that were weaned between July 28 and August 21 exhibited prolonged return to estrus or did not exhibit signs of estrus in both treatment groups. Average maximum and minimum ambient temperatures from July 28 to August 21 were 36.07 ± 0.35^{o}C and 28.71 ± 0.23 ^{o}C, respectively. It is speculated that the sows may have experienced heat stress from the high temperatures during the summer months which could have caused the irregularities seen in the return to estrus.

Discussion

Estrogens are a key component to the development of the female reproductive system. Because phytoestrogens are weak estrogens and have the ability to bind competitively with estrogen receptors, they have the ability to mimic the actions of endogenous estrogens. In animals with reduced concentrations of estrogen, the administration of phytoestrogens has been shown to induce changes in reproductive tissues (Ford *et al*., 2006). In pre-pubertal animals, serum estrogen concentrations have not reached thresholds capable of eliciting an estrus response. The administration of phytoestrogens could provide the needed serum concentrations of estrogen to elicit that response. The present study evaluated the effects of feeding a raw soybean diet, a dietary source of phytoestrogens, on initiation of puberty and reproductive function when fed to gilts.

Age at first estrus was not affected by the use of raw soybeans to the gilts diet. Jefferson *et al.* (2005) reported that pre-pubertal mice receiving doses of 0.5 to 5.0

mg/kg/d of phytoestrogens by subcutaneous injection appeared to exhibit signs of puberty earlier than control mice and mice receiving 50 mg/kg/d of phytoestrogens. However, the mean age at puberty did not differ between the treatments. In the current study, by the second weed of estrus detection in experiment 1, 70% of the gilts consuming the RSB diet exhibited estrus compared to 40% of the gilts consuming the SBM diet. In experiment 2, by the second week of estrus detection 40% of both the RSB and SBM gilts exhibited estrus. The mean age at puberty however did not differ between the treatment groups in either experiment of the current study. Puberty in females has been defined as the age at first estrus and the age at first ovulation (Senger, 1999a). The inability of the prepubertal animal to exhibit estrus is due to the lack of GnRH secreted from the hypothalamus (Senger, 1999a). Gonadotropin releasing hormone is responsible for the release of FSH and LH from the anterior pituitary (Senger, 1999a). Follicle stimulating hormone and LH are essential for the initiation of folliculogenesis and the production of estradiol (Howles, 2000). Estradiol is responsible for the stimulation of the surge center of the hypothalamus (Karsch *et al*., 1997). The administration of exogenous estrogen has the ability to stimulate the hypothalamic surge center and cause the release of GnRH (Senger, 1999a). The age of exposure along with the amount of phytoestrogens consumed could explain why there are discrepancies in the ability of phytoestrogens to influence age to puberty.

In this study, there were no differences in number of CL's present between treatments. In a study conducted by Teague (1955), gilts fed a diet consisting of alfalfa, a legume which contains coumestans (a class of phytoestrogens), possessed increased number of CL's than gilts fed a legume free diet. Coumestans have been shown to have a

greater estrogenic activity than isoflavones and can elicit estrogenic activity at decreased doses (Dixon, 2004). However, Jefferson and others (2005), reported reduced numbers of CL's in prepubertal mice administered genistein at concentrations of 0.5, 5 and 50 mg/kg/d when administered subcutaneously compared to controls. Faber and Hughes (1993) showed that neonatal mice treated with increased doses of genistein (≥ 0.1 mg/kg) had decreased pituitary responses compared to controls. This decrease could explain the reduced number of CL's present in the genistein treated mice. The studies by Jefferson and Teague indicate that neonatal exposure and prepubertal exposure may affect the reproductive system and the number of CL's. One difference in the studies conducted by Teague and Jefferson was the class of phytoestrogens administered. Teague fed gilts alfalfa which is known to contain coumestans and Jefferson administered genistein. Coumestans have been shown to have the greatest estrogenic potency of the phytoestrogens discussed (Whitten and Patisaul, 2001) and therefore could account for the differences seen between the two studies. The length of dietary exposure to phytoestrogens, concentration of phytoestrogens, and age of gilts at exposure could explain why Jefferson and others saw decreased numbers of CLs which were not observed in the present study. There is also the possibility that phytoestrogens are metabolized differently by swine than mice.

In humans, the administration of phytoestrogens in conjunction with progesterone has been shown to increase conception rates by women who have declining serum progesterone and estradiol concentrations (Unfer *et al*., 2004). In the current study, there were no observed adverse effects on pregnancy outcome from consumption of phytoestrogens in either experiment. Woclawek-Potocka *et al*. (2005) found that Holstein

cows fed soybeans exhibited lower pregnancy rates than those fed a non-soybean diet. However, in the same study, the addition of soybeans to heifers diet did not affect pregnancy rates. It appears there might be a possible maturity factor that influences the effects phytoestrogens have on reproductive performance. In the pre-pubertal animal, estradiol concentrations are not as elevated as in mature animals due to the lack of GnRH being released from the hypothalamus. Increased concentrations of estradiol send negative feedback to the hypothalamus causing a stoppage in the secretion of LH and FSH (Griffin and Ojeda, 2000). Therefore, administration of minute doses of exogenous estrogens in prepubertal/young animals may not have the same inhibitory effect as seen by mature animals (Diekman and Anderson, 1982).

Previous research has shown that phytoestrogens may have a positive effect on number of pigs born alive and litter weights. Teague (1955) reported an increase in the number of pigs born alive along with survival rate of pigs to weaning from gilts fed a diet containing alfalfa. Crenshaw and Danielson (1985) saw no differences in the number of pigs born alive to sows fed a raw soybean diet during gestation, but did report that sows which were fed the raw soybean diet forrowed pigs with heavier birth weights. Although the average litter size and weights did not differ in experiment 2 of the present study, there was a decrease in the number of pigs weaned with the RSB gilts compared to the SBM gilts. To gain a better understanding of why this difference was observed, pigs were divided into three birth weight divisions; Light (< 0.9 kg), Medium (0.91 to 1.6 kg), and Heavy (> 1.6 kg). In the light weight group, pigs from RSB fed sows outnumbered pigs from SBM fed sows. Pigs from sows in the light weight group had a 24% survival to weaning versus 45% of the light weight SBM pigs. There was also a significant increase

in the number of SBM pigs in the heavy weight group than the RSB pigs. The finding of this study are consistent with other studies that have reported pre-wean death loss is greatly influenced by pig birth weight (Winters *et al.,* 1947; Rehfeldt and Kuhn, 2006). However due to the uncertainty of the level of phytoestrogen exposure in this study, it cannot be concluded that phytoestrogens are have any responsiblity for the reduction in number of pigs weaned.

Serum progesterone and estradiol concentrations did not differ between treatment groups at standing estrus or diestrus. Progesterone concentrations differed between estrus and diestrus, and were constant with those values reported in the literature for gilts reaching puberty (Esbenshade *et al*., 1982). There has been research that indicates exposure to phytoestrogens may inhibit progesterone production (Hughes *et al*., 1991; Nynca, *et al*., 2006). Hughes and others reported that the method of administration along with metabolic degradation may also play a significant role in the bioavailability of phytoestrogens in systemic circulation. The same researchers also noted that phytoestrogens administered orally did not cause the same inhibitions as parenteral administration.

Estradiol concentrations were increased during estrus than diestrus in both groups with no differences between treatments during each stage. There have been some discrepancies as to whether phytoestrogens stimulate estrogen production. Markarevich and others (1997) reported that phytoestrogens increased the release of estradiol from the ovarian follicles of swine. However, Nynca and Ciereszko (2006) found no differences in estradiol production from porcine follicles. It is possible that sampling time in the current study may not have provided the most accurate sample for analysis. Studies have shown

that estradiol can be found in the highest concentrations around at about 2 days before standing estrus (Esbenshade *et al*., 1982). Serum plasma was collected at standing estrus in the present study to limit the invasiveness of collecting multiple samples and reducing the amount of stress placed on the gilts.

Return to estrus interval did not differ between the two treatment groups. Sows were removed from treatment diets during lactation and placed on a standard corn-soybean meal based lactation diet. Previous research has shown that removal of phytoestrogens from the diet can alleviate symptoms caused by phytoestrogen exposure (Adams, 1995). The half life of plasma phytoestrogens is estimated to be between 5 and 8 hrs (Mazur, 2000). Therefore, sows would potentially be able to metabolically clear any phytoestrogens present in their system. Although sows mean return to estrus interval was not different, sows weaned in after July 28 exhibited prolonged return to estrus intervals or did not exhibit any signs of estrus. Average maximum ambient temperatures from July 28 to August 21 were above 36^{o}C in the present study. Heat stress has been shown to cause increase in days to estrus in swine (Edwards *et al.,* 1968; Teague *et al.,* 1968; Koketsu and Dial, 1997; St-Pierre *et al.,* 2003) and can be attributed to the delay in return to estrus seen in the present study.

References Cited

Adams, N. R., M. R. Sanders, and A. J. Ritar. 1988. Oestrogenic damage and reduced fertility in ewe flocks in south Western Australia. Australian Journal of Agricultural Research. 39:71-77.

Adams, N. R. 1995. Detection of the effects of phytoestrogens on sheep and cattle. Journal of Animal Science. 73:1509-1515.

Crenshaw, M. A., and D. M. Danielson. 1985. Raw soybeans for gestating swine. Journal of Animal Science. 60(1):163-170.

Diekman, M. A. and L. L. Anderson. 1982. Quantification of receptors for estradiol-17β and progesterone in the pituitary and hypothalamus of prepubertal gilts induced to ovulate with pregnant mare's serum and human chorionic gonadotropin. Biology of Reproduction. 27:816-826.

Dixon, R. A. 2004. Phytoestrogens. Annual Review of Plant Biology. 55:225-261.

Drane, H. M., A. E. Wrathall, D. S. Patterson, and C. N. Hebert. 1981. Possible oestrogenic effects of feeding soyameal to prepuberal gilts. British Veterinary Journal. 137(3):283-288.

Dusza, L., R. Ciereszko, D. J. Skarzynski, L. Nogowski, M. Opalka, B. Kaminska, A. Nynca, O. Kraszewska, M. Slomczynska, Il Woclawek-Potocka, A. Korzekwa, E. Pruszynska-Oszmalek, and K. Szkudelska. 2006. Mechanism of phytoestrogens action in reproductive processes of mammals and birds. Reproductive Biology. 6 (Suppl. 1):151-174.

Edwards, R. L., I. T. Omtvedt, E. J. Tuesman, D. F. Stephens and G. W. A. Mahoney. 1968. Reproductive performance of gilts following heat stress prior to breeding and in early gestation. Journal of Animal Science. 27:1634-1637.

Esbenshade, K. L., A. M. Paterson, T. C. Cantley and B. N. Day. 1982. Changes in plasma hormone concentrations associated with the onset of puberty in the gilt. Journal of Animal Science. 54:320-324.

Faber, K. A. and C. L. Hughes, Jr. 1993. Dose-response characteristics of neonatal exposure to genistein on pituitary responsiveness to gonadotropin releasing hormone and volume of the sexually dimorphic nucleus of the preoptic area (SDN-POA) in postpubertal castrated female rats. Reproductive Toxicology. 7:35-39.

FASS. 1999. Guide for the care and use of agricultural animals in agricultural research and teaching. 1st revised Ed.

Ford, J. A, Jr., S. G. Clark, E. M. Walters, M. B. Wheeler, and W. L. Hurley. 2006. Estrogenic effects of genistein on reproductive tissues of ovariectomized gilts. Journal of Animal Science. 84:834-842.

Griffin, J. E. and S. R. Ojeda. 2000. Female reproductive function. Page 220 in Textbook of Endocrine Physiology. Oxford University Press, New York, NY.

Howles, C. M., 2000. Role of LH and FSH in ovarian function. Molecular and Cellular Endocrinology. 161:25-30.

Hughes, C. L., Jr., R. S. Kaldas, A. S. Weisinger, C. E. McCants, and K. B. Basham. 1991. cute and subacute effects of naturally occurring estrogens on luteinizing hormone secretion in the ovariectomized rat: Part 1. Reproductive Toxicology. 5:127-132.

Jefferson, W. N., E. Padilla-Banks, and R. R. Newbold. 2005. Adverse effects on female development and reproduction in CD-1 mice following neonatal exposure to the phytoestrogen genistein at environmentally relevant doses. Biology of Reproduction. 73:798-806.

Kaldas, R. S., and C. L. Hughes, Jr. 1989. Reproductive and general metabolic effects of phytoestrogens in mammals. Reproductive Toxicology. 3:81-89.

Karsch, F. J., J. M. Bowen, A. Caraty, N. P. Evans and S. M. Moenter. 1997. Gonadotropin-releasing hormone requirements for ovulation. Biology of Reproduction. 56:303-309.

Koketsu, Y. and G. D. Dial. 1997. Factors influencing the postweaning reproductive performance of sows on commercial farms. Theriogenology. 47:1445-1461.

Makarevich, A., A. Sirotkin, T. Taradajnik and P. Chrenek. 1997. Effects of genistein and lavendustin on reproductive processes in domestic animals in vitro. Journal of Steroid Biochemistry and Molecular Biology. 63(4-6):329-337.

Nynca, A. and R. E. Ciereszko. 2006. Effect of genistein on steroidogenic response of granulose cell populations from porcine preovulatory follicles. Reproductive Biology. 6(1):31-49.

Rehfeldt C. and G. Kuhn. 2006. Consequences of birth weight for postnatal growth performance and carcass quality in pigs as related to myogenesis. Journal of Animal Science. 84:E113-E123.

Rozman, K. K., J. Bhatia, A. M. Calafat, C. Chambers, M. Culty, R. A. Etzel, J. A. Flaws, D. K. Hansen, P. B. Hoyer, E. H. Jeffery, J. S. Kesner, S. Marty, J. A. Thomas and D. Umbach. 2006. NTP-CERHR Expert panel report on the reproductive and developmental toxicity of genistein. Birth Defects Research (Part B). 77:485-638.

SAS Institute Inc., SAS 9.1.3 Help and Documentation, Cary, NC: SAS Institute Inc., 2000-2004.

Senger, P. L. 1999a. The onset of puberty. In *Pathways to Pregnancy and Parturition*. 1st Revised Edition. 100-114. Ephrata, PA. The Mack Printing Group-Science Press.

St-Pierre, N. R., B. Cobanov and G. Schnitkey. 2003. Economic losses from heat stress by US livestock industries. Journal of Dairy Science. 86(E. Suppl.):E52-E77.

Teague, H. S., 1955. The influence of alfalfa on ovulation rate and other reproductive phenomena in gilts. Journal of Animal Science. 14:621-627.

Teague, H. S., W. L. Roller and A. P. Grifo, Jr. 1968. Influence of high temperature and humidity on the reproductive performance of swine. Journal of Animal Science. 27:408-411.

Unfer, V., M. L., Casini, S. Gerli, L. Costabile, M. Mignosa, and G. C. Di Renzo. 2004. Photoestrogens may improve the pregnancy rate in in vitro fertilization-embryo transfer cycles: a prospective, controlled, randomized trial. Fertility and Sterility. 82(6):1509-1513.

Wang, H., and P. A. Murphy. 1994. Isoflavone composition of American and Japanese soybeans in Iowa: Effects of variety, crop yare, and location. Journal of Agriculture and Food Chemistry. 42:1674-1677.

Whitten, P. L., C. Lewis, E. Russell, and F. Naftolin. 1995. Potential adverse effects of phytoestrogens. Journal of Nutrition. 125:771S-776S.

Whitten, P. L. and H. B. Patisaul. 2001. Cross-species and interassay comparisons of phytoestrogen action. Environmental Health Perspectives. 109(1):5-20.

Winters, L. M., J. N. Cummings and H. A. Stewart. 1947. A study of factors affecting survival from birth to weaning and total weaning weight of the litter in swine. Journal of Animal Science. 6:288-296.

Woclawek-Potocka, I., M. M. Bah, A. Korzekwa, M. K. Piskula, W. Wiczkowski, A. Depta and D. J. Skarzynski. 2005. Soybean-derived phytoestrogens regulate prostaglandin secretion in endometrium during cattle estrous cycle and early pregnancy. Experimental Biology and Medicine. 230:189-199.

CHAPTER IV

COMPARISON OF A GROUND RAW SOYBEAN AND SOYBEAN MEAL DIET ON CARCASS TRAITS OF GILTS

Abstract

As part of an ongoing reproductive efficiency study with gilts fed a raw soybean based diet, an assessment of carcass traits was performed to measure the effect of antigrowth factors present in raw soybeans. Yorkshire x Landrace crossbred gilts (n = 20) were assigned to balanced iso-nitrogenous (CP 14%), iso-caloric diets using either soybean meal (SBM, n = 10) or ground raw soybeans (RSB, n = 10) as the protein supplement. Gilts were fed to between d 35 and 45 of gestation and then harvested for recovery of the reproductive tract and carcass assessment. Carcass trait values included: a decrease (P = 0.05) of hot carcass weight (HCW) of gilts consuming the RSB diet, but no differences for carcass length and back fat thickness (cm) at 1st rib, 10th rib, last rib, last lumbar and average back fat. Loin area cm^2, % fat free lean (FFL), muscle score, and USDA grade scores did not differ (P > 0.05).

Introduction

Soybean meal has been traditionally used in cattle and swine diets because of the protein and fat content. However, raw soybeans contain a number of compounds including allergenic proteins, goitrogens, lectins, phytates, phytoestrogens, protease (trypsin) inhibitors and saponins to name a few (Liener, 1994; Cheeke, 1998). Processing of the beans by proper roasting and cooking, as in micronization and well regulated oil extraction procedures, destroy most of these compounds without detracting from the protein quality (Frape, 2004). Of particular concern when using raw soybeans as a dietary protein supplement is the presence of protease inhibitors that block enterokinase activity on trypsin activation in the small intestine consequently reducing protein digestion and absorption in the gastrointestinal tract. Soybean-derived protease inhibitors are problematic for nonruminants including the pig if raw soybeans form a major part of the animals' diet. One problem associated with consumption of raw soybean diets by some species is the enlargement of the pancreas (Yen *et al.,* 1977). This enlargement is associated with hypersecretion of pancreatic enzymes leading to reduced digestibility of proteins thereby reducing nutrient utilization and pigs appear to be especially sensitive (Yen *et al*, 1977). For these reasons raw soybeans are not commonly used in swine diets due to the effect of antigrowth on young swine (Jimenez *et al*., 1963; Combs *et al*., 1967; Young, 1967).

Raw soybeans are abundant in phytoestrogens (plant estrogens) that are known to have estrogen-like effects under appropriate conditions. For example, raw soybean diets have been used to improve reproductive efficiency by sows (Crenshaw, 1985a), but may possibly alter carcass traits. Very little research has been done with raw soybeans and the

effects on growth and carcass quality in livestock. One recent study reported that incremental increasing the inclusion of raw soybeans (from 0 through to 24%) in the diet of feedlot steers had little effect on overall weight gain, feed efficiency or carcass quality (Felton and Kerley, 2004). Jimenez and others (1963) found no differences in carcass parameters in feeder pigs fed raw soybeans. However, a study conducted at Louisiana State University found that finishing pigs fed ground raw soybeans exhibited reduced carcass weights and smaller loin eye areas (Pontif *et al.,* 1987). These reports suggest that age of pigs and percent of raw soybean content of the overall diet may result in different growth performances and carcass traits in swine.

Thus, as part of an ongoing reproductive efficiency study with gilts fed a ground raw soybean diet, an assessment of carcass traits was performed to measure possible negative effects of the anti-trypsin factor present in raw soybeans on carcass quality.

Materials and Methods

This study followed the FASS (1999) *Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching* and was approved by the Mississippi State University Institutional Animal Care and Use Committee (Protocol No. 04-072).

Animals

Twenty pre-pubertal Yorkshire x Landrace crossbred gilts (BW 79.5 ± 1.4 kg; approx. 144 d, age; Monsanto genetics) were purchased from Prestage Farms (West Point, MS) and housed at the Physiology Research Unit at the Mississippi Agriculture and Forestry Experiment Station (Starkville, MS). Gilts were matched by weight and

assigned to one of two dietary treatments (n = 10/treatment) and initially allowed to acclimatize to their environment on a pre-treatment standard gestation corn soybean meal based diet for eleven days. Gilts were vaccinated for Leptospirosis, Parvovirus and Erysipelothrix (Farrow-Sure Plus B, Pfizer), de-wormed and re-weighed before introducing to the experimental diets. Diet one consisted of soybean meal (SBM; control) as the primary source of protein while the primary protein source of diet two (n = 10) consisted of ground raw soybeans (RSB). Diets were formulated to be iso-nitrogenous at 14% crude protein and iso-caloric for metabolizable energy. Ground raw soybeans accounted for about 75% of the protein source for the first four weeks (phase I) of the study then increased to a 100% dietary protein source thereafter (phase II). The control diet (SBM) was supplemented with poultry fat to equalize the metabolizable energy content of RSB (Table 4.1). Gilts were placed on dietary treatments at approximately 155 days of age with an average body weight of 84.9 ± 1.5 kg and housed in outdoor covered pens with *ad libitum* access to food and water. As part of a larger study, gilts were monitored for estrus from about 160 days of age with a mature teaser boar and bred at the third observed standing estrus by artificial insemination (AI). Following AI, gilts were individually penned and placed on restricted feed of 2.2 kg/day of their respective diets until they were slaughtered between 35 and 45 d of gestation for recovery of the reproductive tract (assessment of ovarian function, conception rates) and carcass evaluation. Gilts were maintained on SBM and RSB for 116.8 ± 5.8 and 112.6 ± 4.0 d, respectively. The slight difference in length of diet exposure is due to the difference in age at which animals were bred and ultimately slaughtered.

Table 4.1

SWINE DIETS USING SOYBEAN MEAL (SBM) OR RAW SOYBEANS (RSB) AS DIETARY PROTEIN SOURCE

	Phase I - 75% RSB		Phase II - 100% RSB	
Ingredients, %	SBM	RSB	SBM	RSB
Corn	75.04	71.46	74.62	69.72
Soybean meal, 48%	17.86	4.68	17.93	
Raw soybean, ground		17.86		24.28
Poultry fat	1.10		1.45	
Sow 120 Premix*	6.00	6.00	6.00	6.00
Total	100	100	100	100
Calculated Analysis				
Protein	14.00	14.00	14.00	14.00
Calcium	1.44	1.45	1.44	1.46
Phosphorous	0.96	0.95	0.96	0.94
Lysine	0.71	0.70	0.71	0.70
ME, kCal/kg	3262	3261	3280	3280
Laboratory Analysis				
Protein	14.10	13.90	14.80	13.80
Calcium	1.28	1.44	1.50	1.60
Phosphorous	0.95	0.95	1.10	1.10

*Sow 120 premix (Multi-Kare, Inc., Tifton, GA) guaranteed analysis: Ca (19% – 22.8%), P (10%), NaCL (7%-8.4%), SE (0.0005%), biotin (8.38 mg/kg), vitamin A (261,242, USP units/kg), vitamin D (40,123 USP units/kg), vitamin E (750.0 INT units/kg)

Carcass Evaluation

Live weight of gilts was recorded prior to transport to the Mississippi State University Meats Laboratory (2.4 km). Gilts were humanely slaughtered the same morning of arrival at the Mississippi State University Meats Laboratory in accordance with the Humane Slaughter act (USDA, 1978). At time of harvest, carcasses were evaluated and measurements recorded included: backfat (taken from the 1st rib, last rib,

last lumbar, and 10th rib) while carcass length and loin eye area were obtained after a 24 hour chill at 2° C. Marbling quality and color rank were assessed by a trained evaluator using the National Pork Producers Council color and marbling standards.

Statistical Analysis

This experiment was analyzed as a complete randomized design with two treatments, consisting of RSB and SBM. Statistical analyses were performed using the GLM procedure of SAS 9.1 (SAS Institute, Cary, NC) to determine if a significant treatment effect existed between diets and carcass attributes. Significance was determined at $P<0.05$.

Results and Discussion

The aim of this investigation was to determine whether gilts (from approximately day 155 to 268 of age) maintained on a diet supplemented with raw soybeans as the main source of dietary protein enhanced reproductive efficiency and how RSB might affect carcass traits compared to gilts (from approximately day 155 to 272 of age) maintained on a control gestation diet supplemented with soybean meal. Throughout the trial, gilts showed a similar rate of weight gain regardless of source of protein (Table 4.2) and no differences for live weights were noted at the time of harvest between the two dietary treatment groups (SBM, 163.2 ± 2.8 kg; RSB 157.6 ± 3.0 kg). Importantly, gestational age at time of slaughter for both groups of bred gilts was similar (SBM, 38.9 ± 2.54 and RSB 43.0 ± 0.92 days post AI) with the variation due to the election to harvest two SBM gilts approximately ten days early due to severe lameness. Furthermore, average daily

gain and gain/feed were also not different during a 4-week period prior to onset of first estrus (SBM, 0.91 kg ± 0.1 and 0.36 ± 0.02; RSB, 0.97 kg ± 0.2 and 0.32 ± 0.05, respectively).

Table 4.2

BODY WEIGHTS AND AGES OF GILTS FED A SOYBEAN MEAL OR RAW SOYBEAN BASED DIET

Dietary Treatments*	SBM[1]	RSB[1]	SBM	RSB	P value[1]
	Mean body weights (kg)		Age (d)		
Phase I - 75% RSB					
Initial	84.7 ± 2.3	85.2 ± 2.1	155	155	0.874
Final	110.3 ± 3.0	112.3 ± 2.7	183	183	0.630
Phase II - 100% RSB					
Puberty			193.0 ± 7.3	184.3 ± 3.9	0.776
Breeding	147.8 ± 6.4	136.3 ± 3.8	232.9 ± 7.3	225.6 ± 3.6	0.396
Final	163.2 ± 2.8	157.6 ± 3.0	271.8 ± 5.8	267.6 ± 4.0	0.159

* Dietary Treatments - SBM, Soybean Meal; RSB, Raw Soybeans

[1] P Values for body weight only; ages were not significantly different

Gilts were maintained on their respective diets through the first 35-45 days of gestation before being harvested for carcass evaluation. Table 4.3 shows the results of the carcass parameters measured. Hot carcass weight ($p = 0.05$) was reduced in RSB compared to SBM fed gilts; 112.6 ± 2.8 vs 120.2 ± 2.3 kg, respectively. However, this may be attributed to the fact that gilts on the RSB diet were taken off *ad libitum* feed at a lighter BW than the control gilts due, in part, to RSB gilts attained puberty earlier, and were placed on a restricted dietary intake (2.3 kg/d) following breeding until harvest. There were no observed differences in backfat thickness and loin eye area between the

two groups (Table 4.3). In addition, no differences were observed in any of the carcass attributes measured including percent fat free lean and marbling quality (Table 4.4). Felton and Kerley, (2004) reported a reduction in hot carcass weight and longissimus muscle area by steers fed whole raw soybeans but in a second experiment with the same parameters no differences were detected between groups.

Incremental addition of raw soybeans to the diet as a supplemental source of protein resulted in a linear decrease in daily weight gain and a quadratic decrease in feed efficiency when fed to pigs with a starting weight of approximately 60 kg and for an average of 61 days (Pontif *et al*., 1987). In contrast, no reduction of longisthmus muscle was detected in this study. Difference of the initial weight and age of pigs as well as genetic improvements in the swine industry could be factors that contributed to these reported differences. It appears from earlier studies that growing and finishing pigs did not perform to their full potential when fed a raw soybean supplemented diet (Jimenz *et al*., 1963; Combs *et al*., 1967; Young, 1967; Hanke *et al*., 1972; Yen *et al*., 1977, Vandergrift *et al*., 1983). Crenshaw and Danielson (1985a) saw no significant differences of the reproductive performance or weight gain of gestating sows for three reproductive parities fed a raw soybean based diet compared to sows fed a traditional soybean meal diet, while the same authors in a similar study reported that finishing pigs were unable to utilize raw soybeans as efficiently as soybean meal (Crenshaw and Danielson, 1985b).

Table 4.3

CARCASS MEASUREMENTS OF GILTS FED A SOYBEAN MEAL OR RAW SOYBEAN BASED DIET

Dietary Treatment*	SBM	RSB	P value
Weights (kg)			
Live, Final	163.2 ± 2.8	157.6 ± 3.0	0.159
Hot Carcass	120.2 ± 2.3	112.6 ± 2.8	0.051
Carcass Length (cm)	89.9 ± 1.2	89.9 ± 0.9	1.000
Loin area (cm^2)	45.0 ± 2.2	43.0 ± 1.7	0.487
Backfat Thickness (cm)			
1^{st} rib	4.7 ± 0.2	5.1 ± 0.2	0.277
Last rib	3.2 ± 0.1	3.0 ± 0.2	0.570
Last lumbar	2.1 ± 0.1	2.4 ± 0.2	0.254
AVG	3.3 ± 0.2	3.5 ± 0.2	0.515
10^{th} rib	3.1 ± 0.2	2.8 ± 0.2	0.372

***** SBM, Soybean Meal; RSB, Raw Soybeans

Table 4.4

CARCASS ATTRIBUTES OF GILTS FED A SOYBEAN MEAL OR RAW SOYBEAN BASED DIET

Dietary Treatment*	SBM	RSB	P value
Fat Free Lean, %	47.6 ± 0.9	48.3 ± 0.8	0.560
USDA Grade	2.6 ± 0.3	2.7 ± 0.3	0.903
Muscle score	2.4 ± 0.1	2.1 ± 0.1	0.145
Marbling quality	2.2 ± 0.1	2.3 ± 0.2	0.628
Color rank	3	3	1.000

***** SBM, Soybean Meal; RSB, Raw Soybeans

Conclusion

This study examined the possible adverse effects of feeding a ground raw soybean diet to pre-pubertal gilts on carcass quality. The only effect observed when feeding ground raw soybeans was a reduction in hot carcass weight. All other growth and carcass parameters measured showed no differences between the treatment groups. Our findings, using the current genetic strain of pigs, suggest that feeding raw soybeans to pre-pubertal gilts through to early gestation has no adverse effects on carcass quality traits.

References Cited

Cheeke, P.R. 1998. Toxins in protein supplements and grain legumes. In Natural Toxicants in Feeds, Forages, and Poisonous Plants. Interstate Publishers Inc., Danville, IL, USA, pp163-199.

Combs, G. E., R. G. Conness, T. H. Berry and H. D. Wallace. 1967. Effect of raw and heated soybeans on gain, nutrient digestibility, plasma amino acids and other blood constituents of growing swine. Journal of Animal Science. 26:1067-1071.

Crenshaw, M. A. and D. M. Danielson. 1985a. Raw soybeans for gestating swine. Journal of Animal Science. 60:725-730.

Crenshaw, M. A. and D. M. Danielson. 1985b. Raw soybeans for growing-finishing pigs. Journal of Animal Science. 60:163-170.

FASS. 1999. Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching. 1st rev. ed. Federation of Animal Science Societies, Savoy, IL.

Felton, E. E. and M. S. Kerley. 2004. Performance and carcass quality of steers fed whole raw soybeans at increasing inclusion levels. Journal of Animal Science. 82(3):725-732.

Frape, D. 2004. Ingredients of Horse Feeds, in Equine Nutrition, Third edition, Blackwell Publishing, Oxford, UK, pp 116-185.

Hanke, H.E., Rust, J.W., Meade, R.J. and Hanson, L.E. 1972. Influence of source of soybean protein, and of pelleting, on rate of gain and gain/feed or growing swine. Journal of Animal Science. 35:958-962.

Jimenez, A. A., T. W. Perry, R. A. Pickett and W. M. Beeson. 1963. Raw and heat-treated soybeans for growing-finishing swine, and their effect on fat firmness. Journal of Animal Science. 22:471-475.

Liener, I.E. 1994. Implications of antinutritional components in soybean foods. Critical Reviews in Food Science and Nutrition. 34:31-67.

NPPC Official Color and Marbling Standards. National Pork Producers Council. Des Moines, IA 50306

Pontif, J. E., L. L. Southern, D. F. Coombs, K. W. McMillin, T. D. Bidner and K. L. Watkins. 1987. Gain, feed efficiency and carcass quality of finishing swine fed raw soybeans. Journal of Animal Science. 64:177-181.

SAS Institute Inc., SAS 9.1.3 Help and Documentation, Cary, NC: SAS Institute Inc., 2000-2004.

Vandergrift, W.L., Knabe, D.A., Tanksley Jr, T.D. and Anderson, S.A. 1983. Digestibility of nutrients in raw and heated soyflakes for pigs. Journal of Animal Science. 57:1215-1224

Yen, J.T., Jensen A.H., and Simon J. 1977. Effect of dietary raw soybean and soybean trypsin-inhibitor on trypsin and chymotrypsin activities in the pancreas and in small intestinal juices of growing swine. Journal of Nutrition. 107:156-

Young, L. G. 1967. Raw soybeans in swine growing-finishing rations. Canadian Journal of Animal Science. 47:227-233.

CHAPTER V

THE USE OF DIGITAL INFRARED THERMAL IMAGING TO DETECT ESTRUS IN THE GILT

Abstract

Yorkshire x Landrace crossbred gilts were evaluated using digital infrared thermal imaging to discriminate between estrus and diestrus phases of the porcine estrous cycle. Gilts (n = 32) were part of an ongoing reproductive efficiency study involving the use of raw soybean versus soybean meal as a source of dietary protein. Diets were iso-nitrogenous (CP 14%) and iso-caloric using either soybean meal (SBM; n = 17) or ground raw soybean (RSB; n = 15) as the protein source. Gilts were monitored daily for signs of estrus using a teaser boar. Thermal images of vulva surface temperatures (TEMP) were recorded at standing estrus and diestrus. Measurements for analysis included minimum (MIN), maximum (MAX), mean (AVG) and standard deviation (SD) of temperature gradients. At imaging, ambient (AMB) and rectal temperatures (RT) were recorded, and blood samples taken for serum progesterone (P4) concentration analysis (by RIA) to confirm stage of cycle. Mean serum P4 values at estrus and diestrus were 1.0 ± 0.1 and 10.9 ± 0.8 ng/ml, respectively. Maximum vulva thermal images were positively correlated with minimum ($r = 0.614$; $P < 0.0001$) and average ($r = 0.849$; $P < 0.0001$) vulva thermal images and was positively correlated with AMB temperature ($r = 0.360$;

P = 0.0002). MAX and AVG vulva thermal temperatures were greater ($P < 0.05$) at estrus than at diestrus (36.6 ± 0.2 and 33.4 ± 0.3°C vs. 35.6 ± 0.3 and 31.8 ± 0.6°C, respectively), while MIN and SD revealed no differences ($P > 0.05$) between stages of the cycle. No differences ($P > 0.05$) in RT were observed between stages of estrous and RT did not correlate with vulva thermal images. Diet had no effect on RT or vulva TEMP.

Introduction

Digital infrared thermal imaging (DITI) is a non-invasive technique that can be used to detect symmetrical and asymmetrical temperature gradients of surface areas. The surface of the skin acts as a cooling system emitting heat (Purohit *et al*., 1985) allowing for accurate measure of temperature gradients through thermal imaging. Changes in blood flow and vascularization of tissues can alter the surface temperature of the skin (Winsor, 1971). The normal body maintains a constant temperature and changes in temperature are easily recognized and detected by DITI and may provide a means for the discovery and monitoring of normal and abnormal physiologic events (Purohit *et al*., 1985).

In the livestock industry, DITI has been used for applications such as injury diagnosis of foot and leg problems in cattle and horses (Cockcroft *et al*., 2000; Purohit, 1980; Schmidt *et al*., 2003; Turner, 1998), assessment of scrotal temperatures as a measure of fertility in bulls and rams (Gabor *et al*., 1998; Kastelic *et al*., 1995; Kastelic *et al*., 1996; Lunstra and Coulter, 1997; Purohit *et al*., 1985) and evaluating heat stress in dairy cattle (Coppola *et al*., 2002; White *et al*., 2006). The successful use of DITI in livestock has prompted its use in wildlife and zoological species due to its non-invasive

capabilities. Researchers at the Frankfurt and Leipzig Zoological Gardens have used DITI for assessment of pregnancy in giraffe and zebra. Thermal images of the abdomen of a black rhinoceros, giraffe and Grevy zebra revealed heat signatures that were not seen in non-pregnant animals of the same species (Hilsberg *et al*., 1997; Hilsberg *et al*., 2002). More recently, thermography has been used to differentiate between pregnancy and pseudo-pregnancy of the giant panda (Durrant *et al*., 2006). However, with each of these studies only a small number of animals were observed. In domestic species, the use of thermal imaging to detect pregnancy has not been as successful. Thermal images taken of the abdomen of domestic female dogs were not able to discriminate between pregnant and non-pregnant females (Durrant *et al*., 2006). The same results were also observed in a study conducted by Jones *et al.* (2005a) using dairy heifers. However in a study using the mare as a model for pregnancy detection by thermal imaging, researchers observed mares in late gestation had greater ($P < 0.05$) average flank temperatures than nonpregnant and recently foaled mares (Bowers *et al*., 2004).

Studies evaluating estrus detection in dairy cattle using thermal imaging have met with mixed results. In a study conducted by Osawa *et al.* (2004), researchers observed an increase in vulva surface area temperature of cows in estrus versus diestrus. However, only a few animals were used for this study. Jones *et al*. (2005b) also evaluated the use of thermal imaging of the vulva as a tool for estrus detection in dairy cattle. In this study, researchers were able to discriminate first estrus from diestrus but not between estrus and diestrus in subsequent cycles.

The purpose of this study was to investigate the use of digital infrared thermal imaging to differentiate between temperature gradients of the vulva during estrus and

diestrus in the pig. The anatomy of the external genitalia of the pig lends itself to the adaptation of thermal imaging as a means of assessing estrus, especially in females prone to silent heat. The results from this study may help to develop a model for estrus detection in domestic and non-domestic species, zoological species and identifying animals that exhibit silent estrus.

Materials and Methods

This study followed the FASS (1999) *Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching* and was approved by the Mississippi State University Institutional Animal Care and Use Committee (Protocol No. 04-072).

Animals

Yorkshire/Landrace crossbred gilts of Monsanto Genetics (Approx. 144 d of age; Mean BW 84.9 ± 0.8 kg) were purchased from Prestage Farms (West Point, MS) and housed at the Physiology Research Unit at the Mississippi Agriculture and Forestry Experiment Station (Starkville, MS). Gilts were housed in outdoor covered pens and were moved into an enclosed building for imaging. Gilts were part of an ongoing two phase study evaluating the effects of a ground raw soybean diet on reproductive performance. Diets were formulated to be iso-nitrogenous (CP 14%) and iso-caloric using either soybean meal (SBM) or ground raw soybean (RSB) as the dietary protein source. The first phase commenced in November, 2005, and phase two was conducted in November, 2006. Thirty two gilts were used for this study, twelve from phase one and twenty from phase two. Gilts were allowed *ad libitum* access to feed and water. Gilts were monitored

daily (0600 to 0700 hrs) for signs of estrus using a teaser boar. At time of first standing estrus and ten days post-standing estrus (diestrus), thermal images of the vulva (Figure 5.1) were taken using a FLIR ThermaCAM S60 camera (FLIR Systems Inc, MA, USA) with the emissivity set at one. Rectal temperatures were collected with the GLA M500 hi-performance digital thermometer (GLA Agriculture Electronics, CA) along with ambient temperatures using the Kestrel 3000 pocket wind meter (Nielsen-Kellerman, PA) at time of imaging. The vulva of gilts were cleaned if soiled with fecal matter and allowed to dry for at least 30 minutes prior to imaging. Distance for imaging ranged from 121.9 to 152.4 cm from the pig. Analysis of vulva thermal images was performed using the ThermaCAM Research Professional 2.7 software (FLIR Systems Inc, MA).

Radioimmunoassay of Serum Progesterone Concentrations

Blood was collected by jugular veinapuncture after imaging for analysis of serum progesterone (P_4) concentrations using a radioimmunoassay (Diagnostic Systems Laboratories, Webster, TX; DSL-3900, DSL-4800) to confirm stage of estrous cycle. Assay sensitivity for P_4 was 0.02 ng/ml and intra- and inter-assay coefficients of variation were 6.13% and 7.40 %, respectively. Thermal images, rectal temperatures and blood serum were collected for at least two consecutive estrous cycles.

Statistical Analysis

Statistical analysis was performed using the GLM procedure of SAS 9.2 (SAS Institute, Cary, NC) to determine differences in thermal signatures at estrus and diestrus. Replicate was included in the GLM model statement to ensure no effect of study

replication was found. Pearsons correlations with Fishers r to z transformations were used to determine relationships among MAX, MIN, AVG vulva, rectal and ambient temperatures. Data with a P value less than 0.05 were considered to be significant.

Results

Thermal images of the vulva (Figure 5.1) were taken during standing estrus and diestrus for two consecutive estrous cycles. Vulva thermal images were analyzed for maximum, minimum and average temperatures and correlated with rectal and ambient temperatures taken at each imaging. Standard deviation of images was also measured to evaluate variation between temperature gradients at each imaging. Ambient temperature was positively correlated with MAX, MIN and AVG vulva temperature ($R = 0.36$, $R = 0.63$, $R = 0.50$, respectively; $P < 0.01$; Table 5.1) regardless of stage of estrous cycle. Rectal temperature was not correlated ($P > 0.05$) with vulva thermal images (MAX, MIN, AVG Vulva) nor ambient temperature during estrus. However, there was a negative correlation between rectal and ambient temperature during diestrus ($R = -0.37$; $P = 0.03$).

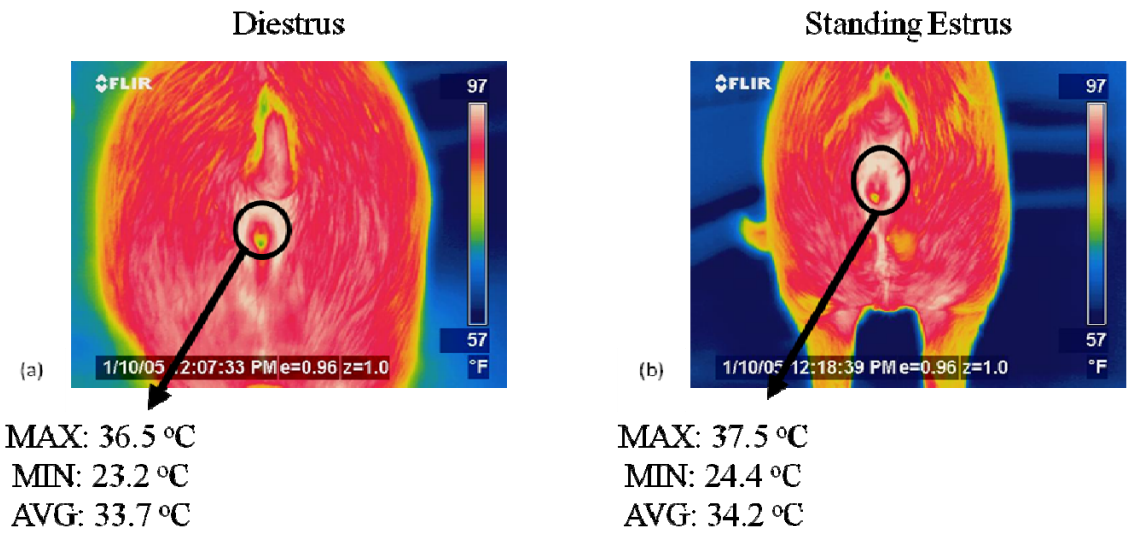

Figure 5.1 THERMAL IMAGES OF THE VULVA

The region of interest from which vulva temperatures were determined is indicated by the circle and the maximum, minimum, and average temperatures of representative gilt (a) in diestrus and (b) in standing estrus are shown in the thermal images below. The color bar on the right side of each thermal image shows the temperature range from 57 to 97 ^{o}F (13.9 – 36.1 ^{o}C). The gilt in estrus has a higher vulva temperature than the gilt in diestrus.

There were no differences ($P > 0.05$) in rectal temperature between estrus and diestrus. During estrus, vulva thermal signatures of gilts had significantly greater ($P < 0.05$) MAX and AVG surface temperatures (36.6 ± 0.2 and 33.4 ± 0.3 ^{o}C, respectively) than during diestrus (35.6 ± 0.3 and 31.8 ± 0.6^{o}C, respectively). Moreover the difference in vulva temperature between estrus and diestrus was greater ($P < 0.05$; Figure 5.2) when the ambient temperature was below 20^{o}C. There were no differences observed in the MIN and standard deviation of vulva surface temperatures between the two stages of the estrous cycle (Table 5.2). Diet had no effect ($P > 0.05$) on MAX, MIN and AVG vulva temperatures of gilts during estrus and diestrus.

Table 5.1

CORRELATION COEFFICIENTS BETWEEN MAX VULVA, MIN VULVA, RECTAL AND AMBIENT THERMAL MEASUREMENTS DURING ESTRUS AND DIESTRUS

Estrus	Ambient	Rectal	AVG Vulva	MIN Vulva
MAX Vulva	0.313*	-0.053	0.870*	0.656*
MIN Vulva	0.596*	0.011	0.842*	
AVG Vulva	0.494*	-0.099		
Rectal	-0.066			
Diestrus				
	Ambient	Rectal	AVG Vulva	MIN Vulva
MAX Vulva	0.402*	-0.068	0.815*	0.602*
MIN Vulva	0.666*	-0.115	0.823*	
AVG Vulva	0.524*	-0.087		
Rectal	-0.365*			

*Ambient temperatures are positively correlated ($P < 0.05$) with each other along with the gilt's vulva maximum (MAX), minimum (MIN) and average (AVG) temperatures regardless of stage of estrus/diestrus.

Blood was also collected at time of imaging by jugular veinapuncture for analysis of serum progesterone concentrations by radioimmunoassay. Progesterone concentrations were decreased ($P < 0.05$) in gilts at the time thermal images were taken corresponding to estrus (1.0 ± 0.1 ng/ml) compared to diestrus (10.9 ± 0.8 ng/ml).

Table 5.2

MEAN MAX, MIN, AVG AND STD OF VULVA TEMPERATURES TAKEN DURING ESTRUS AND DIESTRUS WITH CORRELATING RECTAL TEMPERATURES

	Vulva				
	Max	Min	Avg	Std	Rectal Temp
Estrus	36.64 ± 0.15 [a]	22.28 ± 0.77 [c]	33.36 ± 0.26 [d]	2.25 ± 0.12 [e]	38.83 ± 0.04 [f]
Diestrus	35.61 ± 0.33 [b]	20.64 ± 0.99 [c]	31.78 ± 0.61 [d]	2.66 ± 0.21 [e]	38.76 ± 0.05 [f]
P value	< 0.05	> 0.05	< 0.05	> 0.05	> 0.05
Dietary Treatments*					
Estrus					
SBM	36.74 ± 0.23	22.76 ± 1.15	33.66 ± 0.40	2.09 ± 0.17	38.77 ± 0.05
RSB	36.53 ± 0.19	21.66 ± 0.97	32.98 ± 0.31	2.45 ± 0.16	38.90 ± 0.06
Diestrus					
SBM	35.88 ± 0.38	21.66 ± 1.18	32.01 ± 0.84	2.50 ± 0.26	38.70 ± 0.07
RSB	35.22 ± 0.60	19.14 ± 1.71	31.43 ± 0.87	2.89 ± 0.35	38.85 ± 0.06
[1]P value	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05

*Dietary Treatments – SBM, Soybean Meal; RSB, Raw Soybeans

[1]P Value for differences in diet during each stage

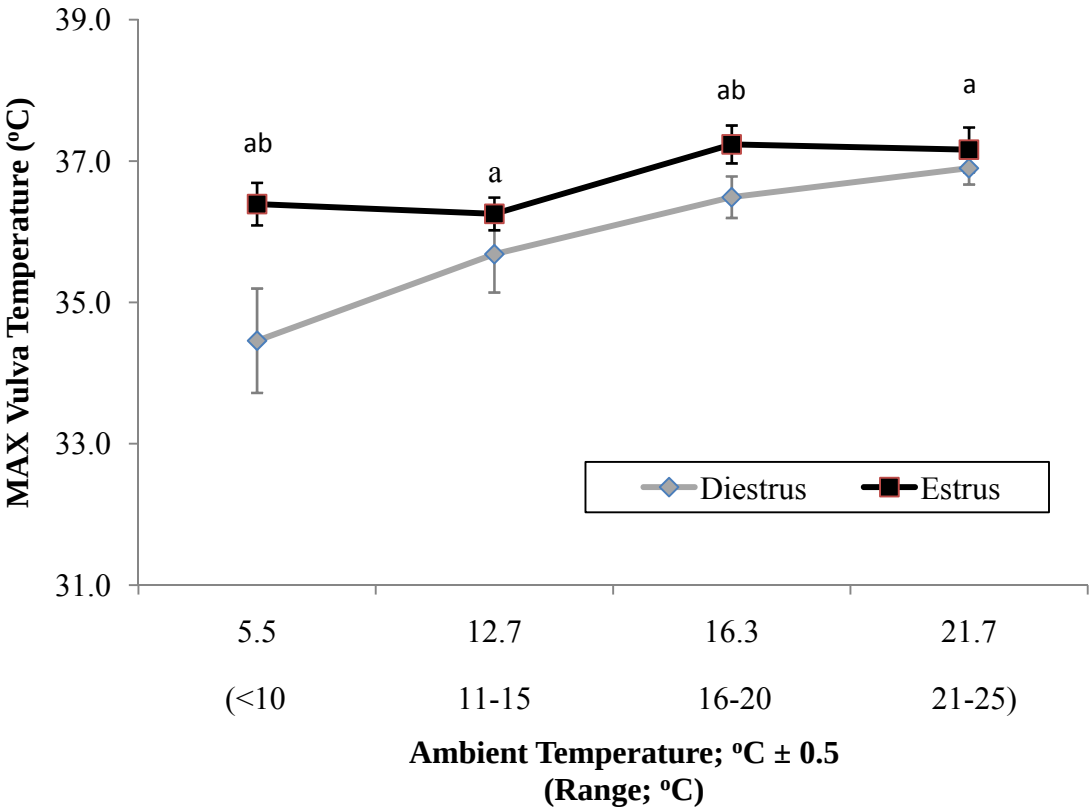

Figure 5.2 MAX VULVA TEMPERATURES AT DIFFERENT AMBIENT TEMPERATURES

Differences in MAX vulva temperature between estrus (a) and diestrus (b) were greater ($P < 0.05$) at an ambient temperature below 20°C. Gilts in estrus had higher ($P < 0.05$) MAX vulva temperatures than those in diestrus. The combined mean MAX vulva temperatures for the ambient temperature range during estrus and diestrus were 36.6 ± 0.2°C and 35.6 ± 0.3°C, respectively.

Discussion

In this study, thermal imaging proved to be an effective way to differentiate between gilts in standing estrus from those in diestrus. During estrus, blood flow to the vulva increases via the internal pudendal artery under the influence of increased circulating estradiol from the developing follicles (Frandson *et al*., 2003). This increase

in blood flow also increases surface area temperature of the vulva. Anatomically, the gilt's vulva is more prominent than other livestock species and is not obstructed by the tail making changes in temperature gradients easily detectable.

Studies using thermography to detect estrus in dairy cattle have been met with mixed results. In 2004, Osawa and colleagues were able to use thermal imaging of the vulva to differentiate between estrus and diestrus in Japanese Black and Holstein-Friesian cows; however, only three animals were used in this study and measurements were only recorded for one cycle. A recent study conducted by Jones et al. (2005b), also evaluated the use of thermal imaging to detect estrus of dairy cows. In this study, researchers were able to use thermal imaging to differentiate first estrus from diestrus after synchronization, but not between estrus and diestrus of subsequent estrous cycles. Possible differences in thermal temperatures may have been attributed to ambient temperature and environment. Debris present on the vulva surface area can also influence thermal temperatures by masking actual thermal signatures and giving false readings. It is essential to ensure the target surface is clean of any foreign material or establish a method of analysis to account for debris present on the surface when applying this technology for estrus detection in species where handling is not feasible or permissive. Target surface should be acclimated to surrounding environment at least 30 minutes prior to imaging (Roy *et al*., 2006).

Environmental conditions such as temperature and air flow can affect the accuracy of thermal images. The suggested ideal temperature for obtaining thermal images of skin surfaces is around 20^{o}C (Love and Linsted, 1975) but a temperature below 30^{o}C is acceptable (Turner *et al*., 1986). Despite the fact that ambient temperatures

remained in the acceptable range in this study (less than 25.8°C), a greater difference between estrus and diestrus was seen at an ambient temperature below 20°C. In the study conducted by Jones *et al.* (2005b), differences in vulva thermal temperature between estrus and diestrus were observed at 21.4°C but not at an ambient temperature of 12.1°C. In our study, it was also difficult to differentiate estrus from diestrus during ambient temperatures between 11 and 15°C. The reason for the inability to differentiate estrus from diestrus at this temperature range is not known. However, one might speculate that the amount of heat being radiated from the body is lower at this ambient temperature. The greatest differences in vulva thermal gradients were observed at ambient temperatures below 10°C. These data lead to the postulation that environment creates the largest obstruction for accurate thermal gradient measurements. Ambient temperature, air flow, moisture and debris are all elements that can affect thermal gradients (Cravello and Ferri, 2008). Therefore more work is needed comparing controlled verses typical management environments to improve and develop new strategies for accurate thermal measurements.

This technology could be a valuable tool for estrus detection; however it may have more value in zoological species where invasive techniques (i.e. ultrasound) or management strategies are not the same as those employed for estrus detection in the domestic setting. The ability to detect estrus non-invasively would allow animals to be observed and monitored in a more natural environment with little human interaction. However variation among species could be problematic in being able to accurately determine estrus in all species. Also estrous cycle length along with temperature gradients of the vulva needs to be mapped out over continuous cycles to evaluate

uniformity. Therefore, more research is needed of both domestic and zoological species to validate thermography as a reliable tool for estrus detection.

These data demonstrate that DITI can discriminate between vulva surface temperature during estrus and diestrus in gilts and could be used to assess the onset of estrus. The use of DITI in the swine industry could provide early detection of onset of estrus and also detecting animals exhibiting silent heat. Further research is needed to determine at what time points of the estrous cycle surface temperature of the vulva begins to increase, reaches its peak surface temperature, and returns to normal. The efficacy of DITI to discriminate estrus and diestrus in the sow also remains to be validated.

References Cited

Bowers, S., S. Gandy, B. Anderson, P. Ryan and S. Willard. 2004. Assessment of pregnancy in the mare using digital infrared thermography. ASAS/ADSA National Meeting, St. Louis, Missouri.

Cockcroft, P. D., F. D. Henson and C. Parker. 2000 Thermography of a septic metatarsophalangeal joint in a heifer. Veterinary Research. 146:258-260.

Coppola, C. L., R. J. Collier and R. M. Enns. 2002. Evaluation of thermal status of cattle using infrared technology. Proceedings of the Western Section American Society of Animal Science, Fort Collins, Colorado.

Cravello, B. and A. Ferri. 2008. Relationships between skin properties and environmental parameters. Skin Research and Technology. 14:180-186.

Durrant, B. S., N. Ravida, T. Spady and A. Cheng. 2006. New technologies for the study of carnivore reproduction. Theriogenology. 66:1729-1736.

FASS. 1999. Guide for the care and use of agricultural animals in agricultural research and teaching. 1st revised Ed.

Frandson, R. D., W. L.Wilke and A. D. Fails. 2003. Anatomy of the Female Reproductive System. In: Anatomy and Physiology of Farm Animals, Troy D (Ed.), Lippincott Williams and Wilkins, p. 394.

Gabor, G. R., G. Sasser, J. P. Kastelic, G. H. Coulter, D. O. Everson, G. Falkay, M. Mezes, S. Bozo, R. B. Cook and J. V. Csik. 1998. Endocrine and thermal responded to GnRH treatment and prediction of sperm output and viability in Holstein-Friesian breeding bulls. Theriogenology. 50:177-183.

Hilsberg, S., R. Goltenboth and K. Eulenberger. 1997. Infrared thermography of zoo animals, first experience in its use for pregnancy diagnosis. Verh Ber Erkrg Zootiere. 38:187-190.

Hilsberg, S., K. Eulenberger and F. Zahorszki. 2002. Application of infrared thermography in pregnancy diagnosis in giraffes. Leipzig. 14-15.

Jones, M., A. Denson, E. Williams, K. Graves, A. Dos Santos, A. Kouba and S. Willard. 2005a. Assessing pregnancy status using digital infrared thermal imaging in Holstein dairy heifers. ASAS/ADSA National Meeting, Cincinnati, Ohio.

Jones, M., A. Denson, S. Bowers, K. Moulton, E. Williams, K. Graves, A. Dos Santos, A. Kouba and S. Willard. 2005b. Thermography of the vulva in Holstein dairy cows: a comparison of estrus vs. diestrus. ASAS/ADSA National Meeting, Cincinnati, Ohio.

Kastelic, J. P., G. H. Coulter and R. B. Cook. 1995. Scrotal surface, subcutaneous, intratesticular and intraepididymal temperatures in bulls. Theriogenology. 44:147-152.

Kastelic, J. P., R. B. Cook, G. H. Coulter and R. G. Saake. 1996. Ejaculation increases scrotal surface temperature in bulls with intact epididymides. Theriogenology. 46:889-892.

Love, T. J. and R. D. Linsted. 1975. Theoretical basis for use of skin temperature as a plethysmographic indicator. Paper 75-WA/Bio-6. ASME Winter Annual Meeting, Houston, TX.

Lunstra, D. D. and G. H. Coulter. 1997. Relationship between scrotal infrared temperature patterns and natural-mating fertility in beef bulls. Journal of Animal Science. 75(3):767-74.

Osawa, T., M. Tanaka, M. Morimatsu, K. Hashizume and B. Syuto. 2004. Use of infrared thermography to detect the change in the body surface temperature with estrus in the cow. Proceedings from the Society for Theriogenology Annual Conference, Lexington, Kentucky.

Purohit, R. C. 1980. The diagnostic value of thermography in equine medicine. Proceedings from the Annual Convention of the American Association of Equine Practitioners. 26:317-326.

Purohit, R. C., R. S. Hudson, M. G. Riddell, R. L. Carson, D. F. Wolfe and D. F.Walker. 1985. Thermography of the bovine scrotum. American Journal of Veterinary Research. 46:2388-2392.

Roy, R. A., J. P. Boucher and A. S. Comtois. 2006. Digitized infrared segmental thermography: time requirements for stable recordings. Journal of Manipulative Physiology and Therapeutics. 29:468e1-468e10.

SAS Institute Inc., SAS 9.1.3 Help and Documentation, Cary, NC: SAS Institute Inc., 2000-2004.

Schmidt, S., S. Bowers, K. Graves, R. Carroll, J. White and S. Willard. 2003. Use of infrared thermography to assess thermal temperature gradients and pathologies of the bovine claw. ASAS/ADSA National Meeting, Phoenix, Arizona.

Turner, A. T., R. C. Purohit and J. F. Fessler. 1986. Thermography: A review in equine medicine. Compendium on continuing education for the practicing veterinarian. 8(11):855-861.

Turner, A. T. 1998. Thermography as an aid in the localization of upper hind limb lameness. Equine Athletics. 11:38-42.

White, M. B., M. Jones, S. Schmidt and S. Willard. 2006. Use of digital infrared thermal imaging (DITI) for monitoring environmental physiology in dairy cattle. Journal of Animal Science. 84(1):212.

Winsor, T. Vascular aspects of thermography. 1971. Journal of Cardiovascular Surgery. 12:379-388.

CHAPTER VI

CONCLUSIONS

Reproductive failure is the second most common reason for culling females in the swine. Genetic selection has a minimal influence on reproductive performance so other potential influences need to be explored. Phytoestrogens are commonly found in soybeans and have been shown to have estrogenic effects in swine. Therefore, in this study prepubertal gilts were fed a diet containing raw soybeans and evaluated for potential phytoestrogenic effects on reproductive performance. In the first experiment, there were no observed effects from feeding raw soybeans on age to puberty, ovarian function or early embryo development. In experiment 2, effects of feeding a raw soybean diet were observed at time of weaning. Gilts consuming the raw soybean based diet weaned fewer pigs than gilts consuming the control diet. There was also a tendency for gilts on the raw soybean diet to have lower pig birth weights. These data differ from other studies that found increases in number of pigs born alive and pig birth weights. The amount of phytoestrogens present in the diets from this study or the previous studies mentioned is not known and therefore could provide some insight into the differences between the findings of the studies.

Further research is needed to evaluate the use of raw soybeans during critical stages of reproductive development.

Raw soybeans have also been associated with the reduction in growth of young swine. A number of research reports suggest that maturity affects the ability of swine to consume raw soybeans. Gilts from the first experiment of this study were examined for effects of consuming a raw soybean based diet on carcass quality. Gilts consuming the raw soybean based diet had reduced hot carcass weights than gilts consuming the control diet. These findings are consistent with the findings reported in the literature when use of raw soybeans in swine diets is associated with decreased growth rate. Although there was a reduction in hot carcass weight, the difference is much less than those reported in the literature for younger swine indicating that maturity may still have an impact on the ability of swine to utilize a raw soybean diet. Other carcass parameters measured did not differ between the treatment groups in this study.

The use of thermal imaging provides a non-invasive technique for monitoring changes in thermal gradients of surface areas of animals. The use of thermal imaging for the detection of estrus in the gilt was explored in the third study of this thesis. The anatomical prominence of the vulva in pigs provides for a useful reproductive target tissue for the measurements of thermal gradients for the purpose of estrus assessment. Thermal images taken during estrus and diestrus revealed different thermal signatures for each imaging period. Gilts in estrus had higher thermal gradient temperatures than during diestrus. The ability of thermal imaging to differentiate estrus from diestrus could provide a means for evaluating estrous activity in non-domestic species.

APPENDIX A

RADIOIMMUNOASSAY PROCEDURES FOR PROGESTERONE

Blood serum is collected by jugular venipuncture and spun down for 30 min at 3000 RPM and 4^{o} C. Serum is pipetted and alloquatted into 1.5 ml plastic test tubes and stored at -20^{o} C.

Step 1 Allow reagents to reach room temperature (~25^{o} C) and invert reagents to ensure proper mixture.

Step 2 Label 2 plain tubes for Total Counts. Label and arrange Anti-Progesterone-Coated Tubes in duplicate.

Step 3 Add 25 µL of the Standards, Controls and unknowns to appropriate tubes.

Step 4 Add 500 µL of Progesterone (I-125) Reagent to each tube.

Step 5 Mix tubes by gently shaking the test tube rack by hand. Incubate all tubes in a water bath at approximately 37^{o} C for 60 to 70 minutes.

Step 6 Decant all tubes except total count tubes and count all tubes in a gamma counter for one minute.

APPENDIX B

RADIOIMMUNOASSAY PROCEDURES FOR ESTRADIOL

Blood serum is collected by jugular venipuncture and spun down for 30 min at 3000 RPM and 4° C. Serum is pipetted and alloquatted into 1.5 ml plastic test tubes and stored at -20° C.

Step 1 Allow reagents to reach room temperature (~25° C) and invert reagents to ensure proper mixture.

Step 2 Label 2 plain tubes for Total Counts. Label and arrange non-specific binding, standards, controls and unknowns tubes in duplicate.

Step 3 Add 200 µL of standards controls and unknowns to appropriate tubes. Add 300 µL of 0 pg/mL Estradiol standard to non-specific binding tubes.

Step 4 Add 100 µL of Estradiol Antiserum to all tubes except non-specific binding and total count tubes.

Step 5 Vortex all tubes and incubate at room temperature (~25 °C) for 1 hour.

Step 6 Add 100 µL of Estradiol (I-125) Reagent to each tube.

Step 7 Vortex all tubes, cover and incubate at room temperature (~25°C) for 2 hours.

Step 8 Add 1 mL of Precipitating Reagent to all tubes except Total Count Tubes.

Step 9 Vortex all tubes and allow to stand at room temperature (~25°C) for 15-20 minutes.

Step 10 Centrifuge all tubes except for Total Count Tubes for 15-20 min at 1500 g.

Step 11 Aspirate all tubes except Total Count Tubes and count all tubes in gamma counter for 1 minute.