Spring 2014

Evaluation of forage soybean yield and quality characteristics and potential as a feed resource for developing replacement beef heifers

Emily G. Taylor
Purdue University

PURDUE UNIVERSITY
GRADUATE SCHOOL
Thesis/Dissertation Acceptance

This is to certify that the thesis/dissertation prepared

By Emily G. Taylor

Entitled
Evaluation of forage soybean yield and quality characteristics and potential as a feed resource for developing replacement beef heifers

For the degree of Master of Science

Is approved by the final examining committee:

Ronald P. Lemenager

Keith D. Johnson

Larry A. Horstman

To the best of my knowledge and as understood by the student in the *Thesis/Dissertation Agreement. Publication Delay, and Certification/Disclaimer (Graduate School Form 32)*, this thesis/dissertation adheres to the provisions of Purdue University's "Policy on Integrity in Research" and the use of copyrighted material.

Ronald P. Lemenager

Approved by Major Professor(s): _____

_____

Approved by: Todd Applegate 04/11/14

Head of the Department Graduate Program Date

EVALUATION OF FORAGE SOYBEAN YIELD AND QUALITY CHARACTERISTICS AND POTENTIAL AS A FEED RESOURCE FOR DEVELOPING REPLACEMENT BEEF HEIFERS

A Thesis

Submitted to the Faculty

of

Purdue University

by

Emily G. Taylor

In Partial Fulfillment of the

Requirements for the Degree

of

Master of Science

May 2014

Purdue University

West Lafayette, Indiana

ACKNOWLEDGMENTS

My success as a teacher, mentor, and overall person are credited to many professors I have had the privilege to work with. I would first like to thank my advisor Dr. Ron Lemenager for taking me on as a M.S. student. Ron, you gave me more support and guidance than I could have asked for and with that every opportunity to succeed in this program. You pushed me past limits I didn't think I had, and I could not be more grateful. I would also like to thank Dr. Larry Horstman for not only being a part of my committee but also allowing me to work with you the past few years. I have truly enjoyed the countless hours spent flushing cows! Also I would like to thank another committee member Dr. Keith Johnson. I can't thank you enough for your time spent with me collecting forage samples and helping me push through a topic a little unfamiliar to me. I appreciate all the mentorship given.

I want to thank my husband Justin, for all the love and support, and help with the animals these past few years! And to the rest of my family who have stood beside me and helped me with all your love and support, thank you!

TABLE OF CONTENTS

Page

Page

LIST OF TABLES

Table Page

Appendix Table

Appendix Table Page

LIST OF FIGURES

Figure Page

ABSTRACT

Taylor, Emily G. M.S., Purdue University, May 2014. Evaluation of Forage Soybean Yield and Quality Characteristics and Potential as a Feed Resource for Developing Replacement Beef Heifers. Major Professor: Ronald P. Lemenager.

Two agronomic studies were conducted to evaluate the yield and quality potentials of forage soybean (*Glycine max* (*L.*)) grown with and without pearl millet (*Pennisetum glaucum* (*L.*)). Results indicate that there were no differences between row spacings of 18 cm or 36 cm and targeted populations of 469,500 seeds ha^{-1} or 234,750 seeds ha^{-1} on dry matter yield or overall quality of the forage soybean. While harvest date had significant effects on quality as the crop matured, forage soybean as a monoculture proved to be a very forgiving crop, and recovered in quality as maturity advanced into the later reproductive stages of plant development. When forage soybean was intercropped with pearl millet as a mixture or in alternating rows, there were no significant differences in DM yield. In general, neutral detergent fiber and acid detergent fiber increased across harvest dates, while crude protein and in-vitro digestibility decreased. Collectively these studies suggest that, depending on equipment availability, row spacings of either 18 cm or 36 cm, and lower seeding rates of 234,750 seeds ha^{-1} would be most cost effective. These results also indicate that one late season harvest of soybean monoculture should reduce harvest cost and maximize both forage yield and quality, while an early season

harvest followed by a late season harvest is needed to maximize both forage yield and quality when soybean is intercropped with pearl millet.

In the feeding trial, yearling beef heifers were fed one of three nutritionally balanced diets; 1) a control diet of alfalfa haylage , 2) soybean silage or 3) soybean and pearl millet silage to evaluate growth performance and reproductive efficiency. While there were differences in dry matter intake and average daily gain, these diets were fed without negatively impacting growth or reproduction of the heifers. All heifers performed adequately on the soybean diets, maturing to at least 63% of their mature equivalent prior to breeding. One of the more significant observations from this study was that heifers consumed a near constant 1% of body weight of neutral detergent fiber per day. This finding should allow more precise dietary formulations by more accurate estimations of dry matter intake.

Collectively, these experiments illustrate that forage soybean is an alternative feed resource for developing replacement beef heifers. Soybean has the potential to have a positive impact on the cow-calf industry by allowing producers to utilize land in a singular and “double crop” manner to improve land use and profitability.

CHAPTER 1 INTRODUCTION

Many management factors impact the economic sustainability of a cow-calf operation including costs associated with heifer development, breeding, feed, equipment, depreciation, and labor. One of the larger expenses affecting cow herd profitability is cost of replacement heifer development, feed cost and forage availability. This leaves producers searching for alternative feeds that optimize performance and maximize profitability.

Production has drastically changed in the last five to ten years, not only for livestock producers, but also for grain crop producers. Management practices have changed due to an increase in both feed (corn and soybean) and cattle prices, as well as wide spread drought in the major cow states, has resulted in pasture/hay land conversion to grain production. In turn, these factors have, and may continue to decrease the number of beef cattle. These changes have left producers not only looking for a way to adjust to feed prices, but also address land use issues. Soybean has been proposed as an alternative feed resource for producers because recent forage cultivars are producing 13 t ha^{-1} with a June planting and 7.5 t ha^{-1} with a July planting. The late planting date simulates a double cropping system (Atkinson et al., submitted to press). This would allow producers to address both the land use issue and the need for forage by growing a forage crop after a cash grain crop such as wheat.

1.1 Forage Soybean

The first domestication of soybean has been traced to the eastern one-half of northern China in the eleventh century B.C. It was thought to be first introduced into the American colonies in 1765 as “Chinese vetches” (Gibson and Benson, 2005; Smith, 2013). In the 1920’s soybean acreage expanded significantly and was mostly used for forage and to a lesser extent, green manure. It was not until 1941 that the acreage of soybean grown for grain first exceeded that grown for forage and other purposes in the United States. The majority of the soybean crop is now processed into oil and meal (Gibson and Benson, 2005).

In 1976, the USDA-ARS began a breeding program to increase the forage potential of soybean (Darmosarkoro et al., 2001). Soybean cultivars were chosen for their high leaf retention, as well as pest and stress resistance (Darmosarkoro et al., 2001). Limited research on use of soybean for forage showed the potential benefits of soybean as a forage crop (Hintz et al., 1992; Sheaffer et al., 2001). Hintz and Albrecht (1994) evaluated the management practices such as cultivar selection, seeding rate and row spacings at the various stages of reproductive maturity of forage cultivars. Soybean selections mostly depend on when and where planting will occur and the ultimate use of the crop either as a cash grain or livestock feed. Recommended seeding rates and row spacings can vary between cultivars, and growth stage can affect the nutrient profile of the plant. Soybean is placed into groups depending on the length of time it takes to reach full maturity (Figure 1.1). Researchers have devised a numbering system (00-8) that allows producers to choose soybean cultivars that best fit their region to maximize production in their growing season. Reproductive maturity of soybean is defined in Table 1.1. Forage soybean cultivars used for forage production do not need to reach reproductive maturity and a higher grouping number is typically recommended to increase tonnage by maintaining vegetative growth later into the season.

Evaluating forages for the purpose of livestock feed can be done with the basic quality analyses which includes dry matter (DM), crude protein (CP), neutral detergent

fiber (NDF), acid detergent fiber (ADF) and either total digestible nutrients (TDN) or in-vitro dry matter digestibility (IVDMD). The formulation of diets relies heavily on these values. Feedstuffs, especially forages, can vary drastically in moisture content. To minimize error due to moisture, all ruminant rations are typically formulated on 100 percent DM basis. Crude protein is calculated by the nitrogen content multiplied by 6.25. An important factor to note is the possibility of heat damage (Lemenager et al., 2011). Heat damage, caused by moisture and resulting microbial and fungal growth, is usually the result of a Maillard reaction, a heat-induced chemical reaction between protein and sugars (Coblentz and Hoffman, 2008). This renders part of the protein unavailable to the animal. Dry matter intake (DMI) is inversely related to NDF (cell wall components; lignin, cellulose, and hemicellulose). Estimation of DMI, expressed as a percent of an animal's body weight, can be estimated from the NDF values. Acid detergent fiber (NDF minus hemicellulose) is directly related to digestibility and can be used to estimate TDN. Net energy for maintenance and gain can be calculated from the TDN values (Lemenager et al., 2011). These values vary in concentration depending on feedstuff, maturity and cultivar.

Sheaffer et al. (2001) evaluated the effects of harvest date on DM yields of grain and forage type soybeans. Grain and forage soybean DM yields were similar at the early and late harvests even though the forage types lagged an average of three reproductive stages behind the grain types at both harvests. These results suggest that forage soybean would have superior DM yields to the grain types if harvested at a similar stage of maturity (Sheaffer et al., 2001). Hintz and Albrecht (1994) investigated the DM partitioning of soybean components grown under a range of management practices. Components of the soybean are defined as leaf, stem, pod and seed. Stage of maturity at harvest had the greatest impact on dry matter partitioning among the various plant components (Hintz and Albrecht, 1994). Row spacing and seeding rate had no significant effect on DM partitioning before R5 (Hintz and Albrecht, 1994). The differences after R5 were explained as interplant competition; however, it was also suggested that plant size may influence dry matter partitioning independent of interplant competition (Hintz and Albrecht, 1994). Ethredge et al. (1989) research supplements this idea when they

concluded that wider row spacing (76cm) resulted in more natural thinning of plants than in narrower row spacing's (25cm). Recommendations have been made from the literature that forage soybean optimum time of harvest is at stages R6-R7 due to increased DM yield. However, at this stage of development, and with such a late group of maturing soybeans, leaf senescence can occur and, therefore, may decrease DM yields (Hintz et al., 1992). Hintz et al. (1992) saw an increase of 5000 kg ha^{-1} DM yield from early reproductive stages (R1) to late reproductive stages (R7).

Results in DM yields can also be affected by the region of the country in which they are grown. Rao et al. (2005) compared the proportion of leaf, stem and pod for forage type soybean cultivars in the Southern Great Plains. Forage soybean total DM yield of all cultivars increased from early to final harvest dates (Rao et al., 2005). By August harvests, minimal differences in leaf to stem ratio on a DM basis among the forage cultivars were observed (Rao et al., 2005). Seiter et al. (2004) evaluated the effect of plant density and row spacing for yield and forage quality in the northeastern United States. Higher yields (32% and 49%) were achieved at narrower row spacings (18 cm vs. 76 cm), although no differences were seen at low, medium or high plant population densities in either the narrow or wide row spacings (Seiter et al., 2004). Egli et al. (1985) suggests that the partitioning of DM into leaf, stem and pod is relatively stable across cultivars, and little difference was observed among cultivars of similar growth habit.

Protein values of forage soybeans can vary depending on variety, stage of harvest maturity, population density and row spacings. Munoz et al. (1983) suggested that the percentage of protein in soybean hay does not decrease with advancing maturity as much as most forages, especially grasses, because seeds contain 35 to 40% protein. Hanway and Weber (1970) support this idea stating that the protein content of leaves and stems decreased with maturity, but the protein of hay increased or remained constant because of the increase in seed content as the plant advanced toward maturity. Hintz et al. (1992) found that CP concentrations declined from stages R1 to R3, remained constant between R3 and R5, and then increased from R5 to R7 for both a grain cultivar and a forage cultivar. The CP concentrations of soybean in wider row spacings (76 cm) were

significantly greater than those grown in narrow row spacings (20 cm), which may be due to the fact that the soybean grown at the wider row spacings had finer stems (Hintz et al., 1992). Sheaffer et al. (2001) found that row spacing effects on quality was consistent with results reported by Hintz et al. (1992), except there was no effect of row spacing on CP concentration. Additionally, as the seed content of soybean increased, Hintz et al. (1992) also measured 126 g kg^{-1} ether extract (EE) for whole-plant forage soybean. This large increase in vegetable fats could have a negative impact when fed to ruminant livestock by decreasing intake and reducing fiber digestion. If the forage is harvested at late reproductive stages, it is recommended to be limited to either no more than 50% of the total ration DM, or consider harvesting at an earlier stage of maturity to reduce total dietary fat (Hintz et al., 1992).

Fiber concentrations vary among forages. Hintz et al. (1992) found that NDF and ADF concentrations increased from R1 to R5 and decreased from R5 to R7. Similarly, Seiter et al. (2004) found NDF and ADF concentrations increased between stages R3 and R5.5. Seiter et al. (2004) suggested this increase was likely a result of increasing fiber concentrations of stem tissues; however increasing amounts of highly digestible pods beyond R5 counteracts the overall increase in fiber concentrations and explains the decrease from R5 to R7 reported by Hintz et al. (1992) and Munoz et al. (1983). Hintz et al. (1992) found increased stem diameter with decreasing row spacings, but no consistent effect of row spacings on NDF and ADF. Seiter et al. (2004) also observed increased stem diameter with decreasing row spacings in the early growth stages. However, sampling at stages R3 to R5.5 changed, and stem diameter in the wider rows was larger than stem diameter in the narrow rows (Seiter et al., 2004). Sheaffer et al. (2001) reported higher NDF and ADF values in cultivars when compared to grain cultivars. This could be explained by the increase in tonnage, later maturity and lesser grain content of the forage crop varieties.

Munoz et al. (1983) found that a grain variety soybean planted at the highest density (291,300 plants/ha) was significantly less digestible than plants from the lowest planting density (97,100 plants/ha). The digestibility of stems decreased substantially beginning

with pod development, but again, the increasing amount of highly digestible pods counteracted this effect (Munoz et al., 1983; Hintz et al., 1992).

Mixed stands having at least one grass and one legume are often desired over pure stands to reduce the potential for bloat and improve seasonal distribution of production (Barnes, 2003). Since soybeans are a potential bloat-causing legume, integrating a grass into the soybean planting should reduce this risk. Plants grown together frequently compete primarily for solar radiation resulting in shading of one or the other plant. Kephart and Buxton (1993) reported that cell-wall concentrations (NDF) decreased under shaded conditions for perennial grasses and increases in digestibility due to lower NDF concentrations have been reported. Kephart and Buxton (1993) reported a 50 g kg^{-1} increase in IVDMD with heavy shading. Redfearn et al. (1999) investigated the yield and quality of forage soybean intercropped with forage sorghum as compared with monocrop forage soybean. Monocrop forage soybean produced 2300 kg ha^{-1} more total DM than the intercropped forage soybean and had 36 g kg^{-1} less stem NDF than the intercropped soybean (Redfearn et al., 1999). This is most likely the reason for a 33 g kg^{-1} increase in digestibility (Redfearn et al., 1999). Ocumpaugh et al. (1981) evaluated forage soybean intercropped with tall fescue. The results were an increase in total DM yield and a 10% increase in CP for the intercropped soybeans and tall fescue (Ocumpaugh et al., 1981) compared to soybean alone.

Another factor that affects yield and nutrient profile of forages is temperature and precipitation. Rao et al. (2005) reported total DM yields over three years with the first year yields being significantly lower compared with the other two. This was attributed to drier conditions during the first growing season. For all cultivars and years, the DM accumulation was minimal until mid- to late-August, because of the late season rains (Rao et al., 2005). Linkemer et al. (1998) reported that the early vegetative period (V2) and the early reproductive stages (R1, R3, and R5) were most sensitive to excess precipitation resulting in reduced crop growth.

1.2 Ruminant Nutrition

Energy is a major nutrient and is divided into two components for ruminant diets, digestible and indigestible energy. Digestible energy (DE) is the energy of the food minus the energy lost in the feces (NRC, 1996). The Beef NRC, (1996) describes DE as a feed evaluation that has some value because it reflects diet digestibility; however, it fails to consider several major losses of energy associated with digestion and metabolism. Because of over-estimations of DE, TDN can be used as a correction for digestible energy and works well in maintenance type diets. Metabolizable energy (ME) is DE minus energy lost as methane during digestion or excreted in the urine (Barnes, 2003). Metabolizable energy minus heat lost during the processes of digestion and synthesis yields net energy (NE) (Barnes, 2003) which is the preferred energy term used in high performance rations for stocker, feedlot and dairy rations.

The ability of feed consumed to meet the NE required for maintenance is expressed as NE_m. The maintenance requirement for energy is defined as the amount of feed energy intake that will result in zero net loss or gain of energy from the tissues of the animal body (NRC, 1996). Cows of similar size and breed may vary in their maintenance requirements (NRC, 2001). It has been suggested that beef breeds vary between maintenance requirements based on their milk production potential (NRC, 2001). The Beef NRC (1996) also makes reference to many researchers who have noted differences in energy requirements, or efficiencies of energy utilization, among breeds of cattle.

The net energy requirements for gain (NE_g) are defined as the energy content of tissue deposited during growth, after maintenance requirements have been met (NRC, 2001). The energy requirements for growth increase as growth rate increases for a given body weight and mature breed size. While increasing energy with growth, it is important to be aware of its effects if fed in excess. Patterson et al. (1992) discussed the influence of age at puberty when using excess energy. This would seem advantageous to a producer as the heifer would breed early and subsequently calve early; however, there are other factors to consider. Sejrsen et al. (1981) found that mammary growth could be adversely affected

by a high plane of nutrition in pre-pubertal heifers. This leads to a decrease in the overall milk production capabilities of that heifer throughout her lifetime (Sejrsen et al., 1981; Capuco et al., 1995).

Beef cattle protein requirements are often reported as either grams of CP/day or as a percent of dry matter intake (DMI). Crude protein values of feeds are calculated by first analyzing nitrogen (N) content and then multiplying by 6.25, since protein contains a near constant of 16% N. Crude protein includes preformed protein in the form of amino acids (AA), peptides, and intake protein, as well as non-protein nitrogen (NPN). Daily crude protein requirements include two fractions, rumen degradable protein (RDP) and rumen undegradable protein (RUP). The RDP fraction is degraded by rumen microbes into ammonia and resynthesized into microbial crude protein (MCP) when an energy substrate (carbon chains) is available, which then supplies a majority of the AA to the small intestine. Bypass protein, or RUP, is the fraction that is not degraded in the rumen and is passed to the small intestine for metabolism (NRC, 2001). Metabolizable protein (MP) is a combination of MCP and RUP (NRC, 1996). Efficient rumen fermentation and MCP production involves both a source of energy and nitrogen. Dewhurst et al. (2000) indicated that ruminants have the ability to survive on the protein provided by microbial crude protein (MCP) created from NPN. In addition, Storm and Orskov (1983) reported that MCP accounts for 60 to 85% of the total AA flow into the small intestine. The addition of preformed protein, in addition to NPN, makes microbial protein production more efficient and increases MCP flow to the small intestine. The RUP fraction adds additional amino flow to the small intestine to meet the higher requirements for performance. Therefore, a combination of dietary NPN, RDP and RUP are needed to supply the correct AA flow to the small intestine to meet the requirements of higher performance animals. Some common sources of RDP are urea, soybean meal, and cotton seed meal while common RUP sources include, dried distillers grains, corn gluten feed, corn gluten meal, feather meal and blood meal (NRC, 2001).

Low-quality forages are an important feed resource for the cow herd segment of the beef industry. To optimize the utilization of these low-quality forages, feed intake and

digestibility must be enhanced. It has been determined that adding CP to meet the animal and microbial requirements will increase low quality forage intake and nutrient flow to the small intestine (Hannah et al., 1991; Lintzenich et al., 1995). Koster et al. (1996) found that supplementation of RDP can dramatically increase the intake of low-quality forage by beef cows. These data suggest that the use of a MP system to formulate diets would be more accurate than formulating on crude protein alone. This is consistent with the Beef NRC (1996) conversion to the MP system, which accounts for true protein absorbed by the intestine as supplied by microbial protein and undegraded intake protein (NRC, 1996).

One of the greatest difficulties in predicting the effect of forage quality on animal response to diets is related to dry matter intake (Mertens, 1994). Another factor affecting intake that should be considered is the NDF of a feedstuff. Neutral detergent fiber is the structural components of the plant, specifically the cell wall, and is a good predictor of voluntary intake because intake of low quality forages is limited by gut fill (Rasby and Martin, 2013). It is assumed that as NDF increases, rumen turn over and DM intake will decrease resulting in a lower amount of total energy consumed by the animal. The logical explanation for this is that intake is limited by digestive tract fill when low quality forage is the primary ration ingredient. Intake is limited by restriction of rumen capacity in the digestive tract when low quality forages are fed (low in digestibility, high in bulk), which results in reduced animal performance because of an energy intake limitation (Mertens, 1994). However, when low fiber diets containing large amounts of readily fermentable carbohydrates are fed, chemostatic mechanisms tend to control DMI (Mertens, 1994; Ruiz et al.,1995).

Ruminal fiber degradation (rate and extent), pH, volatile fatty acid (VFA) production and ammonia nitrogen concentration vary significantly between low and high-quality forages. Varel and Kreikmeier (1999) found that forage utilization was higher in mature cows than in heifers. In that study, heifers consumed less forage organic matter per unit of metabolic BW ($BW.^{75}$) when fed low-quality forage compared to high-quality forage (Varel and Kreikmeier, 1999). In addition, ruminal liquid pool turnover was

slower in the heifers fed the low-quality forage diet, resulting in a lower pH, slower rate of fermentation, increased ammonia nitrogen concentration and decreased VFA production. These data support the recommendations that heifers require a higher quality diet to achieve acceptable performance.

Increased rate of protein synthesis and amino acid flow to the small intestine are required for muscle growth. It has been reported that increased rate of protein synthesis increases with increasing levels of nutrition (Orskov, 1992); however, muscle growth does decline with age. Therefore, early development is important. A study done by Bohman (1955) demonstrated the concept of compensatory growth. Weanling Hereford cattle were allotted to two treatment groups, one of higher protein hay and one of lower protein hay being fed through the winter feeding period. Both groups were grazed on the same pasture through the summer. Results indicated that the higher protein group gained on average three times more rapidly than those fed the lower protein diet during the winter. However, during the summer, cattle fed the lower protein winter diet tended to compensate during the summer by gaining more rapidly than the high protein winter group. This was repeated for two consecutive winters with no differences in gains at the end of the study between the two groups (Bohman, 1955). In addition, Park et al. (1997) reported a 2-fold increase in the efficiency of growth for beef heifers on a stair-stepped compensatory nutrition plan, and had enhanced mammary development with subsequent lactation performance.

Lynch et al. (1997) investigated the reproductive characteristics and total feed input of beef heifers developed from weaning until approximately 45 d before breeding on restricted gain followed by rapid weight gain in the final 45 d to achieve 65% of expected mature body weight by the onset of the breeding season. Heifers fed to gain 0.91 kg/d for the 47-56 d prior to breeding did not differ from heifers fed to gain .45kg/d for the entire 159-d feeding period for body condition score (BCS), frame score, pelvic area or estimated fat thickness. These results suggest that dietary treatments resulted in similar skeletal growth and body composition (Lynch et al., 1997). Yelich et al. (1995) found similar results when a restricted gain (.23 kg/d) was followed by an accelerated

growth phase (1.36 kg/d); however, they found that the heifers on the continuous gain treatment were younger, heavier, and fatter at puberty than those on the restricted/accelerated treatment (Yelich et al., 1995).

1.3 Reproduction

1.3.1 Estrous Cycle

The estrous cycle is a rhythmical change in the reproductive system of mammals. Cattle are considered to be polyestrous, showing a regularly recurring estrous cycle throughout their reproductive life. The length of the estrous cycle has been reported as 21 days (Larson and Randle, 2013) with a range from 17 to 24 days (Salisbury et al., 1978). The range in the estrous cycle length can partly be explained by the presence of follicular waves during their estrous cycles. Each wave consists of the recruitment, selection and dominance of follicles (Ireland et al., 2000). Fortune et al. (2001) explain that recruitment consists of three to six follicles. Selection results in the reduction of follicles from recruitment with one follicle selected for dominance with the others becoming atretic (Ireland et al., 2000). Only the dominant follicle from the final wave is available to respond to the decrease in progesterone and the subsequent luteinizing hormone (LH) surge needed for ovulation (Fortune et al., 2001). This is known as turnover (growth and atresia) of follicles (Ireland et al., 2000). Two and three waves are most common in heifers, while mature cows usually exhibit only two waves (Ireland et al., 2000; Sunderland et al., 1993).

Each cycle consists of two phases, the luteal phase and the follicular phase. Behavioral estrus is displayed during the follicular phase and is identified as the initiation of the estrous cycle and is typically referred to as day 0 of the estrous cycle (Gunn, 2013). The luteal phase is usually from days 1-17 and is the period following ovulation when the corpus luteum (CL) is formed (Forde et al., 2011; Rasby and Vinton, 2013). During this time period, the cycle is under the influence of progesterone created by the presence of a CL on the ovary. The luteal phase is then further broken down into metestrus and diestrus. Metestrus is a period of time (days 2-4 of the estrous cycle) in which the CL

begins to form resulting in increasing progesterone concentrations. Diestrus is a period of time (days 5-17) in which the CL is at maximum size and progesterone production (Merck Animal Health). The follicular phase is days 18-21 following the regression of the CL (luteolysis) and a period where the cycle is under the influence of estrogen (Rasby and Vinton, 2013). Proestrus and estrus make up the follicular phase, during which the presence of a preovulatory dominant follicle is responsible for the secretion of increasing concentrations of estrogen (Gunn, 2013). During the follicular phase, final maturation and ovulation of the dominant ovulatory follicle occurs (Forde et al., 2011)

1.3.2 Hormomal Regulation

The estrous cycle is regulated by hormones of the hypothalamus; gonadotropin releasing hormone (GnRH), which acts upon the anterior pituitary to produce hormones (LH and follicle stimulating hormone (FSH)) with a prominent role in ovarian follicle development (Forde et al., 2011). Increased concentrations of FSH are needed for initial follicular growth and recruitment which is responsible for the synthesis of estrogen from the granulosa cells in the follicle (Hillier, 1994). Hillier (1994) explains as follicular growth continues and approaches dominance (10-20mm diameter) the dependence of the follicle switches from FSH to LH. The binding of LH to receptors of the theca cells (outside of the basement membrane) results in the conversion of cholesterol to testosterone. Testosterone is then diffused into the granulosa cells (lining the inside of the basement membrane) and converted to estrogens by an aromatase enzyme (Forde et al., 2011). LH pulses are necessary to maintain dominance, and allow the dominant follicle to continue estrogen production (Ireland et al., 2000). Christenson et al. (1975) reported a serum LH peak of 67±8 ng/ml just prior to ovulation, which is consistent with Sunderland et al. (1994) and Forde et al. (2011) who state that the dominant ovulatory follicle is subjected to an LH surge prior to ovulation.

Following ovulation, the cells from the ovulatory follicle undergo luteinization to form the CL, which begins in the granulosa cells within 12 hours after ovulation (Alila and Hansel, 1984). The purpose of the CL is to produce sufficient concentrations of progesterone throughout the luteal phase and to maintain early pregnancy (Forde et al.,

2011). While the CL secretes progesterone throughout gestation, the placenta takes over production of progesterone at about 6-8 months (Senger, 2012).

Functionality of the CL can be related to the vigor of the follicle prior to ovulation. Large luteal cells are derived from granulosa cells and small luteal cells are derived from theca cells. Because large luteal cells do not multiply, the total number of granulosa cells in the follicle determines the number of large luteal cells in the newly formed CL. Greater number of large luteal cells can result in an increase in progesterone production (Senger, 2012). If the establishment of pregnancy is unsuccessful, luteolysis will occur. Luteolysis is brought on by the production of prostaglandin $PGF_{2\alpha}$ produced by the uterine endometrium and oxytocin produced by the large luteal cells. It has been established that oxytocin and $PGF_{2\alpha}$ work in a positive feedback mechanism (Gunn, 2013), with $PGF_{2\alpha}$ simulating the ovarian secretion of oxytocin (Flint and Sheldrick, 1982), and oxytocin in turn further stimulates the production of $PGF_{2\alpha}$ (Senger, 2012). As luteolysis occurs, progesterone concentrations decrease, which allows a subsequent LH surge for final maturation and ovulation of an oocyte.

1.3.3 Phytoestrogens

Phytoestrogens are plant-derived compounds that structurally or functionally mimic mammalian estrogens (Ososki and Kennelly, 2003). They have weak estrogenic activity and, therefore, named phyto (plant) estrogens (Brzezinski and Debi, 1999). Estrogens influence multiple aspects of the body including the growth and functionality of the female and male reproductive tissues (Gruber et al., 2002; Ososki and Kennelly, 2003). Considering this relationship, it is not surprising that research has been done to elucidate the effects of phytoestrogens. Phytoestrogens are capable of acting as both agonists and antagonists (Brzezinski and Debi, 1999). Agonists mimic endogenous estrogens and cause estrogenic effects, while antagonists block or alter estrogen receptors and prevent estrogenic activity, causing anti-estrogenic effects (Ososki and Kennelly, 2003).

There are multiple classes of phytoestrogens, however, the most common are isoflavones and lignans (Brzezinski and Debi,1999 ; Durzer and Xu, 1997). Isoflavones

are almost exclusively in legumes and beans, and lignans are found widely in cereals, fruit and vegetables (Brzezinski and Debi, 1999). The most well-known of the phytoestrogen classes are isoflavones. One of the first reported disturbances involving estrogenic effects was noted by Bennetts et al. (1946). In that study, sheep were managed on subterranean clover dominant pastures and observed to have a significant increase in dystocia and death loss of ewes and lambs compared to pastures with minimal subterranean clover (*Trifolium subterraneum L.*) (Bennetts et al., 1946; Ososki and Kennelly, 2003; Brzezinski and Debi, 1999; Kingsbury, 1964).

With increasing need for use of alternative forages, numerous investigators have reported negative effects of phytoestrogens on reproduction in female ruminants when legume forages were included. Adams (1994) discussed that estrogenic and anti-estrogenic activity is determined by the ratio of phytoestrogen to estrogen in the body. This may explain why estrogenic effects predominate in sheep that have low circulating concentrations of estrogen, but anti-estrogenic effects are mainly reported in humans that have with relatively high circulating concentrations of estrogen (Adams, 1994). Cattle also have relatively low circulating concentrations of estrogen, therefore it may be expected that estrogenic effects would dominate in this species (Adams, 1994). Kallela et al. (1984) reported a reduced fertility and cystic ovarian disease in cattle fed a red clover (*Trifolium pretense L.*) silage containing isoflavones. Adams (1994) reported that soybean products contain 2.5 mg g^{-1} isoflavones, but have had no reports of its effects in ruminants. Adams (1994) also reported that alfalfa (*Medicago sativa L.*) contains little phytoestrogen unless suffering from foliar disease. Foliar disease is a fungal infection causing alfalfa to produce estrogenic coumestans (Adams, 1994; Shemesh and Shore, 1994).

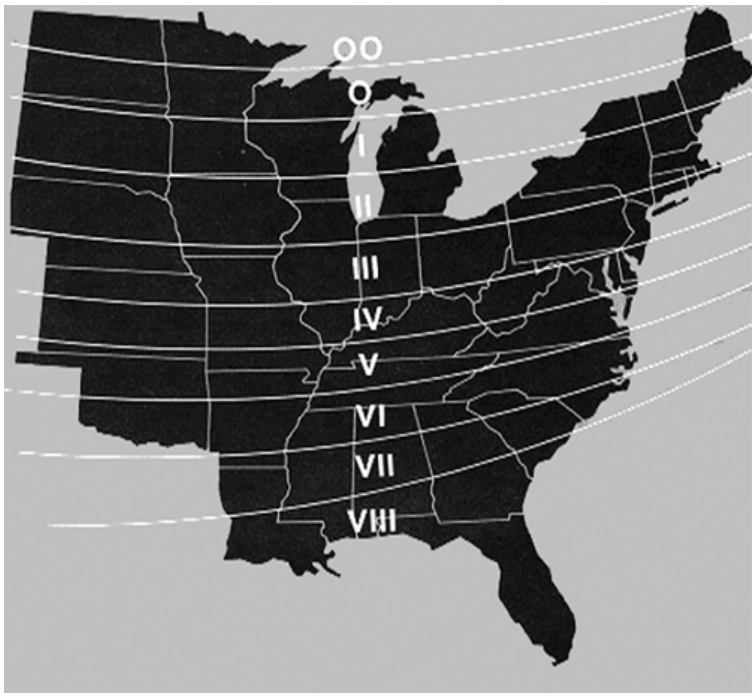

Figure 1.1 Regions of various soybean maturity groups from grain production, adapted for full season growth. Scott and Aldrich (1970)

Table 1.1 Growth stages of soybeans[1]

	Vegetative Stages		Reproductive Stages
VE	**Emergence** - cotyledons have been pulled through the soil surface	**R1**	**Beginning flowering** - plants have at least one flower on any node
VC	**Unrolled unifoliolate leaves** - unfolding of the unifoliolate leaves	**R2**	**Full flowering** - there is an open flower at one of the two uppermost nodes
V1	**First trifoliolate** - one set of unfolded trifoliolate leaves	**R3**	**Beginning pod** - pods are 3/16 inch (5 mm) at one of the four uppermost nodes
V2	**Second trifoliolate** - two sets of unfolded trifoliolate leaves	**R4**	**Full pod** - pods are 3/4 inch (2 cm) at one of the four uppermost nodes
V4	**Fourth trifoliolate** - four unfolded trifoliolate leaves	**R5**	**Beginning seed** - seed is 1/8 inch long (3 mm) long in the pod at one of the four uppermost nodes on the main stem
Vn	**nth trifoliolate** - V stages continue with the unfolding of trifoliolate leaves. The final number of trifoliolate's depends on the soybean variety and the environmental conditions	**R6**	**Full seed** - pod containing a green seed that fills the pod capacity at one of the four uppermost nodes on the main stem
		R7	**Beginning maturity** - one normal pod on the main stem has reached it's mature pod color
		R8	**Full maturity** - 95% of the pods have reached their full mature color

[1]A growth stage begins when 50% or more of the plants are in or beyond that stage. Pedersen (2007)

CHAPTER 2 EVALUATION OF FORAGE SOYBEAN, WITH AND WITHOUT MILLET, AS A FORAGE CROP

2.1 Abstract

Two field studies were conducted to evaluate the yield and quality potentials of forage soybean grown with and without pearl millet. Research was conducted during the 2012 growing season at the Purdue University Agronomy Center for Research and Education, located near West Lafayette Indiana. The soil types included Crosby silt loam (fine, mixed active, mesic aeric eqiaqualfs, Miami fine-loamy, mixed, active mesic oxyaquic hapludalfs, 20%), Rockfield (fine-silty, mixed, superactive, mesic oxyaquic hapludalfs, 30%), Toronto (fine-silty, mixed, superactive, mesic undollic epiaqualfs, millbrook fine-silty, mixed, superactive, mesic udollic endaqualfs 50%). Treatments were designed as a randomized complete block with a split plot arrangement with four replications per treatment. Study one provided results that indicated no differences between row spacings of 18 cm or 36 cm and target populations of 469,500 seeds ha^{-1} (234,750 seeds ha^{-1} achieved) or 234,750 (145,000 seeds ha^{-1} achieved) seeds ha^{-1} oin dry matter (DM) yield or overall quality of the forage soybean ($P > .05$). Harvest dates had significant effects on quality as the crop matured ($P < .05$) Forage soybean, however, proved to be a very forgiving crop, and increased in quality as maturity advanced into the later reproductive stages of plant development. Study two provided similar results to study one for DM yield and forage quality across harvest dates for forage soybean. When forage soybean was intercropped with pearl millet as a mixture or in alternating rows, there were no significant differences in DM yield. In general, neutral detergent fiber (NDF) and acid detergent fiber (ADF) increased across harvest dates, while crude protein

(CP) and in-vitro digestibility (IVTD) decreased. Collectively, these two studies indicate, row spacings of either 18 cm or 36 cm, and a lower seeding rate of 234,750 seeds ha^{-1} would provide excellent yield and quality. These results also indicate that one late season harvest of a soybean monoculture should reduce harvest cost without sacrificing forage yield or quality. In contrast, an early season harvest followed by a late season harvest is needed to maximize both forage yield and quality when soybean is intercropped with pearl millet.

2.2 Introduction

Production has drastically changed in the last five to ten years not only for livestock producers, but also for grain crop producers. Management practices have changed due to high grain prices that have resulted in pasture/hay land conversion to grain production. This shift in land use, along with widespread drought and increased feed cost, has resulted in a decrease in the number of beef cattle in the U.S. These changes have left producers looking for a way to adjust feed prices and address land use issues. Soybean has been proposed as an alternative feed resource for producers because recent forage cultivars have produced 13 t ha^{-1} with a June planting and 7.5 t ha^{-1} with a July planting, simulating a double cropping system (Atkinson et al., submitted to press) This would allow producers to address both the land use issue and the need for forage by possibly seeding and harvesting a forage crop after a cash grain crop such as wheat. The objectives of this study were to evaluate differences in yield and quality when forage soybean was established with and without pearl millet.

2.3 Materials and Methods

2.3.1 Site Description

In the spring of 2012, two studies were initiated at the Purdue University Agronomy Center for Research and Education (ACRE) located in West Lafayette, Indiana. Soil tests were conducted prior to planting and were adequate in fertility for the purposes of this study (Table 2.1). The soil types included Crosby silt loam (fine, mixed active, mesic

aeric eqiaqualfs, Miami fine-loamy, mixed, active mesic oxyaquic hapludalfs, 20%), Rockfield (fine-silty, mixed, superactive, mesic oxyaquic hapludalfs, 30%), Toronto (fine-silty, mixed, superactive, mesic undollic epiaqualfs, Millbrook fine-silty, mixed, superactive, mesic udollic endaqualfs, 50%). Sites of all plot work were in corn prior to initiation of the studies.

2.3.2 Cultural Practices

The soybean cultivar used was Eagle Seed's 'Big Fellow', and the pearl millet cultivar used was Byron Seeds 'Wonderleaf'. Seeding was done using a Tye ® no-till drill with row spacing of 18 cm, at specific seeding rates and row spacings based on treatment. During the growing season, outer broadleaf weeds were controlled by applications of glyphosate (Round up ®) and by hand removal.

Plot dimensions for Study 1 was 20x40m with a planting date of May 18 and for Study 2 was 28x48m with a planting date of June 8. Seeding rates used in this study were based on pure live seed (PLS) and adjusted accordingly. The forage type systems utilized in Study 1(Table 2.2) were: Soybeans planted at 18-cm row spacing and recommended seeding rate of 469,500 seeds/ha (18High), Soybeans planted at 36-cm row spacing and half the recommended seeding rate at 234,750 seeds/ha (36Low). The forage type systems utilized in Study 2 were: Soybean planted at 18-cm row spacing and half the recommended seeding rate at 234,750 seeds/ha (18Low); soybean planted at 18-cm row spacing and recommended seeding rates of 496,500 seeds/ha (18High); soybean planted at 36 cm row spacing and recommended seeding rate of 469,500 seeds/ha (36High); pearl millet planted at 18-cm row spacing and recommended seeding rate of 7.8 kg/ha (PM); mixture of pearl millet and soybean within the same row, with row spacing at 18-cm with recommended half rates (234,750 seeds/ha and 3.5 kg/ha, respectively) of both plants seeded (MIX); alternating rows of pearl millet and soybean at 18-cm spacing with half rates (234,750 seeds/ha and 3.5 kg/ha, respectively) of both plants seeded (ALT).

2.3.3 Sampling

Hand-harvested samples were taken at a height of approximately 10 cm at varied time points for each study and were used for yield and nutrient analysis. In Study 1, four harvests were taken for both treatments at two-week intervals on July 11, July 25, August 10 and August 24. For Study 2: four harvests were taken for all treatments at approximate two-week intervals on July 17, August 3, August 20 and August 31. Treatments 18Low, 18High and 36High were sampled for an additional three harvest dates on September 14, September 28 and October 10. Treatments PM, MIX and ALT were harvested at two-time points for regrowth on September 7 (initial harvest date, July 17) and September 17 (initial harvest date, August 3). Treatments 18Low, 18High and 36High were harvested once for regrowth on October 10 (initial harvest date, July 17; Table 2.2). Harvested areas for 18-cm row spacings were 35 m^2, and for 36-cm row spacings the area harvested was 42 m^2. Total weights were taken for each harvest and subsamples were weighed, dried in a forced-air oven at 60^{o}C, and weighed after drying so dry matter yield could be determined. These samples were used for nutrient analyses.

2.3.4 Nutrient Composition Analyses

Dried forage samples were ground through a 1-mm screen in a Wiley Mill (Arthur H. Thomas Co., Philadelphia, PA). After grinding, samples were stored at room temperature until quality analyses could be run. Samples were then analyzed for NDF, ADF, CP, IVTD, DM and Ash.

NDF was measured using a modified Ankom Filter bag technique (Ankom Technology, Macedon, NY). Ankom protocols were followed with the exceptions of pre-extraction with acetone and the addition of sodium sulfite. No pre-extractions were done since samples did not contain more than 5% fat. Sodium sulfite addition is discouraged for ruminant feeds because the sulfite reaction does not have a biological effect in the rumen (Van Soest et al., 1991). Acid detergent fiber was run sequentially after NDF using the

Ankom Filter bag technique. Again, pre-extractions were not used. Samples were digested (SCP Science, Champlain, NY) and CP (Foss, Denmark) following equipment manufactured protocol. In vitro true digestibility (IVTD) was measured using the Ankom Daisy Incubator technique. DM and ashing followed procedures of Van Soest and Robertson (1985). All forage quality values were reported on a DM basis.

2.3.5 Statistical Analysis

The experiments were designed as randomized complete block with a split plot arrangement. There were four treatment replications for both studies. All statistical analyses used to determine differences between treatments were done using SAS (SAS Inst. Inc., Cary NC). For study 1, the GLM procedure of SAS was used for yield and quality data. For dependent variables, the model included the fixed main effects of block, treatment and harvest, as well as the appropriate treatment * harvest interaction. The treatment * harvest interaction was not significant ($P < .05$) in study 1 for any variable and was removed from the model. For study 2 yield parameters, a log transformation was performed for normalization of the data with all data being presented as back transformed. The GLM procedure of SAS was used for yield and quality data. For dependent variables, the model included the fixed main effects of block and treatment, as well as the appropriate treatment * harvest interaction for soybean alone. Least significant difference tests were performed when the Analysis of Variance F-value had a significance level of $P < .05$.

Table 2.1. Soil fertility prior to planting for Study 1 and Study 2.

Study	Sample	OM[1]	pH[2]	P	K	Mg	Ca
		%		mg kg^{-1}			
1	Whole plot	1.9	6.9	23	109	410	950
2	Rep 1	2.0	6.6	20	99	390	1150
	Rep 2	2.1	6.5	24	105	415	1150
	Rep 3	2.2	6.5	22	122	505	1350
	Rep 4	2.0	7.1	22	132	590	1550

[1]Organic Matter
[2]Buffer pH was 6.9 for both studies.

Table 2.2. Harvest dates for Study 1 and Study 2 by forage system.

Treatments, Study 1		Treatments, Study 2					
18High	**36Low**	**18Low**	**18High**	**36High**	**PM**	**Mix**	**ALT**
11-Jul	11-Jul	17-Jul	17-Jul	17-Jul	17-Jul	17-Jul	17-Jul
25-Jul	25-Jul	3-Aug	3-Aug	3-Aug	3-Aug	3-Aug	3-Aug
10-Aug	10-Aug	20-Aug	20-Aug	20-Aug	20-Aug	20-Aug	20-Aug
24-Aug	24-Aug	31-Aug	31-Aug	31-Aug	31-Aug	31-Aug	31-Aug
		14-Sep	14-Sep	14-Sep			
		28-Sep	28-Sep	28-Sep			
		10-Oct	10-Oct	10-Oct			
					7-Sep[1]	7-Sep[1]	7-Sep
		10-Oct[1]	10-Oct[1]	10-Oct[1]	17-Sep[2]	17-Sep[2]	17-Sep[2]

[1]Regrowth harvest associated with first harvested growth on July 17.

[2]Regrowth harvest associated with second harvested growth on August 3.

2.4 Results and Discussion

2.4.1 Soybean Systems: Study 1

The objective of study one was to compare the forage yield and quality of a forage soybean when grown with different row spacings and plant populations. The two parameters tested were a row spacing of 18 cm and 469,500 seeds ha^{-1} (18High) and a row spacing of 36 cm and 234,750 seeds ha^{-1} (36Low). Stand counts achieved were approximately 145,000 plants ha^{-1} and 69,000 plants ha^{-1} for 18High and 36Low, respectively. Hintz and Albrecht (1994) reported that stage of maturity at harvest had the greatest impact on DM partitioning and that neither row spacing nor seeding rate had a significant effect on DM partitioning before R5. There were no differences in study one between the two soybean treatments for either forage yield or quality within harvest date. However, there were differences in yield ($P < .0001$) and quality, as defined by NDF, ADF, CP and IVTD ($P \leq .02$) across harvest dates. No statistical differences in forage yield were seen when harvested on July 11 and 25 (Table 2.3) when stage of maturity

was V6 to V12. The large increase in yield observed between July 25 and August 10 is in part related to an increase in maturity, but also resulted because of much needed rains on July 20, Aug 6 and Aug 17 to support plant growth (Figure 2.1). Rainfall between July 11 and July 25 was only 1.62 cm but was 4.65 cm between July 25 and Aug 10. The results in the current study are similar to those reported by Hintz and Albrecht (1994) that row spacing and seeding rate have no significant effect on DM partitioning before R5 stage of maturity. Results from the current study also agree with Rao et al. (2005) who observed forage soybean DM yield increased from early to final harvest dates. The optimum time for harvest was recommended by Hintz et al. (1992) to be at maturity stages of R6 to R7 due to the high DM yield. Hintz et al. (1992) also reported an increase of 5000 kg ha^{-1} in DM yields from early reproductive stages (R1) to late reproductive stages (R7). Average yield for the two treatments in the current study increased from 2183 kg/ha with the July 11 harvest to 8498 kg/ha when harvested on August 24. Maturity stages were V6 and R6 on these two dates, respectively.

Similar results to those reported by Munoz et al. (1983) were observed in the current study with a slight increase in CP (194 g kg^{-1} to 213 g kg^{-1}) and digestibility (783 g kg^{-1} to 800 g kg^{-1}) between July 11 and July 25. On these same dates, NDF and ADF held steady and averaged 427 g kg^{-1} and 277 g kg^{-1}, respectively. Major changes in quality took place between July 25 and August 10 as soybean matured from V12 to V17-R1 in this two-week period. Crude protein and digestibility decreased to 158 g kg^{-1} and 751 g kg^{-1} respectively, whereas NDF and ADF increased to 465 g kg^{-1} and 304 g kg^{-1}, respectively, between July 25 and August 10. On August 10, the soybean maturity stage was V17-R1 and progressed to full seed (R6) on August 24. Between the last two harvest dates, overall quality remained steady. There was a slight decrease in CP (26 g kg^{-1}) and no statistical differences in soybean digestibility, NDF or ADF. Munoz et al. (1983) concluded that crude protein in soybean hay does not decrease with maturity as much as most forages because soybean seeds contain 35-40% protein. While the increased yield and small decrease in overall quality at late reproductive stages of soybean maturity observed in the current study suggests that this would be the optimum time for harvest, other factors also need to be considered. Hintz et al. (1992) observed 126 g kg^{-1} ether

extract (EE) for whole-plant forage soybeans. This large increase in vegetable fat could decrease DM intake and reduce fiber digestion in ruminant diets. If the forage is harvested in late reproductive stages of maturity, Hintz et al. (1992) recommends either limiting forage soybean to no more than one half of the total ration DM or harvest at an earlier stage of maturity to minimize any potential negative impacts caused by excess EE.

2.4.2 Soybean Systems without Pearl Millet: Study 2

The objective of study two was to evaluate the yield and quality of forage soybeans grown at different row spacings and plant populations, with and without pearl millet. There were no statistical differences for the three soybean only treatments ($P >$ .05) within harvest date and there were no treatment*harvest (soybean system*harvest) interactions for total DM yield. When the soybean systems were compared across seven harvest dates, there was a linear increase ($R^2 = .97$) in total DM yield between July 17 and October 10 (Figure 2.2). The first DM yield total for the July 17 harvest was 1768 kg ha^{-1}, and regrowth DM yield was 3879 kg ha^{-1} for a total season DM yield of 5469 kg ha^{-1}. Since the July 17 harvest date included regrowth as part of the total DM yield, it was statistically similar to the August 31 harvest date. Results in the current study are in agreement with those reported by Rao et al. (2005). The lack of rainfall between July 11 and July 25 (1.62 cm) may have limited plant growth, but likely did not limit DM yield after the August 3 harvest date (4.8 cm between July 25 and August 10).

There were no statistically ($P > .05$) significant treatment * harvest interactions for NDF, ADF and CP. Forage digestibility as determined by IVTD, did have a significant treatment * harvest interaction. There were statistical differences between treatments 18High and 36High for NDF ($P < .02$; Table 2.4) and ADF ($P < .01$; Table 2.5) for harvest dates September 14, September 28 and October 10. This suggests that row spacings may have an effect on the fiber components of soybean. From harvest date July 17 through August 31, NDF (391 g kg^{-1} to 507 g kg^{-1}) and ADF (241 g kg^{-1} to 366 g kg^{-1}) concentrations within harvest date were statistically similar for both treatments. By September 14, the 18High treatment was slightly higher in NDF (80 g kg^{-1}) and ADF (72

g kg^{-1}) than the 36High treatment. This is in contrast to research reported by Hintz et al. (1992) and Sheaffer et al. (2001) who observed no effects of row spacings on NDF and ADF concentrations. There were no significant differences within harvest date for CP and IVTD ($P > .05$; Table 2.6; Table 2.7).

There were statistical differences for NDF, ADF, CP and IVTD between harvest dates ($P < .0001$) with a significant treatment * harvest interaction ($P < .001$) for IVTD. This interaction, while significant, is the result of small differences in the 18High treatment that do not appear to have practical significance on September 14 and October 10. Between harvest dates July 17 and August 31, there was a significant increase in both NDF (393 g kg^{-1} to 503 g kg^{-1}) and ADF (242 g kg^{-1} to 359 g kg^{-1}), and a significant decrease in CP (236 g kg^{-1} to 136 g kg^{-1}) and IVTD (837 g kg^{-1} to 719 g kg^{-1}). Between August 31 and September 14, NDF decreased, but ADF, CP, and IVTD concentrations remained similar. Maturity increased from V5 on July 17 to V20-R2 by September 14. As soybean continued to mature to mid-reproductive stages (R3-R4) on harvest date September 28, NDF remained constant, ADF declined (33 g kg^{-1}), and both CP (28g kg^{-1}) and digestibility (40 g kg^{-1}) increased. These results can be at least partially explained by the progression toward pod and seed development. Munoz et al. (1983) reported that digestibility of stems decreased rapidly after pod development, but the increasing amount of highly digestible pods and seeds offset the overall effect of forage digestibility. For both NDF and ADF, there was a slight decrease (41 g kg^{-1} and 14 g kg^{-1}, respectively), between harvest dates September 28 to October 10. Crude protein and digestibility during this same time frame decreased (20 g kg^{-1} and 11 g kg^{-1}, respectively) with advancing maturity (R6) by the October 10 harvest date. These results are similar to Hintz et al. (1992) who reported an improvement in forage quality as seed mass increased to offset the decline in forage quality of vegetative structures.

The optimum time for harvest of forage soybean as described by Hintz et al. (1992) is between the stages of R6 and R7 to maximize the DM yield of the crop. Forage soybean is a very forgiving crop compared to many of the more traditional forage species by maintaining overall quality throughout the various stages of advancing maturity.

Therefore, overall harvesting decisions based on yield and quality must be determined based on the animal production system. If harvested at the recommended stage of maturity, quality of the soybean would fit almost any beef production system. Ration formulations would need to be based off of a nutrient analysis and include appropriate supplementation to meet the animal's specific nutrient requirements.

2.4.3 Soybean Systems including Pearl Millet: Study 2

When all six treatments were compared, there were significant differences (P <.0001) for total DM yields; however, with only one exception, there were no significant differences among the three soybean treatments, or between the MIX, ALT and PM treatments, within harvest date on the August 20 harvest date. The PM treatment had significantly higher yields (2575 kg ha^{-1}) than the average of the MIX and ALT treatments (Table 2.8). Additionally, total DM yield at final harvest for the soybean only treatments (October 10) and pearl millet containing treatments (August 31) were not different. Statistically there were no differences in total DM yield between PM containing treatments when an early harvest early plus regrowth harvest (denoted as July 17 in Table 2.8) was compared to a single late season harvest (August 31). These results indicate there is no advantage in total DM yield to an early harvest followed by a late season regrowth harvest compared to a single late season harvest.

When quality parameters were measured, there were significant differences among treatments and harvest dates (P < .0001) for NDF, ADF, CP and IVTD. The soybean only treatments were similar in NDF (Table 2.9; Figure 2.2) and ADF (Table 2.10; Figure 2.3) within the harvest dates of July 17, August 3, August 20 and August 31. The PM treatment was highest (P < .05) in NDF for harvest dates July 17 through August 20 (range = 625 g kg^{-1} to 687 g kg^{-1}), with the MIX and ALT being intermediate (avg. = 534 g kg^{-1} to 585 g kg^{-1}) and soybean only treatments lowest (avg. = 393 g kg^{-1} to 435 g kg^{-1}) on those same harvest dates. The MIX and ALT treatments were similar in NDF and ADF within the harvest dates of July 17, August 3 and August 20. There was no difference in NDF for the PM treatment across harvest dates of July 17 and August 3

(625 g kg^{-1} and 644 g kg^{-1}, respectively). A significant increase ($P < .05$) in NDF occurred when these earlier harvests were compared to PM forage harvested on August 20 and August 31 (687 g kg^{-1} and 685 g kg^{-1}). This increase is most likely due to the advancement in maturity from an early vegetative stage (V5) on July 17 and August 3 to an early reproductive stage (R0-R4) on August 20, and early seed stage (R4-S2) on August 31. Results for ADF followed a similar trend among treatments to those reported for NDF. There was no difference in ADF of the pearl millet treatments between July 17 and August 3, but a significant increase in ADF was observed between the August 3 (range = 293 g kg^{-1} to 312 g kg^{-1}), and August 20 (range = 342 g kg^{-1} to 371 g kg^{-1}) and August 31 (range = 349 g kg^{-1} to 386 g kg^{-1}) harvest dates.

When evaluating forage regrowth for NDF and ADF (Tables 2.9 and 2.10), there were no differences among treatments within harvest date. The regrowth NDF concentrations (avg. = 453 g kg^{-1}) of soybean only treatments were similar to those of late season (avg. = 461 g kg^{-1}) harvests (September 28 and October 10). In general, NDF concentrations of pearl millet treatments' regrowth were similar between regrowth harvest dates (September 7 and September 17; avg. = 661 g kg^{-1}). The ADF concentrations of the pearl millet containing treatments regrowth were not different between harvest dates (September 7 and September 17; avg. = 362 g kg^{-1} vs. 338 g kg^{-1}) and were most similar to the August 20 harvest date. Overall these results indicate that the fiber components of pearl millet and intercropped forage soybean with pearl millet are higher than when forage soybean is grown as a monoculture. When averaged across all harvest dates, the MIX and ALT treatments contained 36% more NDF and 12% more ADF, while the PM only treatment contained an average of 47% more NDF and 17% more ADF than the soybean only treatments.

The three soybean monoculture treatments were similar in CP (Table 2.11; Figure 2.4) within each harvest date, but decreased ($P < .05$) linearly across harvest dates from July 17 to August 31 before increasing during late season harvests as pods developed. There were differences in CP between PM (117 g kg^{-1}), MIX (182 g kg^{-1}), and ALT (158 g kg^{-1}) treatments for the July 17 harvest date, and PM was lower ($P < .05$) in CP than the MIX and ALT treatment means on the August 3 (93 g kg^{-1} and 128 g kg^{-1}), August 20 (61

g kg^{-1} and 102 g kg^{-1}) and August 31 (45 g kg^{-1} and 69 g kg^{-1}) harvest dates. The differences in CP between the MIX and ALT treatments on the July 17 harvest date may be explained by the proportion of soybean and pearl millet in the harvested crop for that date. Calculations were made using the equation reported by Twidwell et al. (1986) that utilizes N concentrations of the individual species to estimate the proportion of each species in a mixed sample. For the MIX treatment, the ratio of soybean to pearl millet was 67:33 and the ALT treatment ratio was 45:55. This could explain the increase in CP for the MIX treatment on the July 17 harvest date, since the CP concentration of soybean is higher than pearl millet. Overall, CP decreased as the pearl millet, and the soybean and pearl millet treatments matured, while the monoculture soybean CP concentrations decreased through mid-season and then increased during late season as pods began to develop. When evaluating the regrowth for the pearl millet containing treatments, there were no differences in CP (avg. = 67 g kg^{-1}) between PM, MIX and ALT treatments across the two harvest dates (September 7 and September 17). In general, the regrowth CP of the MIX and ALT was most similar to the August 31 harvest date for MIX and ALT (avg. = 68 g kg^{-1}) treatments, and the regrowth of PM had similar CP as the August 20 harvest date for the PM treatment (avg. =61 g kg^{-1}). These results suggest that the protein component of pearl millet and intercropped forage soybean with pearl millet are significantly lower than when forage soybean is grown as a monoculture.

Digestibility (IVTD; Table 2.12; Figure 2.5) results for the three soybean treatments followed a similar linear decline across harvest dates from July 17 to August 31 before increasing during late season harvests as pods and seeds developed. There were no differences between the PM, MIX and ALT treatments for digestibility within harvest dates of July 17 and August 20. Pearl millet alone and MIX were similar in IVTD to each other, but lower than the ALT treatments on August 3. On August 31 the PM and ALT treatments were similar to each other in IVTD, but lower than the MIX treatment. While there are statistically significant differences, these differences are small and are considered to have minor importance in practice. Across harvest dates, the pearl millet containing treatments were similar between July 17 and August 3, however, only the

MIX was similar between August 20 and August 31. There was a significant decrease in IVTD between August 3 and August 20 for PM, MIX and ALT treatments.

When evaluating IVTD regrowth, at a harvest date, there were no differences within the soybean only system, or within the PM, MIX and ALT systems. There was, however, a significant increase in IVTD for pearl millet containing treatments between the September 7 and September 17 harvest dates. This can be explained by differences in stage of maturity of the regrowth on September 7 (V6) and September 17 (V2). In general, the regrowth of September 7 (avg. = 766 g kg^{-1}) is most similar to the August 20 (avg. = 759 g kg^{-1}) harvest date, the regrowth of September 17 (avg. = 822 g kg^{-1}) is most similar to the August 3 (avg. = 818 g kg^{-1}) harvest date and the regrowth of October 10 (avg. = 833 g kg^{-1}) is most similar to the July 17 (avg. = 837 g kg^{-1}) harvest date. Overall, digestibility is similar between the intercropped forage soybean with pearl millet and soybean grown as a monoculture for all harvest dates in this study from July 17 to August 31. The last harvest of pearl millet containing treatments (August 31) was determined by stage of maturity (R4-S2), with minimal achieved due to timing in the growing season. In the case of the soybean only treatments, growth continued and quality increased as pod and seed development advanced into late September and early October. Dry matter yields (Table 2.8) were previously discussed as totals. Initial harvests for July 17 and August 3 for PM were 3090 kg ha^{-1} and 4893 kg ha^{-1}, respectively. For MIX it was 3146 kg ha^{-1} and 4303 kg ha^{-1}, respectively and for the ALT it was 3010 kg ha^{-1} and 4402 kg ha^{-1}, respectively.

In conclusion, when evaluating both total DM yield and forage quality, forage soybean was similar in yield, higher in CP and IVTD (digestibility), and lower in fiber at later stages of maturity, when grown as a monoculture compared to either pearl millet or soybean intercropped with pearl millet. There were no significant differences in total DM yield in the final harvest of PM, MIX and ALT treatments which indicates there is no real advantage to a system that includes early harvest plus regrowth harvest based on total DM yields. When determining harvest time based on a combination of both yield and

quality, it may be worthwhile to harvest early and allow regrowth to optimize total forage feed value.

Table 2.3. Study 1 means within harvest date for 18High and 36Low for crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), in-vitro true digestibility (IVTD) and DM yield. Least significant difference (LSD, $P < 0.05$).

Harvest Date		CP	NDF	ADF	IVTD	Yield
		g kg^{-1}				kg ha^{-1}
11-Jul		194	429	286	845	2183
25-Jul		214	428	270	872	2803
10-Aug		159	465	304	800	5369
24-Aug		126	454	323	783	8498
	LSD	12	28	23	26	1273

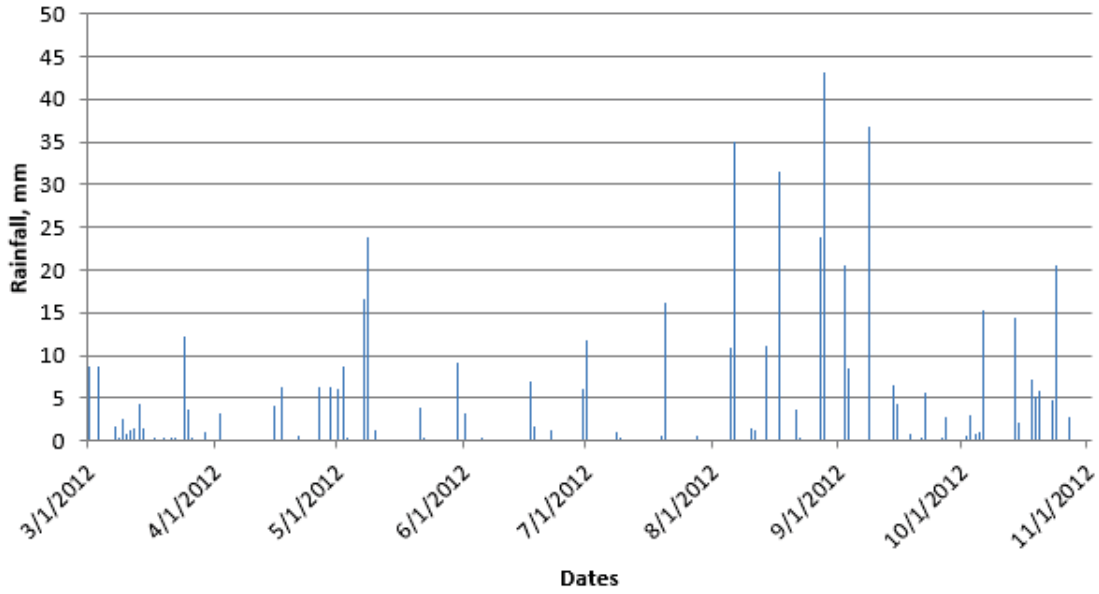

Figure 2.1. Rainfall totals (mm) for 2012 growing season from March 1 through November 1.

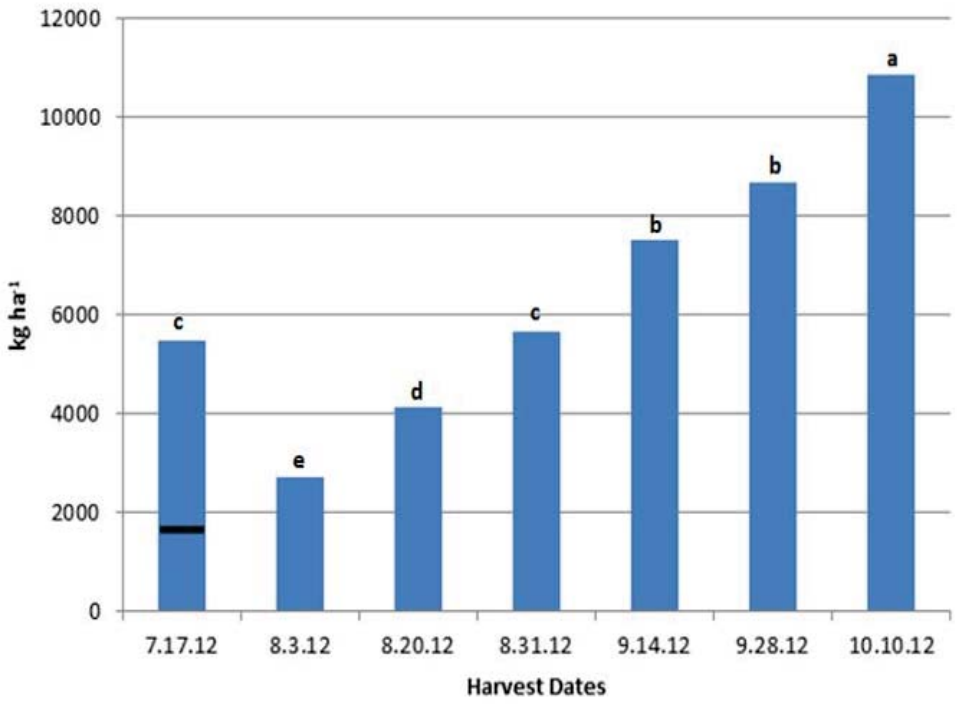

Figure 2.2. Study 2 total DM yield (kg ha^{-1}) for soybean systems without pearl millet at harvest. Data were transformed for statistical analysis and back transformed for this figure so least significant intervals are not appropriate. Means with the same letter designation are not statistically different (P< .05). Harvest date July 17 represents early harvest (below the bar) and early harvest plus second harvest.

Table 2.4. Neutral detergent fiber (NDF) treatment means (g ka^{-1}) for a soybean system within harvest date.

	Harvest Dates							
Treatments	17-Jul	3-Aug	20-Aug	31-Aug	14-Sep	28-Sep	10-Oct	Mean
18Low	392	426	434	494	477	477	443	449
18High	391	418	434	507	518	596	440	458
36High	397	414	438	509	438	451	415	437
Mean	393	419	435	503	478	474	433	

Main effect Soybean System, (P < .05, LSD = 14).

Main effect Harvest Date, (P < .05, LSD = 21).

Table 2.5. Acid detergent fiber (ADF) treatment means (g kg^{-1}) for a soybean system within harvest date.

	Harvest Dates							
Treatments	17-Jul	3-Aug	20-Aug	31-Aug	14-Sep	28-Sep	10-Oct	Mean
18Low	237	269	292	347	341	319	303	301
18High	241	267	298	366	381	323	308	312
36High	247	263	293	366	309	291	279	293
Mean	242	267	295	359	344	311	297	

Main effect Soybean System, ($P < .05$, LSD = 12).

Main effect Harvest Date, ($P < .05$, LSD = 18).

Table 2.6. Crude protein (CP) treatment means (g kg^{-1}) for a soybean system within harvest date.

	Harvest Dates							
Treatments	17-Jul	3-Aug	20-Aug	31-Aug	14-Sep	28-Sep	10-Oct	Mean
18Low	239	207	164	149	158	166	163	178
18High	238	199	167	127	137	173	150	170
36High	231	203	160	132	140	178	145	170
Mean	236	203	163	136	145	173	153	

Main effect Soybean System, ($P < .05$, LSD = 7.8).

Main effect Harvest Date, ($P < .05$, LSD = 12).

Table 2.7. In-vitro digestibility (IVTD) treatment means (g kg^{-1}) for a soybean system within harvest date.

	Harvest Dates							
Treatments	17-Jul	3-Aug	20-Aug	31-Aug	14-Sep	28-Sep	10-Oct	Mean
18Low	845	805	779	724	755	760	767	776
18High	846	812	788	712	695	767	745	766
36High	821	806	768	721	750	791	773	776
Mean	837	808	778	719	733	773	762	

Main effect Soybean System, ($P < .05$, LSD = 10).

Main effect Harvest Date, ($P < .05$, LSD = 16).

Table 2.8. Total DM yields (kg ha^{-1}) for forage system.

	Harvest Dates						
	17-Jul[2]	**3-Aug**[3]	**20-Aug**	**31-Aug**	**14-Sep**	**28-Sep**	**10-Oct**
Treatments	kg ha^{-1}						
18Low	5812^{h,i,j}	2549^{m}	4047^{l}	5999^{g,h,i,j}	7535^{d,e,f,g,h}	8529^{a,b,c,d}	11376^{a}
18High	5459^{i,j,k}	2916^{m}	4205^{k,l}	6294^{e,f,g,h,i,j}	7707^{d,e,f,g,h}	9399^{a,b,c,d}	10763^{a,b,c}
36High	5154^{j,k,l}	2662^{m}	4135^{k,l}	4812^{j,k,l}	7275^{d,e,f,g,h,i}	8173^{c,d,e,f}	10479^{a,b,c}
PM	9047^{a,b,c,d}	8386^{d,e,f,g,h,i}	8560^{a,b,c,d}	11067^{a,b}			
MIX	8054^{c,d,e,f,g}	7096^{b,c,d,e,f}	5778^{h,i,j}	8884^{a,b,c,d}			
ALT	8101^{c,d,e,f}	7331^{d,e,f,g,h,i}	6193^{f,g,h,i,j}	8665^{a,b,c,d}			

[1]Means with unlike superscripts are significant (P < .05), and corresponds with transformed data. Values in table are back transformed, and shown for value familiarity.
[2]Harvest date includes regrowth for total yield. Soybean treatments' regrowth harvest date was on October 10 and pearl millet treatments' regrowth harvest date was on September 7.
[3]Harvest date includes regrowth for total yield. Pearl millet treatments' regrowth harvest date was on September 17.

Table 2.9. Neutral detergent fiber (NDF) treatment means (g kg^{-1}) for a forage system within harvest date.

	Harvest Dates									
Treatments	17-Jul	3-Aug	20-Aug	31-Aug	14-Sep	28-Sep	10-Oct	7-Sep[1]	17-Sep[2]	10-Oct[1]
	g kg^{-1}									
18Low	392	426	434	494	477	477	444			444
18High	391	418	434	507	518	496	440			461
36High	397	414	438	510	438	451	415			455
PM	625	644	687	685				672	655	
MIX	525	580	577	607				684	645	
ALT	544	585	592	651				657	655	

Main effect of Forage System, (P <.05, LSD= 39).
[1]Regrowth from July 17 initial harvest.
[2]Regrowth from August 3 initial harvest.

Table 2.10. Acid detergent fiber (ADF) treatment means (g kg^{-1}) for forage system within harvest date.

	Harvest Dates									
Treatments	17-Jul	3-Aug	20-Aug	31-Aug	14-Sep	28-Sep	10-Oct	7-Sep[1]	17-Sep[2]	10-Oct[1]
	g/kg									
18Low	237	269	292	347	341	319	303			257
18High	241	267	298	366	381	323	308			258
36High	247	263	293	366	309	292	279			272
PM	293	312	371	375				354	343	
MIX	271	305	347	349				381	340	
ALT	285	293	342	386				351	332	

Main effect of Forage System, (P <.05, LSD= 31).
[1]Regrowth from July 17 initial harvest.
[2]Regrowth from August 3 initial harvest.

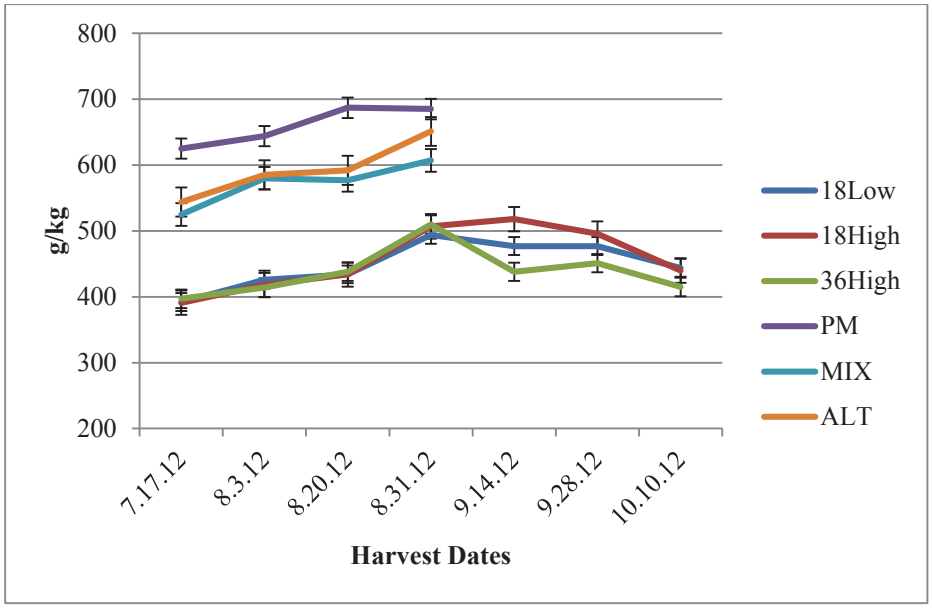

Figure 2.3. Neutral detergent fiber (NDF) treatment means (g kg^{-1}) for a forage system within harvest date

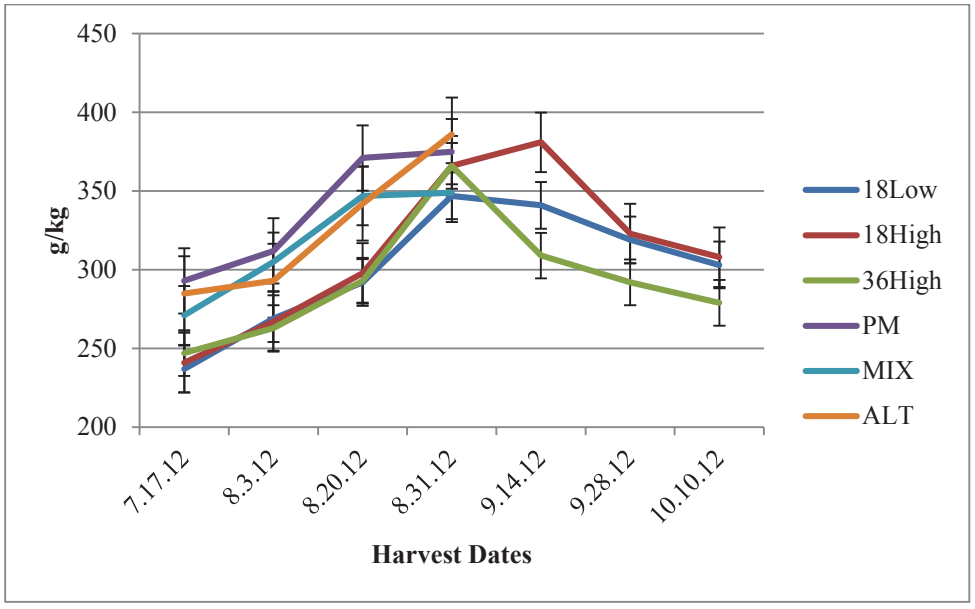

Figure 2.4. Acid detergent fiber (ADF) treatment means (g kg^{-1}) for a forage system within harvest date

Table 2.11. Crude Protein (CP) treatment means (g kg^{-1}) for a forage system within harvest date.

	Harvest Dates									
Treatments	17-Jul	3-Aug	20-Aug	31-Aug	14-Sep	28-Sep	10-Oct	7-Sep[1]	17-Sep[2]	10-Oct[1]
	g kg^{-1}									
18Low	239	207	164	149	158	166	164			198
18High	238	199	167	127	137	173	150			212
36High	230	203	160	132	140	178	145			207
PM	117	93	61	45				70	64	
MIX	182	123	97	77				56	73	
ALT	158	134	108	60				63	75	

Main effect of Forage System, (P <.05, LSD= 21).
[1]Regrowth from July 17 initial harvest.
[2]Regrowth from August 3 initial harvest.

Table 2.12. In-vitro digestibility (IVTD) treatment means (g kg^{-1}) for a forage system within harvest date.

	Harvest Dates									
Treatments	17-Jul	3-Aug	20-Aug	31-Aug	14-Sep	28-Sep	10-Oct	7-Sep[1]	17-Sep[2]	10-Oct[1]
	g kg^{-1}									
18Low	845	805	779	724	755	760	767			828
18High	846	812	788	712	696	767	745			830
36High	821	806	768	721	750	791	773			840
PM	818	804	746	710				776	811	
MIX	830	810	761	778				749	815	
ALT	838	840	769	714				772	840	

Main effect of Forage System, (P <.05, LSD= 30).
[1]Regrowth from July 17 initial harvest.
[2]Regrowth from August 3 initial harvest.

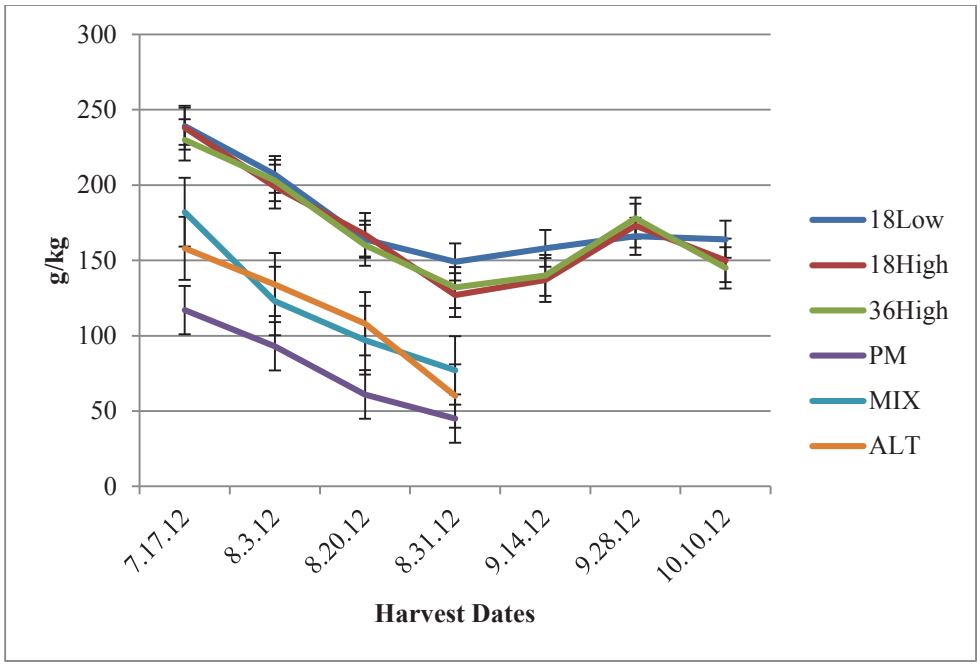

Figure 2.5. Crude protein (CP) treatment means (g kg^{-1}) for a forage system within harvest date.

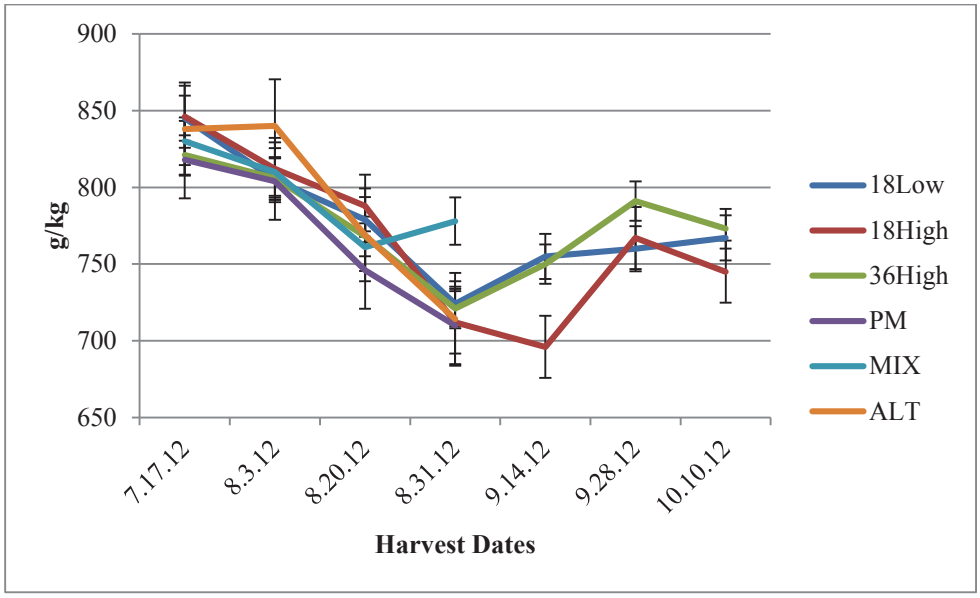

Figure 2.6. In-vitro digestibility (IVTD) treatment means (g kg^{-1}) for a forage system within harvest date.

CHAPTER 3 EVALUATION OF FORAGE SOYBEAN, WITH AND WITHOUT PEARL MILLET, AS AN ALTERNATIVE FORAGE FOR DEVELOPING BEEF REPLACEMENT HEIFERS

3.1 Abstract

Angus-Simmental beef replacement heifers (n=90; BW=366 kg; BCS=5.53) were used to evaluate the effects of feeding forage soybean based silages on heifer body weight, body condition, follicular growth and conception. At 65d prior to timed artificial insemination (TAI), heifers were allotted by genotype, BCS and BW to receive either; 1) a control diet of alfalfa haylage (CON), 2) soybean silage (SB) or 3) soybean and pearl millet silage (SBxPM). All diets were formulated to meet or exceed nutrient requirements (NRC,2000) of replacement beef heifers targeted to gain .79 kg/d. Diets were terminated 21d post-TAI and heifers were commingled and placed on a common diet. Estrous status of the heifers was determined by 2 samples of blood progesterone taken prior to initiation of estrous synchronization. Follicular diameter was determined at time of breeding by ultrasonography. Pregnancy diagnosis was accomplished 35 and 66d post-TAI, respectively, for TAI and end of season pregnancy. Final BW (avg. 414 kg; $P \geq .10$) and BCS (avg. 5.28; $P \geq .07$) for the heifers were similar among treatments. No differences were seen in TAI (avg. = 48%; $P > .19$) or overall breeding season (avg. = 93%; $P > .99$) pregnancy rates. Ovulatory follicle diameters (avg. 11.7mm) were not different ($P > .31$) between treatments. In summary, forage soybean based silages, with and without pearl millet, were an acceptable alternative forage for developing replacement beef heifers in this study.

3.2 Introduction

To maximize profitability in a cow-calf operation, growth rate, body composition and target weight prior to breeding must be optimized to allow reproductive competence. Replacement heifers are developed to replace approximately 15-20% of a producer's herd and, therefore, represent a significant loss if they do not conceive and carry a calf to term. Many factors contribute to age at puberty and reproductive competence such as genetics, environment, nutrition, body composition and health. Patterson et al. (1992) reviewed the importance of nutrition and its effects on puberty attainment in heifers. The expenses associated with replacement heifer development, coupled with recent high feed costs are affecting cow herd profitability. This leaves producers searching for alternative feed alternatives that optimize replacement heifer performance and maximize profitability. The objective of this study was to evaluate forage soybean forage, with or without pearl millet, as an alternative feed resource for developing replacement beef heifers.

3.3 Methods

This study was conducted at the Purdue Animal Sciences Research and Education Center near West Lafayette IN. All animals were handled in compliance with approved protocols from the Purdue Animal Care and Use Committee (PACUC 1303000837).

3.3.1 Animals and Diets

Angus-Simmental heifers (n=90; BW=366 kg ± 25 ; BCS=5.53 ± .35, with a range in age of 55 days) were used in a complete randomized block design study to evaluate the effects of feeding an ensiled forage soybean or soybean x pearl millet forage on heifer body weight, body condition, follicular growth and conception.

Heifers were blocked by genotype, BCS and BW, and randomly assigned to one of three treatments with three replications per treatment. The dietary treatments were: 1) alfalfa haylage (CON), 2) soybean silage (SB), or 3) soybean x pearl millet silage (SBxPM). Diets are shown in Table 3.

All diets were formulated to be isocaloric, isonitrogenous, and either meet or exceed all other nutrient requirements (NRC, 2000) to obtain a target gain of .79 kg and 65% of mature BW prior to initiation of breeding (Table 3.1). Ingredient compositions of feed stuffs used to formulate diets were obtained by wet chemistry methods (AOAC, 1990) before trial initiation (Dairy One, Ithaca, NY). Feed samples were collected from the TMR mixer during the study and frozen. Upon completion of the study, samples were composited, mixed and subsampled for NDF analysis using the Ankom procedure (Ankom Technology, Macedon, NY). Heifers were housed in nine mounded lots with concrete feeding apron immediately adjacent to the concrete feed bunks, and ad libitum access to water. All dietary treatments were fed as a total mixed ration (TMR) beginning 65d prior to TAI breeding and were fed ad libitum once daily at 0800 in concrete bunks. Daily feed delivery adjustments were made based on bunk scores and ingredient DM adjustments were made weekly. Heifers were comingled and placed on a common pasture 21 days post-TAI breeding.

An average of two preprandial BW's and BCS's (1=emaciated, 9=obese; Wagner et al., 1988) were taken on consecutive days at trial initiation and termination were used in this study. A single day preprandial midpoint BW and BCS was also taken. An experienced single investigator was responsible for BCS throughout the study. Final BW and BCS were taken at termination of estrous synchronization. At TAI heifers were commingled by treatment and remained on their treatment diets 21 days post-TAI.

3.3.2 Cyclicity

Cyclicity was determined with the collection of blood samples for progesterone on two 10-day intervals. The first sample collection was taken on d -10 and d 0 relative to trial initiation, and the second sample collection was taken on d -10 days and d 0 relative to initiation of estrous synchronization. Collected blood samples were taken in 6 ml EDTA tubes (BD Vacutiner ^{TM}; Becton-Dicknson, Franklin Lakes, NJ) and placed on ice until processed. Samples were centrifuged at 1750 × g for 25 minutes at 4°C. Plasma was then transferred to polystyrene tubes and frozen at -20°C for subsequent progesterone analysis.

Plasma progesterone concentrations were determined using a radioimmunoassay (RIA) kit (Coat-A-Count, Siemens Medical Solutions Diagnostics, Los Angeles, CA). Heifers with a progesterone concentration ≥ 1ng/ml were determined to be cycling. Across four assays, the average intra-assay CV was .5% and the inter-assay CVs for pooled plasma samples containing 0.5 ng/ml and 6.8 ng/ml of progesterone, were .37% and 2.2% respectively.

A reproductive tract score (RTS) was performed using rectal palpation by a Board Certified Theriogenologist on d-10 relative to initiation of estrous synchronization to determine the pubertal status according to the procedure described by (Anderson et al., 1991).

3.3.3 Estrous Synchronization, Breeding, and Luteal Function

On d 56 of the study, all heifers were started on a 5-day Co-Synch + CIDR protocol to synchronize ovulation. At protocol initiation, all heifers were inserted with a intravaginal progesterone source (CIDR Zoetis, Florham Park, NJ) paralleled with the administration of 100μg of GnRH (Cystorelin, Merial Animal Health, Duluth, GA). Five days after protocol initiation, the CIDR was removed and two separate 25 mg injections of $PGF_{2\alpha}$ (Lutalyse, Zoetis, Florham Park, NJ) were simultaneously given. At CIDR removal, all heifers were tail painted (Tell Tail; FIL, 132 Mount Maunganui, New Zealand) to assist in estrus detection. Estrus detection took place for 72 h post-prostaglandin administration. Heifers exhibiting estrus within 60 h of prostaglandin administrations, were artificially inseminated (AI) following the AM/PM rule. Heifers not exhibiting behavioral estrus were TAI bred at 72 h.

At breeding, all heifers were evaluated by trans-rectal ultrasound for measurements of dominant follicular size. Both ovaries were scanned for follicles, with one follicle being determined as dominant. The dominant follicle was measured (mm in diameter) and recorded. Both breeding and ultrasonography was done by two trained technicians.

On d 7 post-AI, blood samples were collected and processed for progesterone concentrations, as described above, to indirectly measure CL function. Progesterone concentrations were analyzed using the same RIA kit and procedure previously described.

3.3.4 Statistical Analysis

Performance data were analyzed with pen as the experimental unit and reproductive data were analyzed with individual animal as the experimental unit. Differences between treatments for binomial data (estrus, TAI, AI pregnancy, season-long pregnancy, resorbed pregnancy, puberty at start of trial, and puberty at start of estrous synchronization) were analyzed using the GLIMMIX procedure of SAS (SAS Inst. Inc., Cary NC). The remaining parameters; reproductive tract scores, follicle size, estrus interval, progesterone concentration, BW, BCS, DMI, ADG and G:F were analyzed using the MIXED procedure of SAS. The following contrasts were used to test treatment effects: 1) CON diet vs. the average of diets containing soybeans, and 2) SB diet vs. SBxPM diet. For dependent variables, the model included the fixed main effect of treatment. Ovary size, sire and tech were initially included in the model as covariates, but were removed due to insignificance. For all variables analyzed, a P-value $\leq .05$ was identified as significant.

3.3.5 Forages

Cultivars used were Eagle Seeds soybean 'Big Fellow', and Byron Seeds pearl millet 'Wonderleaf'. Soybean as a monoculture was seeded on June 22 and harvested on October 6. Dry matter yield was 5415 kg ha^{-1}. Soybean intercropped with pearl millet was seeded on May 19 and harvested on August 18, with a dry matter yield of 7023 kg ha^{-1}. Soybean and soybean plus pearl millet were planted in 18 cm row spacings. Seeding rates (PLS) were 495,050 seeds ha^{-1} for SB and the soybean and pearl millet seeding rates were 247,500 seeds ha^{-1} and 3.5 kg ha^{-1}, respectively. Seeding of the soybean and pearl millet were in alternating rows. All forages were harvested, ensiled and stored in an Ag-Bag® (Miller-St. Nazianz, Inc. Company, St. Nazianz, WI) for a minimum of four months

prior to feeding. Samples were taken using a forage probe through the plastic for nutrient analysis prior to trial initiation and diets were formulated according to results. The plastic area probed was sealed with recommended tape immediately after samples were taken.

Table 3.1. Formulated (actual) dietary feed ingredients, dietary nutrient composition, and calculated dry matter intake of yearling beef heifer diets.

	Treatment[1]		
Item	CON	SB	SBxPM
Ingredient, formulated % (actual kg/DM/d[2,3])			
Alfalfa haylage	76.1(7.28)	---	---
Soybean silage	---	75.1(6.31)	---
Soybean x pearl millet silage	---	---	63.4(5.24)
Corn stover	16.3(1.45)	6.5(.66)	15.2(1.33)
Dried distiller's grains	6.7(.57)	---	---
Soybean hulls	---	17.2(1.5)	20.3(1.56)
Mineral Supplement	1.11(.10)	1.11(.11)	1.11(.09)
Formulated Nutrient, (actual) Intake[3,4]			
CP, g/d	1365(1295)	1368(1170)	1371(1135)
NE_m,Mcal/d	7.74(7.86)	7.74(7.74)	7.74(7.74)
NE_g, Mcal/d	2.92(2.48)	2.93(1.94)	2.92(1.62)
Ca, g/d	119(114)	87(76)	84(69)
P, g/d	30(28)	30(27)	29(24)
Mg, g/d	23(22)	36(31)	37(31)
K, g/d	149(142)	184(158)	225(187)
NDF, kg/d[5]	(3.84)	(3.95)	(4.27)

[1]CON = control; SB = Soybean silage; SBxPM = Soybean and pearl millet silage.
[2]DMI was measured for CON, SB and SBxPM total mixed rations delivered.
[3]Expressed on DM basis
[4]Calculated dietary chemical composition based on analysis of individual dietary feed ingredients.
[5]NDF concentration of total mixed ration determined by wet chemistry methods (Ankom Technology, Macedon, NY).

3.4 Results

3.4.1 Growth Performance

Initial heifer BW (366 ± 25; P ≥ .70) and BCS (5.53 ± .35; P ≥ .06) did not differ between treatments (Table 3.2). Likewise, final BW (P ≥ .10) and BCS (P ≥ .07) were similar between treatments. Overall, heifers fed the soybean containing diets consumed 10% less (P = .001) DMI than control fed heifers, but the DMI of the soybean diets were not significantly different from each other. Similarly, ADG of the heifers fed the soybean diets were not significantly different from each other, but heifers fed the soybean containing diets gained 21.4% slower (P = .01) than control heifers. Feed efficiencies (G:F) were not significantly different among treatments.

3.4.2 Reproductive Performance

The proportion of pubertal heifers prior to initiation of treatments did not differ (P ≥ .63; Table 3.3) among treatments. Reproductive tract scores evaluated prior to estrous synchronization were not different (P ≥ .24), but progesterone concentrations were significantly higher (P = 0.03) for heifers fed the SB compared to SBxPM diets. This suggests a decrease in the number of heifers cycling prior to estrus synchronization in the SBxPM treatment group, however, there were no differences in the proportion of heifers that exhibited estrus prior to TAI (P ≥.105). Of those heifers that did exhibit estrus prior to TAI, there were no differences in the interval between prostaglandin administration and estrus (P >.13). No differences were seen in ovulatory follicle diameter at time of AI (P > .31), correlation between dominant follicle size and progesterone analysis on d-7 post AI (P > .42) or overall breeding season (*P* > .99) pregnancy rates between treatments.

3.5 Discussion

It has been recognized across the beef industry that replacement beef heifer development is important to puberty attainment. Developing beef heifers to a target

weight of 55% (Funston and Deutscher, 2004) to 65% (Patterson et al., 1988) of their mature BW prior to breeding is key to lifetime reproductive performance. Body weights and BCS did not differ significantly throughout this current study. The mature cow weight in this herd is about 648kg. Using this mature weight, the heifers in this study obtained 65%, 63.7% and 63% of their mature weight, respectively, for CON, SB, and SBxPM treatments. It could be concluded that all treatments performed adequately and all heifers obtained the recommended target weight.

Diets were formulated to provide similar energy and protein concentrations across treatments; however, DMI differed between treatments (P =.01). Previous studies have examined the effects of forage quality on the animals' response related to DMI (Mertens, 1994). Varel and Kreikmeier (1999) reported that DMI was lower for low-quality forages than high-quality forages, specifically low-quality forage that was high in NDF concentrations (Mertens, 1994). Predicted intake for the heifers when formulating the diets was 9.68 kg of DM/d. The actual DMI for the CON diet was 9.37 kg of DM/d which was 5% lower than predicted, while the SB diet was 8.58 kg of DM/d (13% lower than predicted) and the SBxPM diet was 8.22 kg of DM/d (16.6% lower than predicted). Recent studies conducted with dairy heifers have revealed a new insight into predicting DMI using NDF values. Hoffman and Kester (2013) suggested that dairy heifers will consume a near-constant 1.0% of BW in NDF in commercially reared heifers. Analysis of TMR diets in the current study resulted in NDF concentrations of, 41.0%, 46.0%, and 52.7% in the CON, SB and SBxPM diets, respectively. The SB treatment was 12.2% higher in NDF than the CON treatment and the resulting DM intakes were 8.6% lower. The SBxPM treatment was 14.6% higher in NDF than the SB treatment, resulting in a 4%, non-significant difference (P >.05) in DM intake. Using the average pre-prandial body weight mean for each treatment in this study, the NDF values of each diet were compared to DMI. Neutral detergent fiber intake expressed as a percent of BW was calculated to be, .97, 1.01 and 1.10, respectively, for the CON, SB, and SBxPM treatments. This resulted in an average NDF intake of 1.03% of body weight across treatments. Results in the current study with replacement beef heifers support results reported by Hoffman and Kester (2013) with replacement dairy heifers that suggest heifers will consume a near constant 1% of their body weight in NDF per day. Due to the

fluctuation in NDF between diets, it could be concluded that differences in dietary NDF concentration caused the observed differences in DMI across treatments. Average daily gain differed (P =.01) between treatments and can be directly correlated to the differences in DMI. These data would strongly suggest a need to utilize NDF concentration of forage-based TMR diets when formulating replacement beef heifer diets to make sure that nutrient requirements are met.

It is not apparent why there was differences in cyclicity prior to synchronization between the two soybean treatments in the current study, however, this did not significantly affect either TAI or season long pregnancy rates. Reproductively, heifers performed similarly on all dietary treatments, and we conclude that forage soybeans, with or without pearl millet, are an acceptable forage alternative for replacement heifers. Additionally, it is recommended that when forage-based rations are developed, a nutrient analysis that includes NDF be utilized to more accurately predict DM and nutrient intake.

Table 3.2. Effect of yearling beef heifer diet on dry matter intake and growth performance.

	Treatment[1]				Contrast P-value[2]	
Item	CON	SB	SBXPM	SEM	1 vs 2,3	2 vs 3
BW, kg						
Initial	366.0	367.0	365.2	3.90	0.98	0.76
Final	422.3	413.1	407.6	6.07	0.16	0.55
BCS[3]						
Initial	5.53	5.56	5.49	0.02	0.69	0.07
Final	5.33	5.22	5.28	0.04	0.26	0.37
DMI, kg/d						
Overall	9.39	8.58	8.24	0.18	0.01	0.22
ADG, kg						
Overall	0.87	0.71	0.65	0.04	0.01	0.38
G:F , kg/kg						
Overall	0.09	0.08	0.08	0.01	0.06	0.55

[1]CON = control (1); SB = soybean silage (2); SBxPM = soybean and pearl millet silage (3).

[2]A P-value ≤ .05 was identified as significant.

[3]Body condition score on a scale of 1 to 9 (1 = emaciated, 9 = obese; Wagner et al., 1988).

Table 3.3. Effect of yearling beef heifer diet on reproductive performance.

	Treatment[1]				Contrast *P*-value[2]	
Item	CON	SB	SBXPM	SEM	1 vs 2,3	2 vs 3
Estrous[3], %	63.3 (19/30)	46.6 (14/30)	43.3 (13/30)		0.11	0.80
TAI[4], %	36.6 (10/30)	50 (15/30)	56.6 (17/30)		0.14	0.61
AI pregnancy[5], %	53.3 (16/30)	50 (15/30)	66.6 (20/30)		0.66	0.20
Season pregnancy[6], %	93.3 (28/30)	93.3 (28/30)	93.33 (28/30)		1.0	1.0
Resorbed[7], %	6.66 (2/30)	3.33(1/30)	13.33(4/30)		0.97	0.30
Puberty start[8], %	73.3 (22/30)	66.6 (20/30)	70 (21/30)		0.63	0.78
Puberty synch[9], %	100 (30/30)	100 (30/30)	90 (27/30)		0.21	0.03
Tractscores[10]	4.5	4.2	4.2	0.20	0.25	0.91
Follicle size[11], mm	11.21	12.10	11.85	0.63	0.31	0.77
Estrus Interval[12], h	58.0	50.5	56.3	2.81	0.13	0.15
P^4 Con.[13], ng/ml	3.95	3.37	3.66	2.52	0.43	0.83

[1]CON = control; SB = Soybean silage; SBxPM = Soybean and Pearl Millet silage
[2]A P-value ≤ .05 was identified as significant.
[3]Proportion of heifers that exhibited estrus within 72 h after $PGF_{2\alpha}$.
[4]Proportion of heifers that did not exhibit estrus prior to 60 h followimg $PFG_{2\alpha}$ that were TAI.
[5]Number of confirmed pregnant heifers at 35d post AI/number of heifers inseminated.
[6]Number of confirmed pregnant heifers at 66d for end of season pregnancy diagnosis.
[7]Percent of pregnancies lost between 35 d and 66 d of pregnancy.
[8]Percent of heifers reaching puberty prior to initiation of treatments.
[9]Percent of heifers reaching puberty prior to initiation of estrous synchronization.
[10]Reproductive tract scoring estimates of pubertal status; based on Anderson et al., (1991)
[11]Diameter measurements of dominant follicle size at time of breeding.
[12]Heifers exhibiting standing estrus within 72 h after $PGF_{2\alpha}$, defined as the interval from $PGF_{2\alpha}$ to standing estrus.
[13]Progesterone concentration days post-AI breeding.

CHAPTER 4 GENERAL DISCUSSION

The objective of the studies in this thesis was to answer an applied, producer-oriented question: Can soybeans have a positive impact on the cow-calf industry by allowing producers to utilize land, either singularly or in a "double crop" manner, to improve profitability? Since the 1940's, soybean has been mostly utilized as a grain crop and little research has been done to evaluate yield, quality and feeding value of forage cultivars. Three studies were developed to determine the feed value of a forage soybean variety, with and without pearl millet. This included two agronomy studies conducted in small plots to determine optimum seeding rates, row spacing, and harvest date. The forage soybean proved to be a very forgiving crop. While most forage crops tend to decrease in quality as they advance in maturity, soybean increased in quality as maturity advanced into the later reproductive stages of plant development that can be directly related to an increase in pod and seed development. This allows producers to take one late season harvest for tonnage and still maintain a high quality feed for their livestock. A third study was designed using replacement beef heifers as a sensitive nutritional model to evaluated DMI, growth and reproductive response to a forage soybean system.

The literature has suggested that some legume forages contain phytoestrogens than can negatively impact reproduction. While we did not measure phytoestrogens in this study we did have soybean included in the study. We did not see a significant overall treatment effect on reproduction in this study, it is interesting that the SBxPM treatment had fewer heifers cycling at the beginning of the breeding season, but had a 25% higher TAI conception rate than heifers fed the control treatment and 33% higher TAI conception rate than heifers fed the SB treatment. We speculate that the SBxPM diet might have caused a dilution of any potential phytoestrogens in this diet due to the pearl millet (grass) component. If phytoestrogens are having a negative effect on reproduction, one could speculate, due to the dilution from the pearl millet, this is the reason for the

numeric increase in AI pregnancy rates. This numeric increase, if real, would have a significant and positive effect on cow herd profitability. One limitation of this study was lack of animal numbers to obtain significance in the binomial traits such as AI pregnancy rate and duration of the study 60 d prior to TAI and 21 d post-TAI. Without a significant reproductive response in this study, it was determined that the expense of phytoestrogen quantification was not justified. This does not mean, however, that phytoestrogens did not have an effect on reproduction. It would be interesting to have a cooperator beef herd, interested in feeding double crop forage soybean following wheat, which would allow data to be collected on reproductive competence to provide additional evidence.

This study also observed a significant difference in DMI. It was interesting that beef heifers in this study consumed a near constant 1% of BW of NDF per day, which agrees with data collected in commercial dairy development operations and summarized by faculty at the University of Wisconsin. It would be of interest to further evaluate the concept of NDF intake as a predictor of DMI. The implications of having this thumb-rule would be of extreme value in ration formulation for the beef industry. This is true because the equations currently used to predict DM intake of beef animals consuming forage based diets are not accurate across forage qualities.

Overall heifer target weight of 65% of their mature equivalent was met prior to the breeding season; however this was only a 65d feeding study. While not significant, heifers on the SBxPM diet tended to be lighter, and had a lower ADG. Had this been a 120 to 150d feeding period, similar to a winter feeding period fed by producers, the lower numerical growth rates observed on the SBxPM treatment, may have prevented these heifers from reaching the targeted 65% of mature weight by the breeding season This could have a negative impact on reproduction. However, these tendencies may also be explained by the reduction in DMI observed for the SBxPM treatment. While these heifers did consume a near constant 1% of BW of NDF per day, their daily intake was lower than what was formulated, due to the initial underestimation of the actual NDF values of the forages fed. This begs the point that an accurate estimate of DMI is needed

across the wide variation in forage qualities used by the beef industry to properly target performance goals.

Overall conclusions from the three studies would be that forage soybean, with and without pearl millet, are an adequate alternative feed resource for developing replacement beef heifers. I would like to reevaluate the phytoestrogens in the soybean, and potentially conduct a longer feeding trial to further evaluate any "true" impacts that phytoestrogens may have on reproduction. Agronomic recommendations for seeding rate, row spacings and harvest dates are as follows. A) There were no differences seen between high or low seeding rates or row spacings, therefore, I would recommend seeding at 95,000 seeds/acre using either a 7 in or 14 in row spacing. Depending on equipment availability, producers have the options of using either a drill or planter. B) Producers should harvest for tonnage on a monoculture soybean because forage quality is not significantly compromised as the plant progresses toward maturity. C) If soybean is intercropped with pearl millet, results suggest either a mixed stand or alternating row spacings result in the same tonnage and quality. D) While tonnage was not different for one late season harvest vs. early harvest plus a regrowth harvest, quality did suffer due to the advancement in maturity of the pearl millet stand for the one late season harvest date. Because of this decrease in quality, it would be our recommendations to harvest the intercropped soybean with pearl millet early and allow regrowth to optimize both yield and quality.

REFERENCES

REFERENCES

Alila, H. W., and Hansel, W. 1984. Origin of different cell types in the bovine corpus luteum as characterized by specific monoclonal antibodies. Biol. Reprod., 31(5), 1015-1025.

Anderson, K.J., D. G. LeFever, J.S. Brinks, K.G. Odde. 1991. The use of reproductive tract scoring in beef heifers. Agri-Practice. 12(4),123-128

Barnes, R. F., Nelson, C. J., Collins, M. and Moore, K. J. 2003. Forages: An Introduction to Grassland Agriculture. Blackwell Publishing. Ames, Iowa.

Bennetts, H. W., Uuderwood, E. J., and Shier, F. L. 1946. A specific breeding problem of sheep on subterranean clover pastures in Western Australia. Australian Veterinary J., 22(1), 2-12.

Bohman, V. R. 1955. Compensatory growth of beef cattle: The effect of hay maturity. J. Anim. Sci., 14(1), 249-255.

Brzezinski, A., and Debi, A. 1999. Phytoestrogens: the "natural" selective estrogen receptor modulators. European J. of Obstetrics and Gynecology and Reprod. Biol., 85(1), 47-51.

Capuco, A. V., Smith, J. J., Waldo, D. R., and Rexroad Jr, C. E. 1995. Influence of prepubertal dietary regimen on mammary growth of Holstein heifers. J. Dairy Sci., 78(12), 2709-2725.

Coblentz, W. K., and Hoffman, P. C. 2008. Heat damaged forages: Effects on forage quality. Focus on Forage. Vol. 10. No. 8. Cooperative Extension Service, Univ. of Wisconsin, Madison, WI.

Darmosarkoro, W., Harbur, M. M., Buxton, D. R., Moore, K. J., Devine, T. E., and Anderson, I. C. 2001. Growth, development, and yield of soybean lines developed for forage. Agron. J., 93(5), 1028-1034.

Devine, T. E., and Hatley, E. O. 1998. Registration of 'Donegal' forage soybean. Crop Sci., 38(6), 1719-1720.

Dewhurst, R. J., Davies, D. R., and Merry, R. J. 2000. Microbial protein supply from the rumen. Anim. Feed Sci. and Tech., 85(1), 1-21.

Egli, D. B., Guffy, R. D., and Leggett, J. E. 1985. Partitioning of assimilate between vegetative and reproductive growth in soybean. Agron. J., 77(6), 917-922.

Ethredge, W. J., Ashley, D. A., and Woodruff, J. M. 1989. Row spacing and plant population effects on yield components of soybean. Agron. J., 81(6), 947-951.

Flint, A. P. F., and Sheldrick, E. L. 1982. Ovarian secretion of oxytocin is stimulated by prostaglandin. Nature J. of Sci., 587-588

Forde, N., Beltman, M. E., Lonergan, P., Diskin, M., Roche, J. F., and Crowe, M. A. 2011. Oestrous cycles in Bos taurus cattle. Anim. Reprod. Sci., 124(3), 163-169.

Funston, R. N., and Deutscher, G. H. 2004.Comparison of target breeding weight and breeding date for replacement beef heifers and effects on subsequent reproduction and calf performance. J. Anim. Sci., 82(10), 3094-3099.

Fortune, J. E., Rivera, G. M., Evans, A. C. O., and Turzillo, A. M. 2001.Differentiation of dominant versus subordinate follicles in cattle Biol. of Reprod.,65(3), 648-654.

Gibson L., Benson G., 2005.Origin, History, and Uses of Soybean (*Glycine max*), Iowa State University, Dept. of Agronomy. http://agronwww.agron.iastate.edu/Courses/agron212/Readings/Soy_history.htm

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

Gunn, P. 2013. Inclusion of dried distiller's grains with solubles in beef cow diets and impact on reproduction and subsequent development of progeny. (Doctorial dissertation, Purdue University) Retrieved from ProQuest LLC. 3591229

Hannah, S. M., Cochran, R. C., Vanzant, E. S., and Harmon, D. L. 1991. Influence of protein supplementation on site and extent of digestion, forage intake, and nutrient flow characteristics in steers consuming dormant bluestem-range forage. J. Anim. Sci., 69(6), 2624-2633.

Hanway, J. J.,and Weber, C. R.1971. N, P, and K percentages in soybean (*Glycine max (L.) Merrill*) plant parts. Agron. J., 63(2), 286-290.

Hillier, S. G. 1994. Current concepts of the roles of follicle stimulating hormone and luteinizing hormone in folliculogenesis. Human Reprod., 9(2),188-191.

Hintz, R. W., and Albrecht, K. A. 1994. Dry matter partitioning and forage nutritive value of soybean plant components. Agron. J., 86(1), 59-62.

Hintz, R. W., Albrecht, K. A., and Oplinger, E. S.1992. Yield and quality of soybean forage as affected by cultivar and management practices. Agron. J., 84(5), 795-798.

Ireland, J. J., Mihm, M., Austin, E., Diskin, M. G., and Roche, J. F. 2000. Historical perspective of turnover of dominant follicles during the bovine estrous cycle: key concepts, studies, advancements, and terms. J. Dairy Sci., 83(7),1648-1658.

Kallela, K., Heinonen, K., and Saloniemi, H. 1984. Plant oestrogens;the cause of decreased fertility in cows. A case report. Nordisk Veterinary Med., 36(3-4),124.

Kephart, K. D., and Buxton, D. R. 1993. Forage quality responses of C3 and C4 perennial grasses to shade. Crop Sci., 33(4), 831-837.

Kingsbury, J. M. 1964. Poisonous plants of the United States and Canada. Soil Sci., 98(5), 349.

Klopfenstein, T. J., Erickson, G. E., Diedrichsen, R. M., Carr, T.P., Brown, A.W., Buckner, C.D., and Bremer V. R., 2010. Lipid and NDF analysis of ethanol byproduct feedstuffs. University of Nebraska-Lincoln, Dept. of Anim. Sci. http://beef.unl.edu/Lipid201032

Köster, H. H., Cochran, R. C., Titgemeyer, E. C., Vanzant, E. S., Abdelgadir, I and St-Jean, G. 1996.Effect of increasing degradable intake protein on intake and digestion of low-quality, tallgrass-prairie forage by beef cows. J. Anim. Sci., 74(10), 2473-2481.

Kurzer, M. S., and Xu, X. 1997. Dietary phytoestrogens. Annual review of nutrition, 17(1), 353-381.

Larson, R.L. and Randle, R.F. The bovine estrous cycle and synchronizationof estrus. http://www.vet.ksu.edu/studentorgs/bovine/pdf/Estrous_Cycle_physiology1.pdf.

Lemenager, R. P., Jones, D. D., Buckmaster D. R., Field W. E., Glanville T., Horstman L. A. Williams R. E. 2011. Cow-Calf Production InThe U.S. Corn Belt. Ames, IA: MidWest Plan Service.

Linkemer, G., Board, J. E., and Musgrave, M. E. 1998. Waterlogging effects on growth and yield components in late-planted soybean. Crop Sci., 38(6), 1576-1584.

Lintzenich, B. A., Vanzant, E. S., Cochran, R. C., Beaty, J. L., Brandt, R. T., and St Jean, G. 1995. Influence of processing supplemental alfalfa on intake and digestion of dormant bluestem-range forage by steers. J. Anim. Sci., 73(4), 1187-1195.

Lynch, J. M., Lamb, G. C., Miller, B. L., Brandt, R. T., Cochran, R. C., and Minton, J. E. 1997. Influence of timing of gain on growth and reproductive performance of beef replacement heifers. J. Anim. Sci., 75(7), 1715-1722.

Merck Animal Health., 2013. The four phases of oestrus in cows. http://www.partners-in-reproduction.com/reproduction-cattle/four-phases-oestrus.asp

Mertens, D. R. 1994. Regulation of forage intake. In: Fahey, G. C., Collins, M., Mertens,, D. R. and Moser, L. E. (eds) Forage Quality, Evaluation, and Utilization, ASA, CSSA and SSSA, Madison, Wisconsin, pp 450-493.

Miyamoto, A., Shirasuna, K., Wijayagunawardane, M. P. B., Watanabe, S., Hayashi, M., Yamamoto, D., and Acosta, T. J. 2005. Blood flow: a key regulatory component of corpus luteum function in the cow. Domestic animal endocrinology, 29(2), 329-339.

Munoz, A. E., Holt, E. C., and Weaver, R. W. 1983. Yield and quality of soybean hay as influenced by stage of growth and plant density. Agron. J., 75(1), 147-149.

NRC. 2000. Nutrient Requirements of Beef Cattle. 7th rev. ed. Natl. Acad. Press, Washington, DC.

NRC. 2001. Nutrient Requirements of Dairy Cattle. 7th rev. ed. Natl. Acad. Press, Washington, DC.

Ocumpaugh, W. R., Matches, A. G., & Luedders, V. D. 1981. Sod-seeded soybeans for forage. Agron. J., 73(3), 571-574.

Orskov, E.R. 1992. Protein Nutrition in Ruminants. Academic Press, London.

Ososki, A. L., and Kennelly, E. J. 2003. Phytoestrogens: a review of the present state of research. Phytotherapy Research, 17(8), 845-869.

Park, C. S., Danielson, R. B., Kreft, B. S., Kim, S. H., Moon, Y. S., and Keller, W. L. 1998. Nutritionally directed compensatory growth and effects on lactation potential of developing heifers. J. Dairy Sci., 81(1), 243-249.

Patterson, D. J., Corah, L. R., Kiracofe, G. H., Stevenson, J. S., and Brethour, J. R. 1989. Conception rate in Bos taurus and Bos indicus crossbred heifers after postweaning energy manipulation and synchronization of estrus with melengestrol acetate and fenprostalene. J. Anim. Sci., 67(5), 1138.

Patterson, D. J., Perry, R. C., Kiracofe, G. H., Bellows, R. A., Staigmiller, R. B., and Corah, L. R. 1992. Management considerations in heifer development and puberty. J. Anim. Sci., 70(12), 4018-4035.

Pedersen P., 2007. Soybean Growth Stages, Iowa State University, Dept of Agronomy. http://extension.agron.iastate.edu/soybean/production_growthstages.html

Rao, S. C., Mayeux, H. S., and Northup, B. K. 2005. Performance of forage soybean in the southern Great Plains. Crop Sci., 45(5), 1973-1977.

Rasby, R., and Vinton, R. 2013. Estrous Cycle Learning Module. University of Nebraska. http://beef.unl.edu/learning/estrous.shtml

Redfearn, D. D., Buxton, D. R., and Devine, T. E. 1999. Sorghum intercropping effects on yield, morphology, and quality of forage soybean. Crop Sci., 39(5), 1380-1384.

Ruiz, T. M., Bernal, E., Staples, C. R., Sollenberger, L. E., and Gallaher, R. N. 1995. Effect of dietary neutral detergent fiber concentration and forage source on performance of lactating cows. J. Dairy Sci., 78(2), 305-319.

Salisbury, G. W., N. L. VanDemark, and J. R. Lodge. 1978. Physiology of Reproduction and Artificial Insemination of Cattle (2nd Ed.). W. H. Freeman and Co., San Francisco, CA.

Scott, W. O., Aldrich, S. R. 1970. Modern Soybean Prouction 1st Ed. S & A Publ. Inc, Champaign, IL.

Sejrsen, K. 1978. Mammary development and milk yield in relation to growth rate in dairy and dual-purpose heifers. Acta Agriculturae Scandinavica, 28(1), 41-46.

Senger, P. L., 2012. Pathways to Pregnancy and Parturition. Ephrata, PA. Cadmus professional Communications.

Seiter, S., Altemose, C. E., and Davis, M. H. 2004. Forage soybean yield and quality responses to plant density and row distance. Agron. J., 96(4), 966-970.

Sheaffer, C. C., Orf J. H., Devine, T. E, and J.G. Jewett. 2001. Yield and Quality of Forage Soybean. Agron. J. 93:99-106.

Shemesh, M., and Shore, L. S. 1994. Effects of hormones in the environment on reproduction in cattle. Factors Affecting Calf Crop (eds. Fields, MJ and Sand, RS) CRC Press, Florida, 287-297.

Storm, E., Orskov, E. R., and Smart, R. 1983. The nutritive value of rumen micro-organisms in ruminants. British Journal of Nutrition, 50(2), 471-478.

Sunderland, S. J., Crowe, M. A., Boland, M. P., Roche, J. F., and Ireland, J. J. 1994. Selection, dominance and atresia of follicles during the oestrous cycle of heifers. J. of Reprod. and Fertility, 101(3), 547-555.

Twidwell, E. K., Johnson, K. D., and Cherney, J. H. 1986. Indirect estimation of wheat-hairy vetch ratios in a mixed stand. Agron. J., 78(2), 344-346.

Varel, V. H., and Kreikemeier, K. K. 1999. Low-and high-quality forage utilization by heifers and mature beef cows. J. Anim. Sci., 77(10), 2774-2780.

Wagner, J. J., K. S. Lusby, J. W. Oltjen, J. Rakestraw, R. P. Wettemann, and L.E. Walters. 1988. Carcass composition in mature Hereford cows: Estimation and effect on daily metabolizable energy requirement during winter. J. Anim. Sci. 66:603-612.

Yelich, J. V., Wettemann, R. P., Dolezal, H. G., Lusby, K. S., Bishop, D. K., and Spicer, L. J. 1995. Effects of growth rate on carcass composition and lipid partitioning at puberty and growth hormone, insulin-like growth factor I, insulin, and metabolites before puberty in beef heifers. J. Anim. Sci., 73(8), 2390-2405.

APPENDIX

APPENDIX

Table A1: Analysis of variance for Study 1 yield (kg ha $^{-1}$).

		Yield		
Source	DF	SS	F value	Pr > F
Block	3	11581983	2.58	0.081
Treatment	1	2233875	1.49	0.236
Harvest	3	1984702647	44.16	<.0001
Treatment*Harvest	3	3140570	0.70	0.563

Table A2: Analysis of variance for Study 1 NDF, ADF, CP and IVTD (g kg^{-1}).

		NDF		
Source	DF	SS	F value	Pr > F
Block	3	4373.99	2.07	0.14
Treatment	1	2.76	0.00	0.95
Harvest	3	8345.25	3.94	0.02
Treatment*Harvest	3	1430.24	0.68	0.58
		ADF		
Source	DF	SS	F value	Pr > F
Block	3	4826.79	3.35	0.0385
Treatment	1	285.82	0.60	0.4490
Harvest	3	12368.94	8.58	0.0007
Treatment*Harvest	3	1213.28	0.84	0.4861
		CP		
Source	DF	SS	F value	Pr > F
Block	3	682.96	1.62	0.214
Treatment	1	260.80	1.86	0.187
Harvest	3	36037.97	85.70	<.0001
Treatment*Harvest	3	319.31	0.76	0.053
		IVTD		
Source	DF	SS	F value	Pr > F
Block	3	2201.39	1.08	0.381
Treatment	1	29.80	0.04	0.836
Harvest	3	16341.46	7.98	0.001
Treatment*Harvest	3	691.30	0.34	0.798

Table A3: Analysis of variance for Study 2 soybean system for yield (kg ha^{-1}).

		Yield		
Source	DF	SS	F value	Pr > F
Block	3	0.0863	3.46	0.02
Treatment	2	0.0221	1.34	0.21
Harvest	6	5.9580	119.90	<.0001
Treatment*Harvest	12	0.0293	0.29	0.99

Table A4: Analysis of variance for Study 2 soybean system for NDF, ADF, CP, and IVTD (g kg^{-1}).

		NDF		
Source	DF	SS	F value	Pr > F
Block	3	12082.15	6.06	0.01
Treatment	2	5827.58	4.38	0.02
Harvest	6	106158.77	26.62	<.0001
Treatment*Harvest	12	14173.16	1.78	0.07
		ADF		
Source	DF	SS	F value	Pr > F
Block	3	7276.16	5.22	0.01
Treatment	2	5295.55	5.70	0.01
Harvest	6	121234.57	43.5	<.0001
Treatment*Harvest	12	10795.98	1.94	0.05
		CP		
Source	DF	SS	F value	Pr > F
Block	3	1198.64	1.86	0.15
Treatment	2	1273.22	2.96	0.06
Harvest	6	90064.71	69.85	<.0001
Treatment*Harvest	12	2211.10	0.86	0.59
		IVTD		
Source	DF	SS	F value	Pr > F
Block	3	3533.95	3.14	0.03
Treatment	2	1870.20	2.49	0.09
Harvest	6	172097.77	76.41	<.0001
Treatment*Harvest	12	14795.88	3.28	0.01

Table A5: Analysis of variance for Study 2 soybean system for yield (kg ha^{-1}).

		Yield		
Source	DF	SS	F value	Pr > F
Block	3	19234088	5.14	0.04
Treatment	2	398013	0.16	0.86

Table A6: Analysis of variance for Study 2 soybean system NDF, ADF, CP and IVTD (g kg^{-1}).

		NDF		
Source	DF	SS	F value	Pr > F
Block	3	4905.07	3.90	0.07
Treatment	2	618.74	0.74	0.52
		ADF		
Source	DF	SS	F value	Pr > F
Block	3	5554.56	3.18	0.11
Treatment	2	581.30	0.50	0.63
		CP		
Source	DF	SS	F value	Pr > F
Block	3	661.21	2.04	0.21
Treatment	2	426.81	1.97	0.22
		IVTD		
Source	DF	SS	F value	Pr > F
Block	3	1734.59	2.87	0.13
Treatment	2	352.35	0.87	0.46

Table A7: Analysis of variance for Study 2 forage system yield (kg ha^{-1}).

		Yield		
Source	DF	SS	F value	Pr > F
Block	3	0.13956883	5.57	0.0014
Treatment	41	3.84167599	14.38	<.0001

Table A8: Analysis of variance for Study 2 forage system NDF, ADF, CP and IVTD (g kg^{-1}).

		NDF		
Source	DF	SS	F value	Pr > F
Block	3	2115.8	0.92	0.434
Treatment	41	1571731.2	49.94	<.0001
		ADF		
Source	DF	SS	F value	Pr > F
Block	3	1860.81	1.29	0.2807
Treatment	41	294591.34	14.95	<.0001
		CP		
Source	DF	SS	F value	Pr > F
Block	3	1425.66	2.17	0.0954
Treatment	41	499967.64	55.59	<.0001
		IVTD		
Source	DF	SS	F value	Pr > F
Block	3	2147.43	1.54	0.207
Treatment	41	290862.76	15.29	<.0001