Food security and the effects of climatic factors on livestock reproduction

by

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The student author, whose presentation of the scholarship herein was approved by the program of study committee, is solely responsible for the content of this thesis. The Graduate College will ensure this thesis is globally accessible and will not permit alterations after a degree is conferred.

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DEDICATION

This work is dedicated to all the people that had encouraged and supported me in one way or another along the way. It is dedicated to my friends back in Costa Rica; without your support and your honest words, I would have never had the courage to take on this challenge. This thesis is dedicated to my brothers in Zamorano, always making me believe in myself and making me realize that I can achieve great things if I put my heart on them. Most importantly, this thesis and all the hours and work behind it are specially dedicated to the center of my life, my family and my fiancée. Thank you for the continued support, the teachings, the patience.

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ABSTRACT

To maximize efficiency and meet the demand for animal-derived products of an increasingly population, livestock production systems need to evolve and be fine-tuned to increase their efficiency both in the use of resources and output of products. The first manuscript investigated the effects of climatic variables on the quality grade and quantity of in vivo derived cattle embryos in the Midwestern United States. The response variables included the number of ovarian structures, viable embryos, quality grade 1 embryos, quality grade 2 embryos, quality grade 3 embryos, freezable embryos (sum of quality grade 1 and quality grade 2 embryos), transferable embryos (sum of quality grade 1–3 embryos), degenerate embryos and unfertilized ova. A negative effect of greater temperatures during the early embryonic development stage tended ($P < 0.10$) to be associated with a decrease in the quality of embryos recovered. Wind speed during the estrous synchronization period was also associated with a lesser number of embryos recovered ($P < 0.05$). Increased wind speed at the early antral follicular phase 40–45 days prior to ovulation was associated with an increase in the percentage of quality grade 1 embryos recovered ($P < 0.05$). This retrospective study confirms that climatic variables have significant effects on the in vivo production of cattle embryos and that wind speed should be considered in future analyses of factors affecting embryo quality.

The second manuscript aimed to determine the possible existence of any effects of lunar cycles and other variables on boar ejaculate variables and to examine those effects relative to other possible factors. Moon phase, greatest daily temperature (T), least daily T, average daily relative humidity (RH), temperature-humidity index (THI), season and the interaction of moon phase with season were analyzed at the day of collection and 45

days prior to date of collection as a proxy of initiation of spermatogenesis. For both dates analyzed, season and the interaction of season with moon had significant effects ($P < 0.05$) on the volume of the ejaculate, the concentration of sperm in the ejaculate and the number of doses obtained per ejaculate. The significant interaction of season and moon phase on boar semen traits found in this study suggests that to maximize productivity of modern swine production systems determining a collection schedule in some seasons relative to moon phase may be advantageous.

The third manuscript has the objective of developing a manual weight prediction method that can accurately be used on African goats to empower small-holders and improve their food security. One linear model and two quadratic models were developed, and their accuracy was compared to a commonly used equation. In all cases, the models produced smaller mean prediction errors than the commonly used equation.

Overall, this thesis provides insight on different alternatives to adapt and improve the efficiency and output of livestock production systems and small producers in rural Africa with the aim of improving resilience in an era of rapid climatic changes.

Key words: food security, bovine reproduction, boar semen, artificial insemination, climatic factors

CHAPTER 1. GENERAL INTRODUCTION

Mankind as a whole faces important challenges regarding food security in the 21st century, but especially in developing countries. The global population is rapidly growing and becoming wealthier on average. One consequence of these changes is that the demand for land, and foods such as meat and dairy products is increasing (Rojas-Downing et al., 2017). While the demands for the planet's resources keep growing, the challenge of increasing production efficiency becomes more important. According to discussions at the World Food Summit, food security exists when all people, at all times, have physical and economic access to sufficient, safe, and nutritious foods that meet their dietary needs and food preferences for an active and healthy life (Smith et al., 2013).

Estimates suggest that somewhere between a 50 to 100% increase in food productivity will be needed by 2050 to feed an additional two billion people (Godfray et al., 2010; Smith et al., 2013). For this reason, improving access to animal derived products and improving incomes of vulnerable populations in rural areas of developing countries is very important. However, it is key to sustainably manage resources is very important. In these aspects, livestock production will play a key role in food security worldwide. Specially because the livestock sector contributes to the livelihoods of one billion of the poorest population in the world and employs close to 1.1 billion people worldwide (Hurts et al., 2007).

To maximize efficiency and meet the demand for animal-derived products of a fast-growing and increasingly wealthy population, livestock production systems need to evolve and be fine-tuned to increase their efficiency both in the use of resources and output of products. For these reasons, improvements in every field of animal science are needed, as

well as the exploration of "out of the box" approaches. However, maximizing efficiency is not the only challenge faced by the livestock industries around the world. Climate change comes accompanied by increasingly erratic precipitation patterns and rising temperatures around the world. The combination of these factors will increase the negative effects of heat stress on livestock production and reproduction. Because of this, livestock production systems need to find new ways to adapt to heat stress and manage its negative effects.

This thesis aims to provide some insight into the prominent role of livestock in improving food security along with some key aspects that will be of very high importance during the years to come through three manuscripts that describe:

- Analysis of climatic effects such as heat stress on modern livestock reproduction.
- The exploration of new factors associated with maximizing the efficiency of intensive livestock reproduction.
- Empowerment of small-stake holder farmers in developing countries to improve food security.

Thesis organization

This thesis consists of six chapters. Chapter 1 is the general introduction . Chapter 2 covers literature review that aims to address a detailed overview of current literature related with the research areas covered in this thesis. Chapters 3 through 5 consist of three manuscripts that describe the aspects of cattle and pig reproduction and the important role of livestock in improving food security in the near future. Chapter 6 presents implications from the research and conclusions of this thesis.

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CHAPTER 2. REVIEW OF LITERATURE

This literature review aims to provide recent and thorough information about pertinent topics touched on this thesis such as female bovine reproduction, male porcine reproduction and food security issues. It covers and highlights topics of female bovine reproductive physiology that include follicular development dynamics, female reproductive endocrinology, the use and evolution of estrus synchronization and super ovulation protocols and the effects of heat stress in female bovine reproduction and early embryonic development. Topics regarding the male porcine reproduction include spermatogenesis, the effects of seasonal environmental variation and lunar effects on swine behavior and reproduction. The literature review concludes with topics regarding food security, including the role of women and livestock in food security in African countries.

Reproduction in the cow: An overview

Reproductive activity in the cow hinges on the state of the ovaries, which produce both oocytes and hormones. Ovarian functions are regulated by the endocrine hormones of the hypothalamus, the anterior pituitary, the ovary itself and the uterus (Barrell et al., 1984). The estrous cycle represents the cyclical ovarian activity that allows female animals to go from a period of reproductive non-receptivity to receptivity to the male enabling mating and pregnancy events (Forde et al., 2011). The estrous cycle has a duration of about 21 days (Galina and Arthur, 1990) and it is composed of two main phases, the follicular and the luteal

phase (Forde et al., 2011). The follicular phase precedes ovulation and is marked by fast follicular growth while the luteal phase is characterized by the presence of a corpus luteum.

Follicular growth dynamics in the cow ovary.

During gestation, primordial germ cells of the female bovine embryo differentiate into oogonia during the first trimester of gestation (Erickson, 1966). These oogonia enter the first prophase of meiosis and become arrested in the diplotene stage and are known as primary oocytes (Fortune, 2003). When the primary oocytes are found surrounded by one layer of inactive, flattened granulosa cells, these structures are known as primordial follicles (Barrell et al., 1984; Britt, 2008). When primordial follicles leave the resting stage, the granulosa cells become cuboidal and begin to express markers of cell proliferation (Wandji et al., 1996). Follicles at this stage are known as primary follicles. Growth consists of multiplication of the granulosa and theca cells and the accumulation of fluid within the follicles. When the follicles are filled with fluid, they are known as antral follicles (Britt, 2008). Before ovulation, the dominant antral follicle undergoes marked and rapid growth in size. At this stage follicular cells, especially the granulosa cells, produce large quantities of estrogens (Barrell et al., 1984; Hirshfield, 1991). Follicle development is controlled primarily by a feedback system involving gonadotrophin releasing hormone (GnRH), follicle stimulating hormone (FSH), luteinizing hormone (LH), estrogens, androgens, progestin and other proteins (Webb et al., 1992). Follicular stimulating hormone is the main hormone responsible for the growth and development of follicles in the ovary.

The bovine estrous cycle is characterized by two or three waves of follicular growth (Savio et al., 1988; Sirois and Fortune, 1988). These waves involve synchronous

development of a group of follicles. For animals with two follicular waves, these are initiated at approximately the day of ovulation and day 10 after ovulation, for animals with three follicular waves, the waves are initiated approximately at day 0 (day of ovulation), 9 and 16 after ovulation (Sirois and Fortune, 1988; Ginther et al., 1989). The dominant follicle of a follicular wave becomes larger than the other follicles and through the production of steroidal and non-steroidal substances causes atresia of the subordinate follicles of the wave and the static dominant follicle of the previous wave (Ginther et al., 1989; G.A. Bo et al., 1995).

After two or three follicular waves, when the dominant follicle reaches a diameter of around 13mm., a surge of luteinizing hormone will cause ovulation (Sirois and Fortune, 1988).The LH surge is caused by the increasing amounts of estrogens produced by the granulosa cells of the dominant follicle (Fortune, 1986). Once the follicle has released the oocyte, it will undergo luteinization. During the next phase of the estrous cycle, the dominant reproductive hormone is progesterone, produced by the corpus luteum. This hormone inhibits hypothalamic function via a negative feedback signal and consequently impedes any further follicular maturation during the life span of the corpus luteum (Barrell et al., 1984; Hirshfield, 1991).

Endocrinology of female reproduction

Female ovarian activity and thus, the estrous cycle, is managed by a small set of hormones that are coordinated through a system of positive and negative feedback mechanisms. The most important hormones involved are gonadotrophin releasing hormone (GnRH) produced in the hypothalamus, follicle stimulating hormone (FSH) and luteinizing

hormone (LH) produced in the anterior pituitary, progesterone, estradiol and inhibins produced in the ovaries and prostaglandin produced in the uterus (Forde et al., 2011). The survival of large follicles beyond the atresia barrier depends on pituitary gonadotrophin secretion, which in turn relies on pulses of GnRH released by the hypothalamus (Barrell et al., 1984).

Gonadotropin releasing hormone exerts its control on the estrous cycle through its effects on the anterior pituitary, which regulates the secretion of the gonadotrophins LH and FSH (Schally et al., 1971; Forde et al., 2011). Gonadotropin releasing hormone exerts its control binds to specific receptors on gonadotrophs, and causes the release of intracellular calcium, in return, this causes a release of both FSH and LH. The gonadotrophs become insensitive to GnRH, and because of this, the releases of GnRH form the hypothalamus are pulsatile to prevent the negative feedback on the GnRH receptor (Roche, 1996).

During the follicular phase, progesterone is found in basal concentrations as the corpus luteum is undergoing regression (Forde et al., 2011). At this time, the granulosa cells of the dominant follicle are producing estrogens, mainly estradiol, with increasing quantities. The high concentrations of estrogens cause the female bovine to display behavioral estrus for twelve to twenty four hours Barrell et al., 1984), ovulation occurs approximately 12 hours after the display of behavioral estrus (Forde et al., 2011).

When a dominant follicle is selected to ovulate, FSH concentration declines due to the negative feedback of estrogens and inhibin on the secretion of FSH and the follicle becomes dependent on LH for ovulation (Hansel and Convey, 1983; Forde et al., 2011). Estrogens, particularly estradiol, will have a negative feedback on the secretion of LH when in low concentration. However, high concentrations during the late part of the follicular

phase will cause positive feedback on LH secretion (Kesner et al., 1981). An LH surge is responsible for ovulation, and during the next phase of the estrus cycle, the luteal phase, the ovary is controlled by the secretion of progesterone by the corpus luteum (CL).

The beginning of the luteal phase is characterized by the formation of the CL from the vestiges of the antral follicle. Formation of the CL involves the luteinization of both theca and granulosa cells, once the process is completed, these cells produce progesterone as preparation for the maintenance of a pregnancy (Auletta and Flint, 1988). Progesterone has a negative feedback on hypothalamic function and obstructs any further ovulation during the life span of the CL (Hansel and Convey, 1983; Barrell et al., 1984). In other words, progesterone does not have a negative feedback on FSH secretion, therefore there is still follicular growth. Nevertheless, progesterone lessens the frequency of LH pulses and through this mechanism impedes ovulation (Rahe et al., 1980; Forde et al., 2011).

Finally, during the final stage of the luteal phase, progesterone plays a critical role in signaling the expression of genes in the uterus that are required to initiate the secretion of uterine milk (Geisert et al., 1992; Spencer et al., 2008). If there is no pregnancy and if interferon tau(maternal recognition signal of pregnancy in bovine) is not present, luteolysis of the CL occurs through the action of prostaglandin secreted by the uterus. Once the CL has lost functionality and thus, stops producing progesterone, another normal ovulation can be expected within 48 to 72 hours as the secretion of LH is normalized (Hansel and Convey, 1983: Barrell et al., 1984).

Superovulation and estrus synchronization protocols

Commercial embryo transfer programs in North America appeared in the early 1970s, when continental breeds of cattle were introduced and at that point where in short supply. Superovulation and embryo transfer offered a viable option to rapidly increase the number of animals of these breeds (Bó and Mapletoft, 2014). The process of embryo transfer in cattle starts with the superovulation of the donor cow (Hasler, 2014). The main objective of superovulation protocols in embryo transfer programs is to produce the maximum number of good quality embryos that will be transferred to recipients and result in successful pregnancies. The earliest superovulation protocols consisted of gonadotropin application s days before the expected estrus date to induce the development of multiple preovulatory follicles followed by an injection of human chorionic gonadotropin at the expression of estrus to induce ovulation (Gordon, 1975).

The swift widespread adoption of embryo transfer resulted in a rapid advancement of techniques and approaches by researchers (Bó and Mapletoft, 2014). In 1983, it was reported that the use of FSH instead of human or equine chorionic gonadotropin increased the number of transferrable embryos recovered per flush (Monniaux et al., 1983). Later it was proven that that gonadotropins, more specifically equine chorionic gonadotropin increased the incidence of abnormal profiles of LH and progesterone which in return cause lower ovulation and fertilization rates (Greve et al., 1983; Callesen et al., 1986; Bó and Mapletoft, 2014). For these reasons, most cattle currently are superovulated primarily using pituitary extracts that are rich in FSH (Bó et al., 2010; Mapletoft and Bó, 2012; Hasler, 2014).

It is known that the second follicular wave takes place near 10 days after behavioral estrus is displayed independently of the number of follicular waves before ovulation (Ginther

et al., 1989; Bó et al., 2006) and multiple follicles should be available at this time. Nevertheless, it has been shown that superovulatory response was higher when gonadotropin treatments were initiated at the precise starting time of a follicular wave and not before or after it has already taken place (Nasser et al., 1993). Thus, the first step in a superovulation/estrus synchronization protocol consist of controlling the time at which the wave of follicular growth takes place.

Time of follicular wave emergence has been altered by mechanical procedures or hormonal treatments. The main mechanical method consists of transvaginal ultrasound-guided follicle ablation of all follicles with diameters ≥5 mm, all follicles or just the dominant follicle (Bungartz and Niemann, 1994; Bergfelt et al., 1997; Kim et al., 2001; Bo et al., 2002).This procedure aims to eliminate the suppressive effects on follicular growth exerted by the dominant follicle on the subordinate follicles of each follicular wave. Emergence of a new follicular wave takes place approximately 1.5 days after the ablation (G.A. Bo et al., 1995) and the time of ovulation can be accurately synchronized by administering prostaglandins. However, it has been shown that that the timing of estrus could be more accurately controlled when a progesterone/progestogen implant was inserted for the period of superestimulation and two injections of PGF were administered on the day of implant removal (Bo et al., 2002).

On the other hand, hormonal methods aim to cause luteinization or atresia of the follicles present when the treatment is administered. One of the most common protocols involves the use of progestin/progesterone and estradiol. The estradiol treatment causes negative feedback on the release of FSH and induces follicle atresia while progesterone suppresses follicle growth (Adams et al., 1992; G.A. Bo et al., 1995).Once estradiol has been

metabolized, FSH concentrations increase and a new follicular wave emerges around 4 days after the administration of estradiol (G. A. Bo et al., 1995). However, the use of estradiol has been outlawed in many countries and therefore other hormonal methods have been developed.

Another hormonal approach is to use porcine LH or GnRH to induce ovulation of the dominant follicle (Macmillan and Thatcher, 1991), and a new follicular growth wave starts approximately 2 days later. Nonetheless, the uncertainty of the time from GnRH treatment to wave emergence may be too inconsistent for superestimulation (Bó et al., 2010; Mapletoft and Bó, 2012) .

An alternative approach is to combine the use of gonadotropin and a progestin device (Bó et al., 2010). A persistent follicle is induced by the strategic use of prostaglandin along with a progestin device, and gonadotropin or porcine LH is used to induce the persistent follicle to ovulate, with superovulation treatment starting at ovulation (Mapletoft and Bó, 2012).

Follicle stimulating hormone is key for the growth of preovulatory follicles. When follicular growth takes place, the subordinate follicles undergo regression/atresia because of the decrease secretion of FSH caused by the negative feedback of estradiol and inhibin from the dominant follicle (Barrell et al., 1984; G.A. Bo et al., 1995; Bó et al., 2010; Forde et al., 2011). For this reason, most protocols enable the maintenance of dominant and subordinate follicles and induction of ovulation is based on the application of multiple injections of exogenous FSH during multiple days (Mapletoft and Bó, 2012).

Heat stress in female bovine reproduction

Although modern cooling systems or extensive shading may be used for livestock, heat stress remains as one of the principal effectors of lowered reproductive performance during the warmer seasons of the year. Even though heat stress is generally associated with the tropics and the southern areas of the United States, it still poses an issue in cooler regions, particularly to grazing cattle.

Hyperthermia can impair cellular function in various tissues of the reproductive system, affect steroid production, alter follicular wave dynamics and lower progesterone concentrations (Wolfenson et al., 2000). Disruption of the follicle and its enclosed oocyte seems, however, to be a pivotal factor in the complex mechanism in which heat stress impairs fertility (Roth, 2008). More specifically, greater than optimal temperature-humidity index (THI) affects reproductive performance by altering the concentrations of FSH and inhibin in plasma and thus reducing follicular dominance. These endocrine effects may have physiological significance that could be associated with lower fertility of cattle (Roth et al., 2000; Hansen et al., 2001; Argov et al., 2005; Ferreira et al., 2016).

Heat stress also may have delayed effects on reproduction. Autumn fertility of dairy cows is lower than in winter, although ambient temperatures drop and cows are no longer exposed to thermal stress (Wolfenson et al., 2000). It has been shown that follicular wave dynamics can be altered in subsequent cycles even though cows are no longer under conditions of heat stress because of an impairment of theca cells to produce androstenedione, which is used by granulosa cells to produce estradiol (Wolfenson et al., 1997; Roth et al., 2000).

Heat stress is also considered to be one of the primary factors decreasing the effectiveness of using multiple ovulation technologies (Putney et al., 1989a; Hansen et al., 2001; Al-Katanani et al., 2002; Jordan, 2003). The use of *in vivo* techniques results in lesser embryo recovery numbers and poorer quality grades of embryos during the warmer months of the year. Greater temperatures affect the oocyte by altering the patterns of ovarian follicular development, steroid production, and gene expression (Roth et al., 2000; Hansen et al., 2001; Argov et al., 2005; Ferreira et al., 2016). There is also evidence that heat stress before insemination is associated with decreased fertility in cattle, possibly reflecting detrimental effects on the developing oocyte. In addition, heat stress ranging from 12 to 72 hours post-breeding can negatively affect conception rates of cattle (Ulberg and Burfening, 1961; Ambrose et al., 1999).

Heat stress in early embryonic development

Although mechanisms that are responsible for the embryonic resistance and adaptation to heat stress are not defined, there is evidence that suggests that these could involve changes in embryonic cell function or in the microenvironment of the embryo or in the cow's reproductive track (Ealy et al., 1993) since there is evidence that heat stress alters protein secretion in the female reproductive track (Malayer et al., 1988). In many cells, the synthesis of heat shock proteins during periods of elevated temperatures limits the negative effects associated to heat stress (Mirkes, 1987). Thus, another possibility is that embryos develop the capacity to produce proteins and other molecules that limit effects of heat on cellular function.

It has been suggested that maternal heat stress during the first 3-7 days of gestation and maternal heat stress during the final oocyte maturation and ovulation causes retarded embryonic development and an increased incidence of embryonic mortality (Putney et al., 1989b, 1998; Ealy et al., 1993). Embryos recovered from superovulated cows during the hot months of the year tend to be less able to develop in culture than similar embryos collected from superovulated cows in the colder months (Monty and Racowsky, 1987; Hansen et al., 2001). It is interesting to note that the embryo becomes more resistant to maternal heat stress as pregnancy advances, with heat stress resistance starting at day 3, the time at which bovine embryonic genome is activated, between the 8 to 16-cell stage (Barnes and Eyestone, 1990). It has been shown that it has been shown that heat stress during days 8-16 of pregnancy can reduce embryonic size (Biggers et al., 1987); nonetheless, severe heat stress can impair embryonic development later in pregnancy (Ealy et al., 1993; Hansen et al., 2001), . Therefore, the best approach to preserving embryo quality during periods of heat stress is to provide cooling to the pregnant cow during the period of time when embryos are most sensitive to the detrimental effects of heat stress.

Spermatogenesis in boars

Spermatogenesis occurs in the testes, more specifically in the seminiferous tubule (Knox, 2003) and can be defined as the process by which Spermatogonial cells produce spermatozoa (Clermont, 1972). This process is supported by specialized cells in the testicle called Leydig and Sertoli cells. In pigs, this process is composed of three distinct stages: spermatogonial, spermatocytary, and spermiogenic phases with each of these phases

corresponding to the events of mitosis, meiosis and differentiation, respectively (França et al., 2005).

During the Spermatogonial phase, one Spermatogonial stem cells enter into the proliferative pool and undergoes mitosis with one daughter cell returning to the Spermatogonial stem cell pool and the other daughter cell (spermatogonia A1) undergoing further rounds of mitosis. The spermatogonia A1 cell divides once, giving rise to two type A2 spermatogonia. These two type A2 cells divide again, producing four A3, eight A4, 16 intermediate spermatogonia, 32 type B spermatogonia, and finally 64 primary spermatocytes.

The spermatocytary phase is characterized by the transit of the spermatocytes through the blood teste barrier and the initiation of meiosis. Meiosis 1 produces 128 spermatocytes and meiosis 2 produces 256 round spermatids (França et al., 2005; Parrish et al., 2017). Finally, during the spermiogenic period, the haploid spermatids develop further into spermatozoa. The spermatozoa are then released into the seminiferous tubules and are stored in the epididymis where they will undergo maturation (Parrish et al., 2017). Each Spermatogonial stem cell that enters the proliferative pool will produce 256 spermatozoa, but somewhere around 70% to 90% of the spermatozoa are lost (Knox, 2003; Costa et al., 2013; Parrish et al., 2017). Spermatogonial stem cells take approximately 34-36 days to change from a round cell on the outer wall of the tube to its release into the seminiferous tubules as a sperm with tails and an additional 12 to 14 days undergoing maturation as they travel through the epididymis. Once \mature, spermatozoa will have full motility and fertilizing capability when diluted with seminal plasma in the ejaculate (Knox, 2003).

Endocrinology of spermatogenesis

As in females, the endocrinology of spermatogenesis is controlled by the Hypothalamic-pituitary-gonadal axis with the production of gonadotropic releasing hormone (GnRH), luteinizing hormone (LH) and follicular stimulating hormone (FSH). In males, it controls key functions including the production of spermatozoa in the seminiferous tubules and the synthesis of androgens by Leydig cells in the testes (Boron, 2005). In males, LH stimulates the production of testosterone by the Leydig cell (França and Cardoso, 1998). On the other hand, FSH stimulates Sertoli cells to produce androgen-binding protein (ABP) which is secreted in the luminal space and helps to keep local testosterone concentrations high (Boron, 2005).

Sertoli cells also play key roles in sex differentiation and spermatogenesis. During early gestation, Sertoli cells play a key role in testes differentiation by producing Anti-Müllerian hormone, which suppresses the development of the female reproductive tract (França and Cardoso, 1998). Nonetheless, the development of the male reproductive tract and secondary sex characteristics is controlled by steroids secreted by Leydig cells (Merchant-Larios and Moreno-Mendoza, 2001). Also, Sertoli cells provide support to Spermatogonial germ cells. Groups of germ cells are supported by one Sertoli cell and therefore, the total number of Sertoli cells present in the testicle dictates sperm production. The Sertoli cells line the seminiferous tubules that produce sperm (Knox, 2003).

Follicle stimulating hormone also stimulates germ cells to start cell division and development. Also, the FSH molecule binds to Sertoli cells that nurse the forming sperm cells (Knox, 2003). Follicle stimulating hormone also causes the synthesis of aromatase, which converts testosterone from the Leydig cells to estradiol in the Sertoli cells (Clermont,

1972; Knox, 2003; Boron, 2005). Interestingly, even though estradiol is generally associated with female reproduction, it has been recently shown that estrogen is present in the ejaculate. Estrogen in the ejaculate may be responsible for inducing uterine contractions to aid in sperm transport and may alter the time of ovulation through uterine prostaglandin release (Knox, 2003). Moreover, FSH triggers the production of growth factors by the Sertoli cells that support sperm cells and spermatogenesis and inhibin. In males, inhibin plays an important feedback role in the hypothalamic-pituitary-testicular axis (Boron, 2005).

Factors that influence semen production in boars

Heat stress is one of the main factors that negatively affects semen production in boars. High temperatures have been shown to reduce both sperm production and motility of ejaculated sperm for up to 8 weeks after the exposure to heat stress. Heat stress can either be acute or chronic in terms of how quickly it occurs and the time course over which spermatogenesis is affected (Flowers, 2015). Temperatures above 95° F for as little as 3 days cause ejaculates beginning 2-6 weeks after the stress to show abnormalities in spermatozoa. The reason for the delay and length of the appearance of abnormalities depends upon the 14-day transport in the epididymis and the sperm cell stages during the 34-36 days development period (Knox, 2003).

Slight changes to testicular temperatures can affect the survival of sperm cells and cause abnormal spermatozoa to be produced (Parrish et al., 2017). Even though in modern artificial insemination (AI) studs boars are maintained in mechanically ventilated facilities which are well equipped to prevent acute heat stress, temperature and humidity conditions

consistent with chronic exposure tend to be common in these facilities, particularly during the warmer months of the year (Flowers, 2015).

It is also known that testicular size and libido in the male are all influenced by breed (Rothschild, 1996), and genetic diversity within as well as among swine breeds affects the performance of boars used in AI (Flowers, 2008). Kennedy and Wilkins conducted one of the most comprehensive studies on this topic. They found that Yorkshire boars produced higher amounts of spermatozoa than Hampshire or Landrace boars (Kennedy and Wilkins, 1984). Differences in ejaculate volume, concentration, motility and even percent of live/dead sperm cells between European breeds of swine has also been reported (Knox, 2003; Smital et al., 2004; Smital, 2009). However, the clearest example of the effects of breed on boar fertility may be illustrated when comparing Chinese Meishan boars with conventional breeds of European origin (Knox, 2003). Furthermore, it is well recognized that crossbred boars have higher libido and higher sperm production than purebreds (Wilson et al., 1977).

Season also affects the reproductive performance of boars. It has been reported that sperm concentration varies according to the month of the year due to differences in both temperature and length of the natural photoperiod (Kennedy and Wilkins, 1984; Ciereszko et al., 2000; Smital, 2009). When ambient temperatures exceed the thermoneutral zone, ejaculates of boars have a greater incidence of morphological abnormalities, reduced sperm motility and compromised fertility when semen from these ejaculates are used for AI (Flowers, 2015). Furthermore, it is generally assumed that the shorter photoperiod in fall and winter causes physiological changes invigorating reproduction functions in boars resulting in increased variability of several semen characteristics (Claus and Weiler, 1985; Trudeau and Sanford, 1990; Sancho et al., 2004; Smital, 2009). Nonetheless, it has been reported

previously that exposure of boars to long periods of light results in a reduction in sperm numbers and semen quality while there are other reports where sperm numbers/mL of semen and semen quality are greater as a result of exposure of boars to longer periods of light during the day (Flowers, 2015).

One hypothesis is that the retention of residual sensory capacities to environmental cues that were present in wild boars prior to domestication and origin of the modern pig throughout evolution of the porcine species are the origins of these differences. There are marked seasonal influences on reproductive performance in wild boars and sows. Adult sows show sexual rest in summer and early fall and become sexually active predominantly during late fall and winter (Mauget and Boissin, 1987). In adult boars, the testes mass and testosterone concentrations in blood are greater in winter, compared to summer (Schopper et al., 1984; Mauget and Boissin, 1987; Kozdrowski and Dubiel, 2004).

Another factor that may have an effect on semen production in the boar is moon phases but there is no research to support or dispute this claim. There is evidence that the lunar cycle influences reproduction via the hypothalamus-pituitary-gonadal axis in fish, and it causes hormonal changes in insects and causes daily fluctuations in melatonin and corticosterone concentrations in birds (Zimecki, 2006). It has also been shown that moon phases have an effect on human birth rate and moreover, spontaneous human births have occurred at a greater frequency during the full moon phase (Criss and Marcum, 1981).

Even though it is known that lunar cycles affect wild animal behavior (Fitzgerald and Bider, 1974; Steiner et al., 2014) there is very limited information on the effect of lunar cycles on reproduction of domesticated farm animals. In Holstein bulls, lunar periods had no effect on any measure of semen output (Everett and Bean, 1982). Conversely, it has been

shown that overall calf sex ratio and the sex ratios of calves conceived during either a new, first quarter or last quarter moon phase differed from the expected 1:1 ratio, however, no effect of lunar cycle or season on sex ratio of sheep or pigs was found in the same study(Abecia et al., 2017). In horses, peak numbers both in mare mating (estrus) and fertility (percentage of successful coverings) have tended to synchronize on or immediately following a full moon phase with a minimum number in the first moon quarter phase (Kollerstrom, 2004). One hypothesis is that the intensity of lunar effects vary according to the angle of the moon relative to the sun as the earth orbits around the sun. There have been reports of lunar modulation effects that are likely to occur, or be more pronounced, during the eclipse season (Schneider, 1967). Eclipse seasons are the only times during a year eclipses can occur due to the moon's orbit. At these points, the moon will be at its closest to Earth. Each season lasts for approximately 34 days and there is repetition at slightly less than 6 months periods, thus there are always two full eclipse seasons each year (Littmann et al., 2008). Another hypothesis is that some lunar effects such as release of neurohormones may be affected by the strength of the electromagnetic field or the gravitational pull of the moon (Zimecki, 2006).

The role of women and small ruminants in food security

Traditionally, food security policies have focused on health, nutrition and other aspects of women in their reproductive roles; neglecting them as productive farmers (Quisumbing et al., 1995). In Africa, women traditionally play the role of food-crop producers and are under high risk of being food insecure (Gladwin et al., 2001).

However, women play key roles as farm managers and farm workers all around the world. Nowadays there is strong evidence that shows that income in the hands of women contributes more to household food security and child nutrition than income that is controlled by men (Quisumbing et al., 1995). Therefore, the goal of food security policies has shifted from increasing agricultural production to increasing the economic independence of women (Okali and Sumberg, 1985) and therefore, the implementation of small livestock which are often managed by women in household economies can prove to be a very effective strategy to improve food security.

Livestock plays a key role in food security of small-stake holders in eastern and southern African countries where the vast majority of people in rural areas, especially women, rely on investment in their livestock, with small ruminants serving as "current accounts" and larger species as "saving accounts" (Lebbie 2004). Livestock accounts for the 13% of the energy and 28% of the protein consumed globally in developing countries (FAO, 2009; Smith et al., 2013). Moreover, livestock systems represent almost 30% of Earth's usable surface (FAO, 2006) and have a value of at least $1.4 trillion while directly and indirectly supporting around 1.3 billion people globally and directly supporting 600 million smallholder farmers in developing countries (Thornton et al., 2006).

Native African breeds of livestock are known for their resilience (Kouakou et al 2008), breeding capacity under harsh conditions (Simela and Merkel, 2008), and most importantly their capacity to perform well under adverse conditions with minimal input of resources (Olivier et al 2002). Of the 223 million goats in Sub Saharan Africa (SSA), about 64% are found in arid (38%) and semi-arid (26%) agro-ecological zones (Lebbie and Ramsay 1999) with more than 90% being owned by smallholder farmers (Lebbie and Ramsay 1999;

Gwaze et al 2009). Goats are a very effective species as an agent to improve food security in rural Africa. However, rural goat production in Africa faces multiple challenges that include high disease and parasite prevalence, low levels of management, limited forage availability and poor marketing management (Gwaze et al 2009) which are responsible for poor overall productivity.

Goats represent an important livestock component across all agroecological zones in sub-Saharan Africa. Moreover, goats are found in all production systems ranging from pastoral and agro- pastoral systems through ranching range systems to small holder mixed-crop-livestock systems (Lebbie 2004). However, in countries like South Africa, 50% of the country's goat population are kept under small-scale conditions (Shabalala and Mosima 2002). Smallholder farming systems in developing countries are characterized by minimal resources in terms of land and capital, low income, poor food security, diversified agriculture and informal labor arrangements derived from family members (de Sherbinin et al., 2008). Therefore, goats are an ideal vehicle for cash generation to improve food security and welfare among communal families (Gwaze et al 2009).

Prediction of live weight of livestock using body measurements

Body measurements are a simple, economically viable and effective way to predict live weight of animals in rural scenarios where access to scales might be limited. Accurate estimation of live weight of animals may improve the capacity of producers to negotiate the right price when selling animals to traders (Walugembe et al., 2014) since larger sized animals usually are heavier and produce more income than smaller animals (Alemu Yami et al., 2009). Accurate measurement also allows producers to more accurately estimate

medication doses and feed supply as well as monitoring growth and choosing replacement animals and making better management decisions overall (Mahieu et al., 2014). These methods are particularly important for small ruminants like goats, that are generally sold to traders in rural Africa, who often underestimate weight, which adversely affects farmers because they receive less money for their animals than they are really worth (Walugembe et al 2014).

Nevertheless, it is important to note that body conformation and the correlation between body measurements and live weight might vary according to species, breed and environmental conditions that affect performance of the animal; and therefore, all these factors should be taken in consideration when predicting live weight of livestock. A good example of this is a commonly used equation (Horner, 2013), that was developed for dairy goats managed in production systems with high input levels in developed countries. Since rural goat production in Africa faces challenges that include high disease and parasite prevalence, low levels of management and limited forage availability (Gwaze et al., 2009), this equation loses accuracy due to the differences in body conformation of the breeds and overall health and fitness status of the animals.

Body measurements have been previously used to predict live weight of multiple livestock species including pigs (Walugembe, 2017), horses (Takaendengan et al., 2012), cattle (Ozkaya and Bozkurt, 2009) and sheep (Alemu Yami et al., 2009), among several others. In many of these species, body length and heart girth are the two body measurements that have the highest correlation with live weight (Khan et al., 2006; Sowande and Sobola, 2008; Walugembe et al., 2014), making this two measurements key for accurate predictions of live weight. Correlations within body measurements can also be high. A study on West

African Dwarf (WAD) sheep found high correlations between body length and chest girth (Sowande and Sobola, 2008). Nonetheless, it is important to note that correlations within body measurements and correlations among body measurements and live weight may change with age and sex of the animals (Khan et al., 2006). Therefore, when developing or using models to predict live weight, both factors should be taken in consideration.

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CHAPTER 3. CLIMATIC FACTORS AFFECTING QUANTITY AND QUALITY GRADE OF *IN VIVO* DERIVED EMBRYOS OF CATTLE

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Abstract

The present study investigated the effects of climatic variables on the quality grade and quantity of in vivo derived cattle embryos in the Midwestern United States. Climatic information included greatest and least daily temperature, average daily wind speed and average temperature-humidity index for each of the 765 records. The response variables included the number of ovarian structures, viable embryos, quality grade 1 embryos, quality grade 2 embryos, quality grade 3 embryos, freezable embryos (sum of quality grade 1 and quality grade 2 embryos), transferable embryos (sum of quality grade 1–3 embryos), degenerate embryos and unfertilized ova. Measures for variables among the breeds of donors and sires grouped by geographical origin were compared. A negative effect of greater temperatures during the early embryonic development stage tended ($P < 0.10$) to be associated with a decrease in the quality of embryos recovered. Interestingly, the greater the Temperature-Humidity Index (THI) during the early ovarian antral follicular development stage 40–45 days prior to ovulation was associated with a tendency for greater numbers of total number of freezable and transferable embryos recovered per uterine flushing ($P < 0.10$). Increased wind speed at the early antral follicular phase 40–45 days prior to ovulation was

associated with an increase in the percentage of quality grade 1 embryos recovered ($P < 0.05$). Wind speed during the estrous synchronization period was also associated with a lesser number of embryos recovered ($P < 0.05$). This retrospective study confirms that climatic variables have significant effects on the in vivo production of cattle embryos and that wind speed should be considered in future analyses of factors affecting embryo quality.

Keywords: Beef cattle; Climate; Dairy cattle; Heat stress; Temperature-humidity index; Wind

Introduction

Reproductive biotechnology has a key role in the rapid genetic and productive improvement of beef and dairy cattle herds in the last century (Broom, 2004). Artificial insemination, multiple ovulation, and embryo transfer technologies have been widely used allowing cattle producers to synchronize the estrous cycle of genetically superior groups of embryo donors and recipients with desirable results (Mapletoft and Bó, 2012; Hasler, 2014; Wiltbank and Pursley, 2014). One of the primary factors decreasing the effectiveness of using multiple ovulation technologies is the overall decreased fertility associated with heat stress in cattle (Putney et al., 1989; Hansen et al., 2001; Al-Katanani et al., 2002; Jordan, 2003;). The use of in vivo techniques results in lesser embryo recovery numbers and poorer quality grades of embryos during the months of the year when ambient temperature is greater, often extending into early fall in the northern hemisphere.

Greater temperatures affect the oocyte by altering the patterns of ovarian follicular development, steroid production, and gene expression (Roth et al., 2000; Argov et al., 2005; Hansen et al., 2001; Ferreira et al., 2016). Heat stress alters the duration of estrus, conception

rate, and uterine function in cattle. There is also evidence that heat stress before insemination is associated with decreased fertility in cattle, possibly reflecting detrimental effects on the developing oocyte. In addition, heat stress ranging from 12 to 72 h post-breeding can negatively affect conception rates of cattle (Ambrose et al., 1999; Ulberg and Burfening, 1961). Heat stress may be defined as any combination of environmental variables that result in conditions that are greater than those of animal's thermal neutral zone (García-Ispierto et al., 2007). Heat stress is, therefore, an environmental condition that affects body temperature to increase it above a set-point temperature (Hansen, 2009). One of the most accurate methods of estimating heat stress is the temperature-humidity index (THI) that can be calculated using ambient temperature (T) and relative humidity (RH) in measuring the amount of heat stress an animal experiences (García-Ispierto et al., 2007). Environmental temperature, radiant energy, relative humidity, and wind speed all contribute, however, to the extent of heat stress (García-Ispierto et al., 2007; De Rensis et al., 2015). Even though heat stress is usually associated with zones of tropical or subtropical weather, heat stress poses an important challenge when conducting embryo transfer, especially in grazing cattle in the mid-western areas of the USA (Mader et al., 2006). The objective of this research was to identify the effects of the important weather variables in mid-western USA that may influence the development of cattle embryos from the activation of primordial ovarian follicles until embryo recovery in donor cows. This research is based on the hypothesis that independent climatic factors such as temperature, relative humidity, and wind speed affect the incidence of detrimental effects of heat stress on cattle in mid-western USA.

Materials and methods

Database characteristics

The retrospective data used in this study were composed of 1420 records of superovulatory uterine flushings performed by the Iowa State University (ISU) College of Veterinary Medicine that were conducted from March 2005 to December 2016. All uterine flushings evaluated occurred as a result of superovulation protocols being implemented to induce multiple embryo development.

These protocols consisted of observations for behavioral estrus and use of the timing of estrus to ablate/remove dominant follicles combined with use of timed controlled internal drug release (CIDR; Zoetis, Kalamazoo, MI) device protocols for control of the estrous cycle. Dosages of total FSH (Folltropin-V®, Vetoquinol, Quebec, Canada) ranged from 240 to 320 mg. The donors represented 20 different breeds and some crossbreds. The breeds included were Ayrshire, Santa Gertrudis, Charolais, Chianina, Dexter, Shorthorn, Gelbvieh, Milking Shorthorn, Jersey, Red Angus, Black Angus, Simmental, Limousin, Maine Anjou, Senepol, Simmental/Angus Cross, Polled Hereford, Horned Hereford, club-type cattle, and crosses of unknown breeds that were grouped together. The number of uterine flushings per donor ranged from 1 to 12. For the statistical analyses, the beef breeds were grouped according to the geo- graphical origin; thus, categorizations were British or continental, dairy breeds constituting one group, and all the crossbreds combined being assigned to the other group. There was no separation between heifers and cows in the statistical analyses because the majority of the dairy donors were heifers. In addition, lactational status was not taken into consideration because to the best of our knowledge only one study has been conducted assessing the influence of this variable on embryo quality (Leroy et al., 2005). The records

included uterine flushing date, service date, technician that performed the flushing, sire, sire breed, including the number, quantity, quality grade, and stage of the embryos recovered. Housing conditions were not recorded, thus, were not included in the dataset. Collection of these data would have been of value in assessing climatic factors on embryo quality in the present study. Similar to the way breed data of the donors were managed, breeds of AI sire were categorized according to the origin or use of the breed as continental, British, dairy and crosses. All embryo-related classifications were conducted in ways consistent with the International Embryo Transfer Society (IETS) standards and embryo quality classifications were assigned by American Embryo Transfer Association (AETA) certified personnel. All records with missing or unknown information were not included in the data analyses for the present study.

Climatic variables

The climatic data used in this study were obtained from the Iowa Environmental Mesonet (IEM) Website using the ISU AgClimate database for dates of uterine flushing from 2000 to 2013 and the ISU Soil Moisture Network for dates of uterine flushing from 2013 to 2016. All records were collected by weather stations located in or near Ames, Iowa where all uterine flushings were conducted. Data for mean monthly measurements averaged during the periods of the study are included in Table 1. The greatest daily temperature (T), least daily T, average daily wind speed at 3m of height and average daily relative humidity (RH) data were downloaded from the previously described websites. Wind speed was converted from miles per hour to kilometers per hour by dividing by 1.6. The THI was calculated as proposed by García-Ispierto et al. (García-Ispierto et al., 2007; Vieira et al., 2014).

Data management and statistical analyses

After removing records with obvious errors or omissions, climatic information was added for 765 records. The response variables included the number of structures, viable embryos, quality grade 1 embryos, quality grade 2 embryos, quality grade 3 embryos, embryos assessed to be worthy of freezing based on quality assessments (sum of quality grade 1 and quality grade 2 embryos), transferable (based on quality assessments) embryos (sum of quality grade 1–3 embryos), degenerated embryos and unfertilized ova. All the variables were analyzed as the average number obtained per uterine flushing and as a proportion of recovered structures within the fluid from the flush. The time points analyzed were: (1) 1 day after AI through time of uterine flush; (2) 4 days prior to AI through the AI date; (3) 4 days prior to and including the day of initiation of the estrous synchronization/superovulation protocol (referred as synchronization date); (4) 40–45 days prior to ovulation; and (5) 80–85 days prior to ovulation. The climatic variables for each period were averaged to simplify analyses. The 40-to-45-day period preceding time of ovulation was chosen because at this time the follicles start to become antral follicles (Lussier et al., 1987). The 80-to-85-day period preceding ovulation was chosen because this is the approximate time primordial follicles become activated (Britt, 2008). Data were analyzed using the "car" package version 2.1-4 (John et al., 2015) from R to perform type-III ANOVA, while the least squares means where obtained using the "lsmeans" package version 2.26-3 (Lenth, 2015) on R. All linear models took into consideration the fixed effects of technician performing the uterine flush, donor breed, and sire breed and fitted simultaneously THI, greatest temperature, least temperature and wind speed as covariates. Each climatological variable was included as a different covariate for each period analyzed. Least squares means of embryos produced in vivo breed-of-sire and breed-of-donor group were

estimated with the objective of identifying possible differences in the efficiency of embryo production.

Results

In Table 2, the values are included for regression coefficients for the effects of different climatic variables on the number and percentages of in vivo recovered embryos. Greater wind speed during the period of estrous synchronization tended (P=0.07) to be negatively associated with the total number of embryos recovered at the time of uterine flushing. Greater wind speed is also associated (P=0.03) with a decreased percentage of degraded and unfertilized ova, referred as discardable-quality embryos. An increase of 1 km/h in the wind speed was associated with a reduction of 0.16 ± 0.07 degraded embryos per uterine flush. An increase in THI during the 40–45 days prior to the period during which ovulation occurred (Table 2) tended (P=0.07) to be associated with an increase the number of freezable transferable embryos recovered. An increase of one unit in the THI during the early period of antral follicular stage was associated with a 0.22 increase in the number of freezable and transferable embryos recovered. The THI during the early embryonic development tended (P=0.06) to be negatively associated with the number of transferable embryos recovered. An increase of one unit in the THI was associated with a reduction of 0.24 on the number of recovered transferable quality grade embryos. In Table 3, values are presented for the regression coefficients on the effects of different climatic variables on the number and percentages of in vivo recovered embryos according to the quality grade assigned. The number of quality grade 1 embryos recovered per uterine flushing was associated (P=0.03) with greater temperatures during the early antral follicular stage. An

increase in ambient temperature of 1 °C was associated with a reduction of approximately 0.33 quality grade 1 embryos per uterine flush. The THI was also associated (P=0.03) with the number of quality grade 1 embryos recovered. An increase in 1 THI unit during the period of early antral follicular development was associated with collection of 0.22 more quality grade 1 embryos per uterine flush. During the early embryonic development stage, however, the greater THIs tended (P=0.06) to be negatively associated with the production of quality grade 1 embryos with an increase of 1 THI unit being associated with a decrease of 0.22 quality grade 1 embryos per flush. Wind speed during the 40–45 days period before the time of AI was associated with the percentage of transferable (P=0.03) and freezable (P=0.04) quality grade 1 embryos recovered per flushing. Interestingly, greater temperatures during early follicular development were not associated with the number of quality grade 2 embryos collected as a percentage of freezable embryos recovered. Also, the number of quality grade 3 embryos recovered per flushing was associated (P=0.01) with wind speed during the period of activation of the primordial follicle. An increase of 1 km/h on wind speed was associated with a decrease of 0.02 ± 0.008 quality grade 3 embryos recovered per uterine flush. Data included in Table 4 for the geographical origin of sire breed used for AI indicate site of breed origin was not associated with the number of embryos recovered. Interestingly, only the number of discarded embryos recovered was different (P=0.01) among breed groups of dams, with individuals of dairy breeds having the least number of embryos categorized as quality grade 4 and British breeds having the greatest number of embryos in this category.

Discussion

Hyperthermia can impair cellular function in various tissues of the reproductive system. Disruption of the follicle and its enclosed oocyte seems, however, to be a pivotal factor in the complex mechanism via which heat stress impairs fertility (Roth, 2008). In males, an increase in testicular temperature leads to reduced sperm output, decreased sperm motility and an increased proportion of morphologically abnormal spermatozoa in the ejaculate. In females heat stress affects ovarian follicular development, hormonal secretion, oocyte maturation and embryonic and fetal development (Hansen, 2009). More specifically, in cattle the efficiency of production of embryos using superovulation technologies is often less in periods when there is the greatest ambient temperature. The increased ambient temperature is associated with a reduction in the number of transferable embryos resulting from use of super- ovulation techniques and is due to a reduced super ovulatory response, lesser fertilization rate and reduced embryo quality grade (Gordon et al., 1987; Monty and Racowsky, 1987; Alfuraiji et al., 1996; Hansen et al., 2001). Even though the negative effects of heat stress on reproduction are widely known and studied, there is a limited amount of literature related to the effects of climatic factors such as wind speed on heat stress of grazing cattle. Findings with the retrospective non-randomized present study indicate there is an effect of ambient temperatures when these result in body temperatures that are greater than thermal neutral zone of cattle during the early embryonic development stage. An increase in temperature tended to be negatively associated with the number of quality grade 2 embryos collected as a proportion of freezable embryos. Results of previous studies indicate the embryo is particularly susceptible to heat stress during early development because it lacks the biochemical responses to effectively limit the deleterious effects of heat stress (Lindquist,

1986; Mirkes, 1987). Findings with the present study are not consistent with the premise that a maximal THI is associated with compromised ovarian follicular development. There have been previous reports that a greater than optimal THI affects reproductive performance by causing an increase in the concentrations of FSH and decreased inhibin concentrations in plasma. These endocrine effects may have physiological significance that could be associated with lesser fertility of cattle during the summer and autumn as compared with other seasons of the year (Monty and Racowsky, 1987; Hansen et al., 2001; Argov et al., 2005; Ferreira et al., 2016). Greater than optimal THIs during the period of early antral follicular development in the present study tended to be associated with an enhanced quality of embryos recovered per uterine flushing as a result of a greater percentage of freezable and transferable embryos recovered. This finding could be the result of heat stress accelerating oocyte maturation and suppressing follicular dominance possibly by affecting the production of Anti-Müllerian hormone by the granulosa cells. Anti-Müllerian hormone has been associated with the suppression of follicle activation in mice and cattle (Gigli et al., 2005). This possible effect of the THI on embryo development, combined with FSH administration during the superovulation protocol may contribute to a greater number of antral follicles developing to a stage where ovulations from more follicles can be induced (Edwards et al., 2005; Roth, 2015). Mader et al., 2006 reported that a lesser wind speed decreased the capacity for cattle to dissipate heat (Mader et al., 2006). These previous findings are consistent with the finding in the present study that wind speed during the early antral follicular phase 40–45 days prior to ovulation was associated with the percentage of quality grade 1 embryos recovered. The finding in the present study that wind speed during the estrous synchronization period was associated with percentage of quality grade 1 embryos further indicates the importance of

wind speed on the physiological responses of cattle. In the present study, the increase in total number and decrease in number of poor quality grade embryos recovered by uterine flushing was associated with an increased wind speed. This finding implies an increased wind speed may result in an improvement in the quality grade of recovered embryos. This could be explained by the beneficial effect of increased air movement on dissipating body heat when the THI is greater than optimal. Increased air speed over the body surface results in a disruption of the layer of air near the skin surface. Disruption of this airspace allows for the removal of warm air as it is replaced by cooler air. Body heat of the animal is then transferred to the cool air and removed via continuous air movement (Robertshaw, 1985; Mader et al., 2006). Additionally, Arkin et al. (1991) reported that thermal conductivity of the boundary layer of air adjacent to the hair increased linearly with wind velocity even though the increased ability of the animal to dissipate heat reached a maximum when wind speed approached 7 km/h (NRC, 1981; Arkin et al., 1991; Mader et al., 2006). An important finding in the present study was the significant effect of donor breed on many of the response variables analyzed.

Because the data used in this study originated from 22 donor breeds, it is logical to assume that there would be breed differences for embryo production. Interestingly, inconsistent with what was anticipated for embryo production in dairy breeds, the donor animals in the present study of dairy breeds had similar efficiencies for embryo production as those from beef breeds. Intense selection for greater milk production has had negative effects on reproductive performance of dairy breeds (Al-Katanani et al., 2002; De Rensis and Scaramuzzi, 2003; Roth, 2008). It is acknowledged that limitations of the present study in assessing embryo production dairy breeds was that >90% of the dairy cattle were heifers and

lactation and parity status was not available on multiparous cows. Results of the present study indicate embryo transfer could be an important technique to improve reproductive performance of dairy herds with negative effects of heat stress on reproduction. It has been previously reported in research with dairy cattle that transferring in-vivo- derived frozen-thawed embryos increased pregnancy rates of recipient cows relative to traditionally-inseminated cows (Putney et al., 1989a; Drost et al., 1999). In conclusion, the findings in the present study suggest that climatic variables early in the activation period of ovarian follicular development to and through the time of fertilization can affect embryo production when superovulation protocols are used for embryo transfer. Thus, it may be prudent for producers and ET professionals to alter timing of embryo recovery protocols based on both prior and current weather patterns to optimize return on investment when using these reproductive technologies.

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Tables

Table 3.1 *Mean Monthly weather variable averages from 2000 to 2016*

Month	High T (°C)[1]	Low T (°C)[2]	Mean T (°C)[3]	RH (%)[4]	Wind (km/h)[5]	THI[6]
January	-1.64	-11.22	-6.43	81.42	8.57	24.3
February	0.18	-9.8	-4.81	81.18	8.68	26.96
March	8.09	-2.67	2.71	75.24	8.68	39.77
April	16.26	3.8	10.03	65.92	9.48	51.54
May	21.89	10.09	15.99	67.88	8.32	60.27
June	26.92	15.69	21.31	72.61	6.46	68.46
July	28.65	17.59	23.12	79.34	5.03	71.81
August	27.65	16.44	22.05	81.74	4.85	70.29
September	24.34	11.53	17.93	73.98	5.83	63.36
October	16.59	4.58	10.58	71.98	7.02	52.12
November	8.96	-1.95	3.5	76.36	8.23	40.88
December	0.02	-8.94	-4.46	84.31	8.42	26.93

[1]High T (°C): average daily highest temperature in degrees Celsius

[2]Low T (°C): average daily lowest temperature in degrees Celsius

[3]Mean T (°C): mean daily temperature in degrees Celsius

[4]RH (%): average daily relative humidity

[5]Wind (km/h): daily average wind speed in km/h

[6]THI: Temperature-humidity index

Table 3.2. *Regression coefficients and SE of weather variables on number and percentage of embryos according to quality grade*

Effect	Total Embryos[1], *n*	Freezable[2], *n*	Freezable[2], %	Transferrable[3], *n*	Transferrable[3], %	Discarded[4], *n*	Discarded[4], %
High T 80s (ºC)[a]	0.20 ± 0.23	0.12 ± 0.17	-0.009 ± 0.014	0.13 ± 0.17	-0.01 ± 0.014	0.06 ± 0.17	0.002 ± 0.01
High T 40s (ºC)[b]	-0.13 ± 0.25	-0.28 ± 0.19	-0.005 ± 0.015	-0.29 ± 0.19	-0.003 ± 0.015	-0.02 ± 0.19	-0.002 ± 0.01
High T Sync (ºC)[c]	-0.25 ± 0.21	-0.04 ± 0.16	0.001 ± 0.013	-0.06 ± 0.16	-0.004 ± 0.013	-0.04 ± 0.16	-0.0004 ± 0.01
High T AI (ºC)[d]	-0.05 ± 0.22	-0.15 ± 0.16	-0.006 ± 0.013	-0.13 ± 0.17	-0.005 ± 0.013	0.09 ± 0.16	0.01 ± 0.01
High T Flushing (ºC)[e]	0.27 ± 0.26	0.28 ± 0.20	-0.024 ± 0.016	0.25 ± 0.20	-0.03 ± 0.02	0.08 ± 0.20	0.02 ± 0.01
Wind speed 80s (km/h)[f]	-0.04 ± 0.09	0.05 ± 0.06	0.005 ± 0.005	0.03 ± 0.06	0.005 ± 0.005	-0.03 ± 0.06	-0.002 ± 0.004
Wind speed 40s (km/h)[g]	0.09 ± 0.10	-0.06 ± 0.08	0.004 ± 0.006	-0.06 ± 0.08	0.003 ± 0.006	0.09 ± 0.08	0.007 ± 0.005
Wind speed Sync (km/h)[h]	-0.22 ± 0.10**	-0.02 ± 0.07	-0.0008 ± 0.006	-0.03 ± 0.08	-0.001 ± 0.006	-0.16 ± 0.07**	-0.01 ± 0.005*
Wind speed AI (km/h)[i]	0.09 ± 0.10	0.03 ± 0.07	-0.002 ± 0.006	0.04 ± 0.08	-0.002 ± 0.006	0.01 ± 0.07	-0.002 ± 0.005
Wind speed Flushing (km/h)[j]	-0.08 ± 0.11	-0.01 ± 0.08	0.0009 ± 0.007	-0.004 ± 0.08	0.002 ± 0.007	-0.004 ± 0.08	0.002 ± 0.006
THI 80s[k]	-0.18 ± 0.15	-0.11 ± 0.11	0.005 ± 0.009	-0.20 ± 0.12	0.006 ± 0.009	-0.06 ± 0.11	-0.002 ± 0.008
THI 40s[l]	0.16 ± 0.17	0.22 ± 0.13*	0.005 ± 0.01	0.22 ± 0.13*	0.003 ± 0.01	0.05 ± 0.13	0.002 ± 0.008
THI Sync[m]	0.17 ± 0.14	0.05 ± 0.10	0.00008 ± 0.008	0.06 ± 0.11	0.003 ± 0.009	0.02 ± 0.10	0.0007 ± 0.007
THI AI[n]	0.02 ± 0.15	0.13 ± 0.11	0.006 ± 0.009	0.13 ± 0.11	0.006 ± 0.009	-0.11 ± 0.11	-0.009 ± 0.008
THI Flushing[o]	-0.22 ± 0.18	-0.25 ± 0.13	0.012 ± 0.011	-0.24 ± 0.14*	0.01 ± 0.01	-0.03 ± 0.13	-0.009 ± 0.009

Footnotes on the next paged

*Represents $0.10 > P > 0.05$

**Represents $0.05 \geq P > 0.01$

[1]Total Embryos: Embryos graded as quality grade 1,2,3 and 4

[2]Freezables: Embryos graded as quality grade 1 and 2

[3]Transferrable: Embryos graded as quality grade 1, 2, and 3

[4]Discarded: Degenerated embryos and unfertilized ova

[a]High T 80s (ºC): average daily greatest temperature in degrees Celsius 80-85 days prior to ovulation

[b]High T 40s (ºC): average daily greatest temperature in degrees Celsius 40-45 days prior to ovulation

[c]High T Sync (ºC): average daily highest temperature in degrees Celsius 4 days prior to and including estrous synchronization date

[d]High T AI (ºC): average daily greatest temperature in degrees Celsius 4 days prior to AI up to and including AI date

[e]High T Flushing (ºC): average daily greatest temperature in degrees Celsius one day after AI up to flushing date

[f]wind speed 80s (km/h): daily average wind speed in km/h 80-85 days prior to ovulation

[g]Wind speed 80s (km/h): daily average wind speed in km/h 40-45 days prior to ovulation

[h]Wind speed 80s (km/h): daily average wind speed in km/h 4 days prior to and including estrous synchronization date

[i]Wind speed 80s (km/h): daily average wind speed in km/h 4 days prior to AI up to and including AI date

[j]Wind speed 80s (km/h): daily average wind speed in km/h one day after AI up to flushing date

[k]THI 80s: Average Temperature-Humidity Index 80-85 days prior to ovulation

[l]THI 40s: :Average Temperature-Humidity Index 40-45 days prior to ovulation

[m]THI Sync: Temperature-Humidity Index 4 days prior to and including synchronization date

[n]THI AI: Temperature-Humidity Index 4 days prior to AI up to and including AI date

[o]THI Flush: Temperature-Humidity Index one day after AI up to flushing date

Table 3.3. *Regression coefficients and SE of weather variables on number of embryos according to quality grade*

Effect	Q1 recovered per flush[1], *n*	Q1 Transferrable[2], %	Q1 Freezable[3], %	Q2 recovered per flush[4], *n*	Q2 Transferrable[5], %	Q2 Freezable[6], %	Q3 recovered per flush[7], *n*	Q3 transferrable [8], %
High T 80s (ºC)[a]	0.02 ± 0.14	-0.02 ± 0.01	-0.02 ± 0.01	-0.004 ± 0.05	0.0003 ± 0.009	-0.001 ± 0.010	0.01 ± 0.02	-0.002 ± 0.004
High T 40s (ºC)[b]	-0.33 ± 0.15**	-0.02 ± 0.01	0.02 ± 0.02	0.003 ± 0.06	-0.003 ± 0.01	-0.002 ± 0.01	-0.01 ± 0.02	0.0006 ± 0.004
High T Sync (ºC)[c]	0.03 ± 0.13	0.003 ± 0.01	0.002 ± 0.01	-0.07 ± 0.05	-0.006 ± 0.008	-0.01 ± 0.009	-0.02 ± 0.02	-0.003 ± 0.003
High T AI (ºC)[d]	-0.16 ± 0.13	-0.0004 ± 0.01	-0.00001 ± 0.01	-0.02 ± 0.05	-0.001 ± 0.009	0.0003 ± 0.009	0.01 ± 0.02	0.004 ± 0.003
High T Flushing (ºC)[e]	0.23 ± 0.16	-0.006 ± 0.01	-0.006 ± 0.02	-0.003 ± 0.06	-0.02 ± 0.01	-0.02 ± 0.01*	-0.02 ± 0.03	-0.005 ± 0.004
Wind speed 80s (km/h)[f]	0.004 ± 0.05	0.001 ± 0.0052	0.0002 ± 0.005	-0.003 ± 0.02	-0.002 ± 0.003	-0.003 ± 0.004	-0.02 ± 0.008**	-0.002 ± 0.001
Wind speed 40s (km/h)[g]	0.0004 ± 0.06	0.014 ± 0.006**	0.014 ± 0.006**	0.007 ± 0.02	0.0009 ± 0.004	0.001 ± 0.004	-0.005 ± 0.01	-0.0006 ± 0.002
Wind speed Syncs (km/h)[h]	-0.004 ± 0.06	-0.010 ± 0.006	-0.01 ± 0.006	-0.04 ± 0.02	0.004 ± 0.004	0.004 ± 0.004	-0.003 ± 0.009	-0.0002 ± 0.002
Wind speed AI (km/h)[i]	0.06 ± 0.06	0.005 ± 0.006	0.007 ± 0.006	0.01 ± 0.02	-0.003 ± 0.004	-0.003 ± 0.004	0.006 ± 0.009	0.0009 ± 0.002
Wind speed Flushing (km/h)[j]	-0.022 ± 0.07	-0.009 ± 0.007	-0.009 ± 0.006	-0.02 ± 0.02	-0.004 ± 0.004	-0.003 ± 0.005	0.01 ± 0.01	0.002 ± 0.002
THI 80s[k]	-0.034 ± 0.10	0.01 ± 0.009	0.01 ± 0.009	-0.008 ± 0.03	-0.002 ± 0.006	-0.001 ± 0.006	-0.01 ± 0.01	0.0007 ± 0.002
THI 40s[l]	0.22 ± 0.10**	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.04	0.004 ± 0.007	0.003 ± 0.007	0.006 ± 0.02	-0.0004 ± 0.003
THI Syncs[m]	0.02 ± 0.09	-0.002 ± 0.008	-0.001 ± 0.009	0.04 ± 0.03	0.004 ± 0.005	0.007 ± 0.006	0.01 ± 0.01	0.002 ± 0.002
THI AI[n]	0.13 ± 0.09	0.0003 ± 0.009	0.008 ± 0.009	0.01 ± 0.03	0.002 ± 0.006	0.0003 ± 0.006	-0.008 ± 0.01	-0.002 ± 0.002
THI Flush[o]	-0.22 ± 0.11*	0.003 ± 0.01	-0.003 ± 0.01	-0.006 ± 0.04	0.008 ± 0.007	0.01 ± 0.007	0.01 ± 0.02	0.003±0.003

Footnotes on the next page

*Represents $0.10 > P > 0.05$

**Represents $0.05 \geq P > 0.01$

[1]Number of quality grade 1 embryos recovered per uterine flushing

[2]Quality grade 1 embryos recovered per uterine flushing as a percentage of transferrable embryos (quality grade 1, 2 and 3)

[3]Quality grade 1 embryos recovered per flushing as a percentage of freezable embryos (quality grade 1 and 2)

[4] Number of quality grade 2 embryos recovered per uterine flushing

[5]Quality grade 2 embryos recovered per uterine flushing as a percentage of transferrable embryos (quality grade 1, 2 and 3)

[6]Quality grade 2 embryos recovered per uterine flushing as a percentage of freezable embryos (quality grade 1 and 2)

[7]Number of quality grade 3 embryos recovered per uterine flushing

[8]Quality grade 3 embryos recovered per uterine flushing as a percentage of transferrable embryos (quality grade 1, 2 and 3)

[a]High T 80s (ºC): average daily greatest temperature in degrees Celsius 80-85 days prior to ovulation

[b]High T 40s (ºC): average daily greatest temperature in degrees Celsius 40-45 days prior to ovulation

[c]High T Sync (ºC): average daily greatest temperature in degrees Celsius 4 days prior to and including estrous synchronization date

[d]High T AI (ºC): average daily greatest temperature in degrees Celsius 4 days prior to AI up to and including AI date

[e]High T Flushing (ºC): average daily greatest temperature in degrees Celsius one day after AI up to flushing date

[f]Wind speed 80s (km/h): daily average wind speed in km/h 80-85 days prior to ovulation

[g]Wind speed 40s (km/h): daily average wind speed in km/h 40-45 days prior to ovulation

[h]Wind speed Synchs (km/h): daily average wind speed in km/h 4 days prior to and including synchronization date

[i]Wind speed AI (km/h): daily average wind speed in km/h 4 days prior to AI up to and including AI date

[j]Wind speed Flushing (km/h): daily average wind speed in km/h one day after AI up to flushing date

[k]THI 80s: Average Temperature-Humidity Index 80-85 days prior to ovulation

[l]THI 40s: :Average Temperature-Humidity Index 40-45 days prior to ovulation

[m]THI Sync: Temperature-Humidity Index 4 days prior to and including synchronization date.

[n]THI AI: Temperature-Humidity Index 4 days prior to AI up to and including AI date.

[o]THI Flush: Temperature-Humidity Index one day after AI up to flushing date.

Table 3.4. *LS means for number of embryos produced by breed of sire and breed of dams when grouped by geographical origin.*

	Sires					Dams				
# of Embryos	**British (n= 159)**	**Continental (n= 100)**	**Cross (n= 35)**	**Dairy (n= 472)**	**P-value**	**British (n= 122)**	**Continental (n= 74)**	**Cross (n= 75)**	**Dairy (n= 493)**	**P-value**
Embryos[1]	5.73 ± 0.50	5.74 ± 0.54	5.39 ± 0.81	4.57 ± 0.69	0.27	5.50±0.56	4.82±0.69	5.92±0.67	5.20±0.65	0.07
Freezable[2]	5.32 ± 0.63	5.74 ± 0.57	5.25 ± 0.93	4.80 ± 0.80	0.84	5.35±0.64	5.45±0.79	5.64±0.77	4.66±0.75	0.85
Transferrable[3]	5.70 ± 0.58	6.07 ± 0.64	5.39 ± 0.95	5.05 ± 0.81	0.81	5.57±0.66	5.65±0.81	6.08±0.79	4.92±0.76	0.8
Discarded[4]	5.05 ± 0.57	5.62 ± 0.63	4.90 ± 0.93	3.80 ± 0.80	0.39	6.56±0.65[b]	5.24±0.79[ab]	4.24±0.77[a]	3.33±0.75[a]	0.01

*Different letters across the same row indicate significant differences (0.05≥P).

[1]Total Embryos: Embryos graded as quality 1,2,3, and 4

[2]Freezables: Embryos graded as quality 1 and

[3]Transferrable: Embryos graded as quality 1, 2, and 3.

[4]Discarded: Embryos graded as quality 4 and 5

CHAPTER 4. LUNAR AND CLIMATIC EFFECTS ON BOAR EJACUALTE TRAITS

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Abstract

There is evidence that phases of the moon affect wild animal behaviors including reproduction. There is, however, little evidence of moon phase effects on domestic livestock reproduction. This study investigated the effects of moon phase and climatic variables on boar ejaculate traits. Records of 4149 semen collections from boars of nine different breeds at one boar stud were used. The response variables were volume of ejaculate, concentration of sperm in the ejaculate, and number of doses obtained per ejaculate. Moon phase, greatest daily temperature (T), least daily T, average daily relative humidity (RH), temperature-humidity index (THI), season and the interaction of moon phase with season were analyzed at the day of collection and 45 days prior to date of collection as a proxy of initiation of spermatogenesis. For both dates analyzed season and the interaction of season with moon had significant effects ($P < 0.05$) on the volume of the ejaculate. Moon phase had a significant effect ($P < 0.05$) on volume of ejaculate at the day of collection. Sperm concentration was affected ($P < 0.05$) by the interaction of moon phase with season, high and low temperature, THI, RH and breed. Season had an effect ($P < 0.01$) on concentration of sperm at the initiation of spermatogenesis. For doses that could be used for AI that were obtained/ejaculate, there were effects of moon phase, season, the interaction between season

and moon phase and breed ($P < 0.05$) at collection day and at the initiation of spermatogenesis. There was an interaction ($P < 0.0001$) between season and moon phase for volume of ejaculate, sperm concentration and number of doses obtained per ejaculate at date of collection and at day of initiation of spermatogenesis. The significant interaction of season and moon phase on boar semen traits suggests that to maximize productivity of modern swine production systems determining a collection schedule in some seasons relative to moon phase may be advantageous.

Keywords: Moon phase; Boar; Semen; Reproduction; Lunar effect

Introduction

Novel developments and popularity of artificial insemination (AI) in pigs have led to an improvement of high-performance insemination dose production intended for distribution around the world. Greater knowledge of the factors affecting the quantity and quality of ejaculates can result in the improved biological efficiency, organizational structures of AI companies and economical aspects for inseminations (Knecht et al., 2014). There is the belief among wildlife experts that the moon phase influences animal behavior and reproduction. This is supported by scientific evidence that the lunar cycle influences reproduction via the hypothalamus-pituitary-gonadal axis in fish, causes hormonal changes in insects and causes daily fluctuations in melatonin and corticosterone concentrations in birds (Zimecki, 2006).

Furthermore, moon phases have an effect on human birth rate and spontaneous human births have occurred at a greater frequency during the full moon phase (Criss and Marcum, 1981). Even though it is known that lunar cycles affect wild animal behavior

(Fitzgerald and Bider, 1974; Steiner et al., 2014) there is very limited information on the effect of lunar cycles on reproduction of domesticated farm animals.

In one rare study, lunar periods had no effect on any measure of semen output in Holstein bulls (Everett and Bean, 1982). There, however, is evidence that the overall calf sex ratio and the sex ratios of calves conceived during either a new, first quarter or last quarter moon phase differed from the expected 1:1 ratio. In the same study, there was no effect of lunar cycle or season on sex ratio of sheep or pigs (Abecia et al., 2017). In horses, peak numbers both in mare mating (estrus) and fertility (% successful coverings) have tended to synchronize on or immediately following a full moon phase with a minimum number in the first moon quarter phase (Kollerstrom, 2004)

Season is also an important factor that affects the reproductive performance of boars. Reports in the literature suggest that sperm concentration is different according to the month of the year and that presumably this occurs due to differences in both temperature and length of the natural photoperiod in different seasons of the year (Kennedy and Wilkins, 1984; Ciereszko et al., 2000; Smital, 2009). When ambient temperatures increase to those outside the thermoneutral zone, ejaculates of boars have a greater incidence of morphological abnormalities, reduced sperm motility and there is a compromised fertility when semen from these ejaculates are used for AI (Flowers, 2015). Also, the general conclusion is that decreasing daylight in the fall causes physiological changes stimulating reproduction functions in boars resulting in increased variability of several semen characteristics (Claus and Weiler, 1985; Trudeau and Sanford, 1990; Sancho et al., 2004; Smital, 2009). There, however, have been reports that exposure of boars to long periods of light results in a reduction in sperm numbers and semen quality while there are other reports where sperm

numbers/mL of semen and semen quality are greater as a result of exposure of boars to longer periods of light during the day (Flowers, 2015). These differences probably result from retention in boars throughout evolution of the porcine species of residual sensory capacities to environmental cues that were present in wild boars prior to domestication and origin of the modern pig. There are marked seasonal influences on reproductive performance in wild boars and sows. Adult sows are anestrous in summer and early fall, with the rutting time occurring mainly in late fall and early winter (Mauget and Boissin, 1987). In adult boars, the testes mass and testosterone concentrations in blood are greater in winter, compared to summer (Schopper et al., 1984; Mauget and Boissin, 1987; Kozdrowski and Dubiel, 2004). Furthermore, annual cyclic phenomena of endocrine testicular function has been documented in many other mammalian species such as camels (Yagil and Etzion, 1980), mink (Boissin-Agasse et al., 1981), red foxes (Maurel and Boissin, 1981), hedgehogs (Saboureau et al., 1982), Rhesus monkeys (Wickings and Nieschlag, 1980) and red deer (Lincoln, 1971).

Several reproductive traits including testicular size and libido in boars are all influenced by breed (Rothschild, 1996). Genetic diversity within as well as among swine breeds affects the performance of boars used in Artificial Insemination (AI) (Flowers, 2008). However, no reports of differences of lunar phase effects on breed performance been reported.

The main objective of this study was to determine the possible existence of any effects of lunar cycles and other variables on boar ejaculate variables and to examine those effects relative to other possible factors.

Materials and methods

Boar housing and semen collection

All boars were from one boar stud and housed in individual open pens (8’ × 15’). Windows let in natural light with relative same light intensity throughout the barn, supplemented with fluorescent bulb lighting during daytime hours. The boar stud was heated during the winter and cooled by fresh air ventilation and individual boar water drippers for cooling of boars during the summer months. Boars were tested for disease status before entry into the boar stud and had blood drawn twice/week to monitor for Porcine Reproductive and Respiratory Syndrome (PRRS) virus. The sperm-rich fraction of the boar ejaculate was collected using standard 2- gloved hand technique and data recorded by established standard operating procedures of International Boar Semen (Eldora, IA). Semen was collected from boars no more than three times per 14 days and minimally collected once every 2 weeks. Semen collection materials included a standard collection “dummy” for boars to mount and a thermos that allowed for temperature control of semen. Terminal breed boars were replaced after 14 months and maternal breed boars were replaced after 3 years in International Boar Semen. Boar semen quality had to be a minimum of 75% motility and < 20% abnormal morphology to be placed in an extender for use in AI doses that would be marketed by the stud. Cell concentration was determined by spectrophotometry; and sperm morphology and motility were determined by assessment using visual phase contrast microscopy evaluation at 60× magnification, but such information was not routinely recorded. The same technician collected all ejaculates and the same laboratory technician processed all semen. An AI dose is defined as a total of 80 mL (semen plus extender). Semen doses were calculated to contain minimally 2 billion sperm cells per dose, with a total of 1.5 billion morphologically normal,

motile sperm (calculated from percent motility and percent normal morphology), therefore, doses/boar are reflective of ejaculate quality.

Data collection

The retrospective data used for these analyses resulted from 4149 semen collections, described previously, coming from 127 different boars housed at International Boar Semen during the period from January 2014 to April 2017. Response variables were total concentration of sperm, volume of ejaculate and number of ejaculates.

In addition to the response variables, the records contained the ID of the boar, breed, date of collection, and the moon phase, season and climatic variables that are subsequently described. All records with missing or unknown information were discarded. Data from boars with ten or more collections were included in the analyses. The breeds included Chester White (n = 253), Berkshire (n = 1209), Duroc (n = 671), Landrace (n = 182), Yorkshire (n = 636), Musclor (French stress negative Pietrain; (n = 894) and a group of heritage breeds (n = 304) composed of Gloucestershire Old Spots, Mangalitsa and Meishan.

Moon phase and climatic variables

Moon phase information was obtained from lunar calendars. A specific moon phase included the day when the phase was observed and the 2 days before and 2 days after the phase. The moon phase at 45 days prior to collection was calculated in the same manner. Four moon phases were analyzed. The full moon phase was considered to be the phase at which the moon appears to be completely illuminated by direct sunlight. The new moon phase was considered to be the period when the moon's dark side was facing the earth so that the moon was not visible. The first quarter moon phase was considered to be the phase at

which one-half of the moon appeared to be illuminated by direct sunlight and the fraction of the moon's disk that was illuminated was increasing. The last quarter phase was considered to be when one-half of the moon appeared to be illuminated by direct sunlight and the fraction of the moon's disk that was illuminated was decreasing.

The climatic data used in this study were obtained from the Iowa State University Soil Moisture Network (https://mesonet.agron. iastate.edu/agclimate/hist/dailyRequest.php). All records were obtained from weather stations located near the facility where the boars were housed. To provide a perspective of the monthly mean temperatures that existed during the study, these data are provided in Table 1.

The greatest daily temperature (T), least daily T, and average daily relative humidity (RH) data were downloaded from the previously described website. Temperatures where converted from Fº to Cº using the formula (Fº − 32)*5/9. The Temperature Humidity Index (THI) was calculated using a standard formula (García-Ispierto et al., 2007).

Statistical analyses

Two separate analyses were performed for this research. The first analysis was performed to evaluate the effects of moon phase, season, climate and breed on the day of collection on the boar semen variables assessed in the present study. The second analysis focused on the effects on the semen variables assessed of moon phase, season, climate 45 days prior to the date of collection as well as boar breed while taking into account that boar spermatogenesis and maturation of sperm requires approximately 45 days (Knox, 2003; Parrish et al., 2017).

For both analyses the response variables included volume of the ejaculate defined as the volume of the sperm-rich fraction of the ejaculate, total concentration of sperm in the

ejaculate as the number of sperm per mL in the non-diluted ejaculate collected and number of doses obtained per ejaculate. All doses were designed to contain 2 billion or more sperm. The analyses were performed using a mixed linear model that included the fixed effects of moon phase, season and breed along with covariates of greatest daily temperature, least daily temperature, mean daily relative humidity and mean daily THI along with the interaction of season and moon phase and the random effect of boar nested within breed. Least squares means for the two analyses were produced for main effects moon phases, seasons and breeds for each of the three response variables. Also, the interaction between moon phases and seasons was examined. All the statistical analyses were conducted using SAS® 9.4 (SAS, 2013).

Results

Data are included in Table 2 that resulted from the Analysis of Variance (ANOVA) for the main effects on the day of collection and the day of initiation of spermatogenesis for volume of ejaculate, concentration of sperm in the ejaculate and number of doses obtained per ejaculate. At the initiation of spermatogenesis, concentration of sperm was affected by season ($P < 0.0001$), high temperature ($P < 0.01$), relative humidity ($P < 0.001$), THI ($P < 0.001$), the interaction of moon phase with season ($P < 0.001$) and breed of the boar ($P < 0.0001$).

Least squares means for moon phase are included in Table 3. For concentration of sperm in the ejaculate, there were no effects of moon phase at any date analyzed. At the initiation of spermatogenesis, there were no significant differences in volume of the ejaculate. At this stage, however, the number of doses obtained per ejaculate during the first

quarter of the moon phase was greater ($P < 0.05$) than those obtained during the new phase of the moon. Intriguingly, at day of collection the trends differ. Volume of ejaculates collected during the full phase of the moon were less ($P < 0.05$) when compared to ejaculates collected during any other moon phase and the number of doses obtained per ejaculate were greater ($P < 0.05$) during the last quarter than first quarter phases and the full moon phase.

The least squares means are included for Table 4 for the different seasons at both dates analyzed. At the initiation of spermatogenesis ejaculates collected in the fall were of a larger volume ($P < 0.05$) than the ejaculates collected in summer. Ejaculates collected in summer had greater ($P < 0.05$) sperm concentrations than those collected during the other seasons of the year. The number of doses obtained per ejaculate was less ($P < 0.05$) for ejaculates collected during spring and winter when compared to those collected during summer. When data for the collection date were analyzed, volume of the ejaculates was less ($P < 0.05$) during the summer and winter when compared to ejaculates collected during the fall. Concentration of sperm in the ejaculates was not different throughout the year and the number of doses obtained per ejaculate was greater during ($P < 0.05$) the fall when compared to the rest of the year.

The interaction plots between season and moon phase at initiation of spermatogenesis and on day of collection for volume of ejaculate are depicted in Figs. 1 and 2, respectively. At the initiation of spermatogenesis, the ejaculate volume was greatest during the full and first quarter moon phases in the winter and throughout fall while there was the least volume of ejaculate during the summer with the new moon and full moon phases. When the day of collection was analyzed, the greatest ejaculate volume was obtained during fall when the

moon was in the last quarter phase and there were lesser volumes during winter when the moon was in its full phase.

For sperm concentration in ejaculates, the interaction between season and moon phase at the initiation of spermatogenesis, 45 days before the date of ejaculation are depicted in Fig. 3. Ejaculates that were collected 45 days after a first quarter moon phase during summer had the greatest concentration of sperm. Ejaculates collected 45 days after the moon was in the first quarter phase during the winter had the least concentration of sperm. Sperm concentrations were greater throughout summer than the other seasons.

The interaction of semen doses per ejaculate is depicted in Fig. 4 between moon phase and season at the initiation of spermatogenesis. There were more semen doses that could be used for AI in ejaculates obtained 45 days after a first quarter moon phase when semen collections occurred during the winter when the full moon phase was present. Ejaculates obtained during the spring had the least variation during all the moon phases.

The interaction is depicted in Fig. 5 between moon phase and season on the day of collection for number of semen doses that could be used for AI per ejaculate as the response variable at date of semen collection. The number of semen doses were the least that could be used for AI from ejaculates obtained during the fall when the moon was in the last quarter phase. Ejaculates obtained during the spring as compared with the other seasons of the year had the least variation for number of doses of semen per ejaculate that could be used for AI.

The least squares means for the different breeds of boars for the three response variables are included in Table 5. For both collection date and initiation of spermatogenesis the results follow the same trend. For volume of ejaculate, Yorkshire boars had the greatest value and Duroc boars had the least value. Interestingly, Duroc boars had the greatest

concentration of sperm/mL of ejaculate on both dates when the volume of the ejaculate was least, while Yorkshire boars had the least concentration/mL of ejaculate while having the greatest ejaculate volume. Concentrations of sperm/mL of ejaculate in Musclor boars did not differ in the ejaculates compared to Duroc boars. There were no significant differences among breeds for number of semen doses that could be used for AI that were produced per ejaculate at the collection date. The Heritage boars, however, had more ($P < 0.05$) doses of semen that could be used for AI when the date analyzed was that at the time of initiation of spermatogenesis.

Discussion

There have been very few reports on moon phase effects on domestic livestock reproduction. Previous research suggested a moon phase effect on mare fertility. Peaks both in mare mating (estrus) and fertility (% pregnancies) tended to synchronize on or just after the full moon phase (Kollerstrom, 2004). Kollerstrom also indicated that the magnitude of the effect appeared to depend on lunar latitude, disappearing almost completely during the eclipse seasons. Eclipse seasons are the only times during a year eclipses can occur, due to the moon's orbit. Each season lasts for approximately 34 days and there is repetition at slightly less than 6 months periods, thus there are always two full eclipse seasons each year (Littmann et al., 2008).

For this analysis, interactions between moon phase and season were significant for all the variables analyzed for both collection date and start of spermatogenesis. Because of the interactions, the individual effects should be considered but primary consideration should be given to the interactions between these effects. The significant interaction between moon

phase and season that appeared to exist from the data analyzed in the present study could represent the different intensity of the moon's effect during the year, caused by the change in the angle of the moon relative to the sun as the earth orbits around the sun. There have been other reports of lunar modulation effects that are likely to occur, or be more pronounced, during the eclipse season (Schneider, 1967).

Volume of ejaculate did not vary during different moon phases and this may be explained by the fact that spermatogenesis starts approximately 45 days prior to when the sperm that are produced can become a part of an ejaculate, however, seminal plasma that is produced can be a component of semen at any time after the time it is produced (Knox, 2003), thus the amount of seminal plasma is not affected by conditions 45 days before ejaculation. When measurements were on collection day, the largest ejaculate volume occurred during the first quarter moon phase and there was the least ejaculate volume during the full moon phase.

Season had a significant effect on the three response variables analyzed (Table 2). With change of seasons, there is an associated change in temperature and photoperiod (Kennedy and Wilkins, 1984), which greatly affects semen quality of boars. As a result of high ambient temperatures, there is an increase in the incidence of morphological defects, reduced sperm motility and overall lesser fertility when sperm from these ejaculates are used for AI (Flowers, 2015). During the summer and winter in the present study, there were the least volumes of ejaculates (Table 5). This finding is consistent with previous results where there was an assessment of 31 boars of different breeds (Knecht et al., 2014).

Previous research suggests that pigs have decreased fertility during the summer months due to the relatively greater temperatures, however, there could be other

environmental factors that influence the decreased fertility during the summer months. The ancestors of these boars that gave rise to the modern pig obviously perceived seasonal cues that resulted in seasonality of breeding patterns that were associated with a decreased testosterone content in blood and smaller testicles than during winter as compared with during the summer and early fall (Kozdrowski and Dubiel, 2004; Mauget and Boissin, 1987). There is also evidence that semen production and quality are greater during the fall and winter seasons in boars where semen is collected and used for AI and that this is due to the shorter light period of the days and the lesser ambient temperatures (Claus and Weiler, 1985; Sancho et al., 2004; Smital, 2009). This improvement in semen quantity and quality that is induced by the shorter light period of the days and lesser ambient temperatures is thought to be associated with a greater reproductive physiological activity of boars during the fall of the year (Smital, 2009). These environmental cues may be associated with a greater production of vitamin D which is an important factor in estrogen biosynthesis of both female and male gonads (Blomberg Jensen et al., 2011; Kinuta et al., 2000; Knecht et al., 2014). Even though boars are housed in modern boar stud facilities in commercial pork production enterprises and should not be affected by the varying photoperiod, temperature, and humidity conditions heat stress is a common factor influencing semen quantity and quality variables in boars (Flowers, 2015).

In the present study, there was not a significant effect of season on sperm concentration at day of collection. This finding may imply that with smaller ejaculate volumes there are fewer sperm and vice versa. In previous research, there were greater concentrations of sperm in ejaculates in the winter and fall than spring and summer (Ciereszko et al., 2000; Knecht et al., 2014; Zasiadczyk et al., 2015), however, other research

suggests that there is no consistent effect of photoperiod on spermatogenesis in boars (Flowers, 2015).

It is well known that breed has a significant effect on the semen quantity and sperm quality of boars used for AI (Flowers, 2015). The findings in the present study indicate there is a difference between volume of ejaculate, concentration of sperm per ejaculate and number of doses produced that could be used for AI per ejaculate among boars of different breeds at the approximate date of initiation of spermatogenesis and at the day of semen collection.

In conclusion, the results from the present study suggest that there may be an effect of the phase of the moon on livestock reproduction. The effect of season and moon phase on boar semen traits may be a remnant of the seasonal reproductive behavior in earlier evolutionary development of swine and should be investigated further to ascertain whether understanding phase of the moon effects on reproduction could be important for maximizing semen production and quality in modern swine production systems.

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Tables and figures

Table 4.1 *Mean monthly measurements of climatic data.*

Month	high T(C˚)[1]	low T(C˚)[2]	mean T(C˚)[3]	RH(%)[4]	THI[5]
January	-1.49	-11.4	-6.44	48.44	31.5
February	0.22	-10.38	-5.08	49.84	33.05
March	7.52	-3.49	2.01	53.11	41.31
April	13.51	1.67	7.59	55.05	48.73
May	13.28	1.79	7.53	58.13	49.16
June	27.83	15.92	21.87	64.38	68.72
July	28.19	16.6	22.39	63.33	69.49
August	28.00	16.61	22.31	65.67	69.54
September	25.77	13.13	19.45	62.42	65.27
October	16.65	4.22	10.43	55.94	52.6
November	8.87	-2.69	3.09	48.04	43.75
December	0.48	-7.72	-3.62	41.5	36.27

[1]Average greatest daily temperature in degrees Celsius

[2]Average least daily temperature in degrees Celsius

[3]Average daily temperature in degrees Celsius

[4]Relative humidity

[5]Temperature-humidity index

Table 4.2 *Results of analysis of variance for effects on volume of the ejaculate, concentration of sperm in the ejaculate and number of doses that could be used for AI obtained per ejaculate*

		Initiation of Spermatogenesis						Day of Collection					
		Volume (ml)[1]		Concentration ($x10^6$/ml)[2]		Doses (#)[3]		Volume(ml)		Concentration ($x10^6$/ml)		Doses (#)	
Effect	DF	*F* Value	*P* > F	*F* Value	*P* > F	*F* Value	*P* > F	*F* Value	*P* > F	*F* Value	*P* > F	*F* Value	Pr > F
Breed[4]	6	8.85	<.0001	8.83	<.0001	3.15	0.0066	9.20	<.0001	7.29	<.0001	2.46	0.0279
Season[5]	3	3.92	0.0083	7.25	<.0001	4.57	0.0034	6.62	0.0002	1.58	0.1924	9.48	<.0001
Moon Phase[6]	3	1.65	0.1747	1.41	0.2385	4.13	0.0062	4.19	0.0058	0.90	0.4417	9.09	<.0001
HighT	1	1.10	0.2938	8.28	0.004	1.39	0.2390	1.73	0.1886	4.26	0.0391	0.32	0.5706
LowT	1	0.01	0.9382	3.53	0.0603	0.37	0.5447	0.59	0.4420	4.56	0.0328	0.04	0.8442
RH	1	0.91	0.3411	12.10	0.0005	0.08	0.7839	0.47	0.4908	6.79	0.0092	0.01	0.9340
THI	1	1.20	0.2742	13.29	0.0003	0.96	0.3260	3.00	0.0832	8.21	0.0042	0.00	0.9727
season*moon	9	8.44	<.0001	8.77	<.0001	2.37	0.0115	4.15	<.0001	4.81	<.0001	4.16	<.0001

[1]Total volume (ml) of ejaculate collected

[2]Concentration of sperm in the non-diluted ejaculate collected

[3]Number of doses obtained from the ejaculate

[4]Breed of collected boar

[5]Season of year

[6]Moon phase on the day of collection of semen

Table 4.3 *Least Squares Means for the different moon phases for volume of the ejaculate, concentration of sperm in the ejaculate and number of doses obtained per ejaculate*

	Initiation of Spermatogenesis			Day of Collection		
Moon Phase	Volume (ml)	Concentration ($x10^6$/ml)	Doses (#)	Volume (ml)	Concentration ($x10^6$/ml)	Doses (#)
First [2]	235.03±7.46[a1]	372.77±14.14[a]	19.90±0.62[a]	238.91±7.83[a]	378.93±14.44[a]	19.38±0.62[bc]
Full [3]	232.75±7.55[a]	370.78±14.32[a]	19.35±0.63[ab]	228.57±7.85[b]	387.43±14.48[a]	18.78±0.63[c]
Last [4]	231.12±7.54[a]	378.29±14.29[a]	19.02±0.63[ab]	239.09±7.86[a]	389.34±14.51[a]	20.84±0.63[a]
New [5]	226.48±7.48[a]	386.84±14.18[a]	18.66±0.63[b]	234.09±7.97[a]	378.94±14.73[a]	20.02±0.64[ab]

[1]Different letters across the same column indicate significant differences ($P<0.05$)
[2]First quarter moon phase
[3]Full moon phase
[4]Fast quarter phase
[5]New moon phase

Table 4.4 Least Squares Means for the different seasons for volume of the ejaculate, concentration of sperm in the ejaculate and number of doses obtained per ejaculate

	Initiation of Spermatogenesis			Day of Collection		
Season	Volume (ml)	Concentration ($x10^6$/ml)	Doses (#)	Volume (ml)	Concentration ($x10^6$/ml)	Doses (#)
Spring	229.54 ± 8.42^{ab}	381.66 ± 16.10^{b}	18.60 ± 0.71^{b}	239.88 ± 9.36^{ab}	360.49 ± 17.46^{a}	18.55 ± 0.76^{b}
Summer	213.90 ± 9.29^{b}	427.80 ± 17.65^{a}	20.91 ± 0.79^{a}	221.87 ± 10.12^{b}	393.63 ± 19.01^{a}	19.96 ± 0.84^{b}
Fall	246.79 ± 10.05^{a1}	358.02 ± 19.35^{bc}	19.88 ± 0.87^{ab}	257.53 ± 11.06^{a}	410.56 ± 20.94^{a}	22.93 ± 0.93^{a}
Winter	235.16 ± 9.22^{ab}	341.19 ± 17.71^{c}	17.60 ± 0.79^{b}	221.39 ± 10.12^{b}	369.97 ± 18.99^{a}	17.57 ± 0.84^{b}

[1]Different letters within the same column indicate differences ($P<0.05$)

Table 4.5 Least Squares Means for the different breeds of boars for volume of the ejaculate, concentration of sperm in the ejaculate and number of doses obtained per ejaculate

	Initiation of Spermatogenesis			Day of Collection		
Breed	Volume (ml)	Concentration ($x10^6$/ml)	Doses (#)	Volume(ml)	Concentration ($x10^6$/ml)	Doses (#)
Musclor	227.96±13.86^{ab1}	435.37±26.11^{ab}	23.13±1.12^{a}	229.47±14.86^{b}	433.01±26.36^{ab}	23.18±1.13^{a}
Heritage [2]	194.67±21.90^{bc}	358.41±42.06^{bc}	16.14±1.89^{b}	206.35±23.01^{bc}	348.89±42.86^{bc}	15.64±1.92^{b}
Berkshire	228.28±10.58^{ab}	395.70±19.98^{b}	20.73±0.87^{ab}	237.06±11.44^{b}	395.95±20.25^{b}	20.79±0.87^{ab}
Chester W	225.47±26.03^{abc}	317.77±49.05^{bc}	15.60±2.11^{b}	254.41±27.85^{ab}	318.26±49.53^{bc}	15.68±2.10^{b}
Duroc	167.55±13.21^{c}	527.10±24.98^{a}	19.22±1.09^{ab}	154.66±14.40^{c}	527.56±2523^{a}	19.24±1.09^{ab}
Landrace	269.52±28.43^{a}	321.73±53.55^{bc}	19.79±2.31^{ab}	261.73±30.76^{ab}	319.75±54.06^{bc}	19.77±2.32^{ab}
Yorkshire	305.96±15.20^{a}	284.10±28.76^{c}	20.13±1.25^{ab}	298.90±16.24^{a}	286.03±29.09^{c}	20.12±1.26^{ab}

[1]Different letters within the same column indicate differences (P<0.05)

[2]Group of heritage breeds composed of Meishan, Mangalitsa and Gloucestershire Old Spots

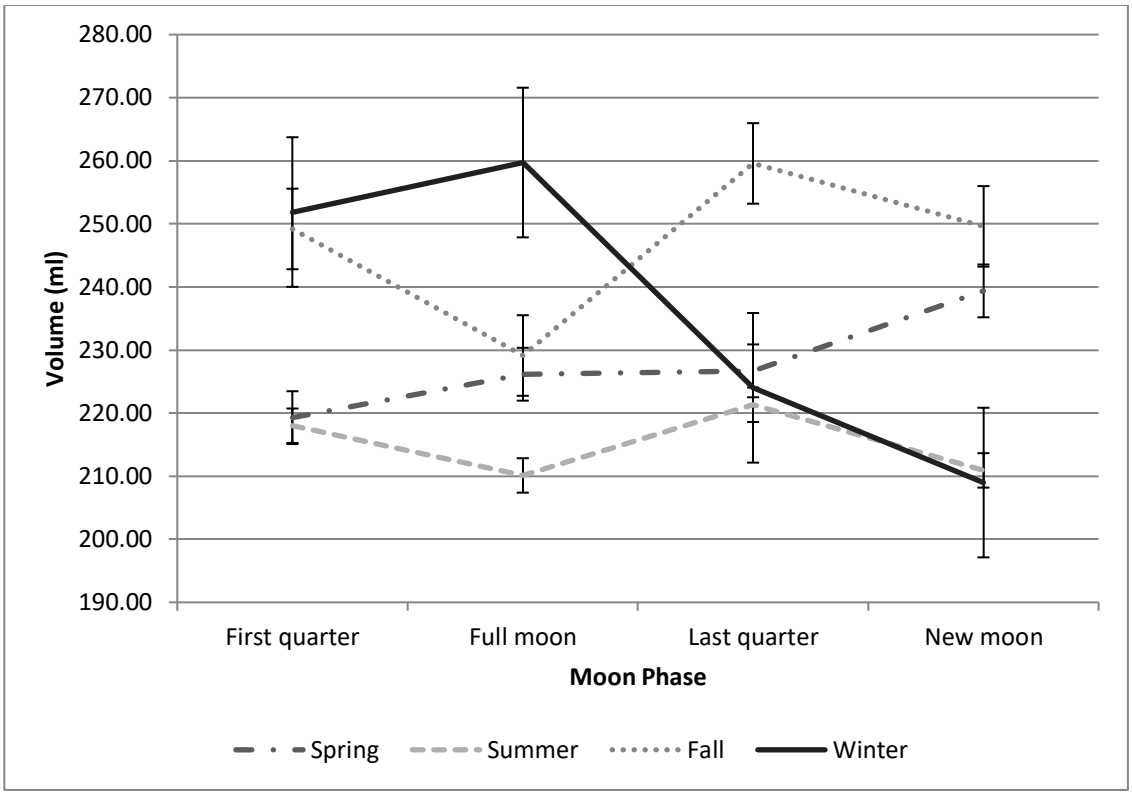

Figure 4.1**.** Interaction plot of season and moon phase at initiation of spermatogenesis for volume of ejaculate (ml). Non-overlapping error bars denote significance.

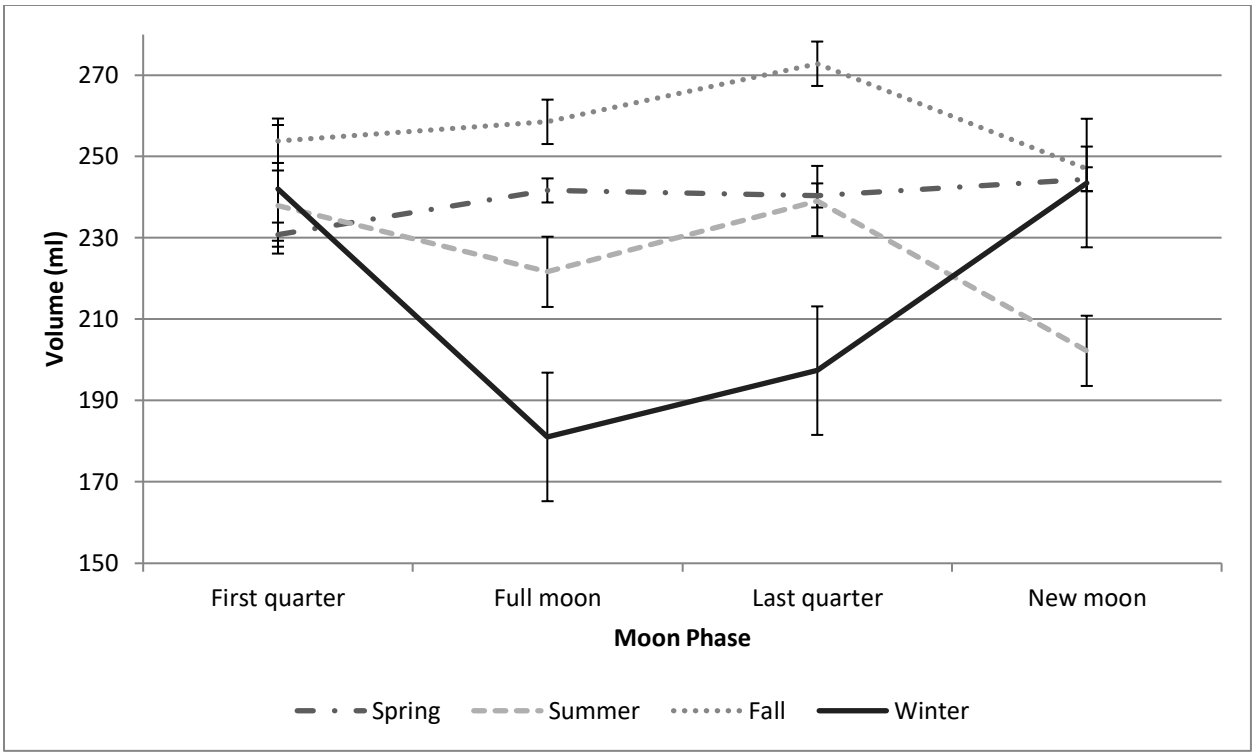

Figure 4.2 Interaction plot of season and moon phase at day of collection for volume of ejaculate (ml). Non-overlapping error bars denote significance.

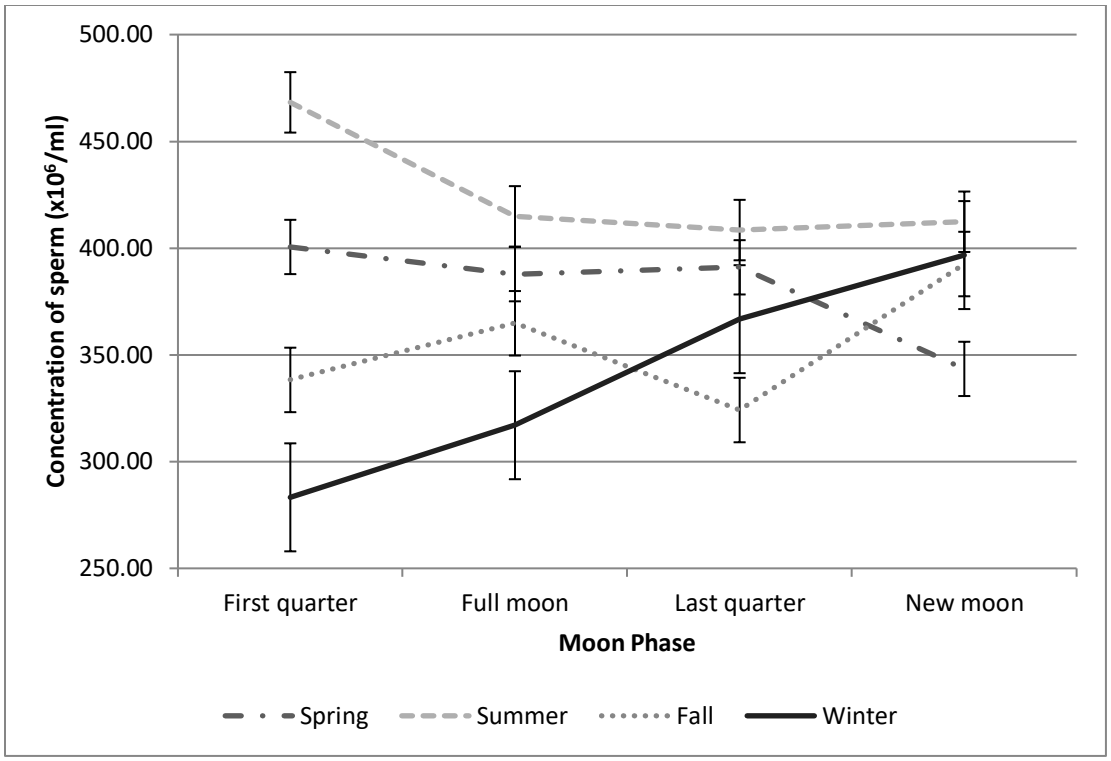

Figure 4.3 Interaction plot of season and moon phase at initiation of spermatogenesis for sperm concentration per ejaculate ($x10^6$/ml). Non-overlapping error bars denote significance.

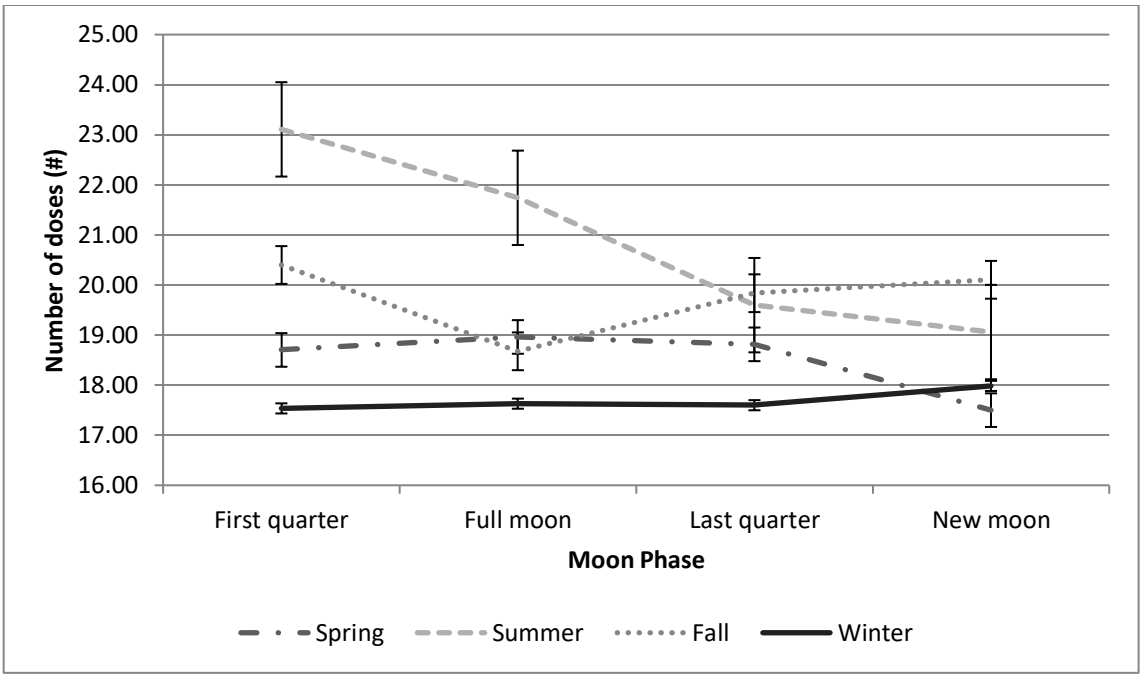

Figure 4.4 Interaction plot of season and moon phase at initiation of spermatogenesis date for number of doses obtained per ejaculate (#). Non-overlapping error bars denote significance.

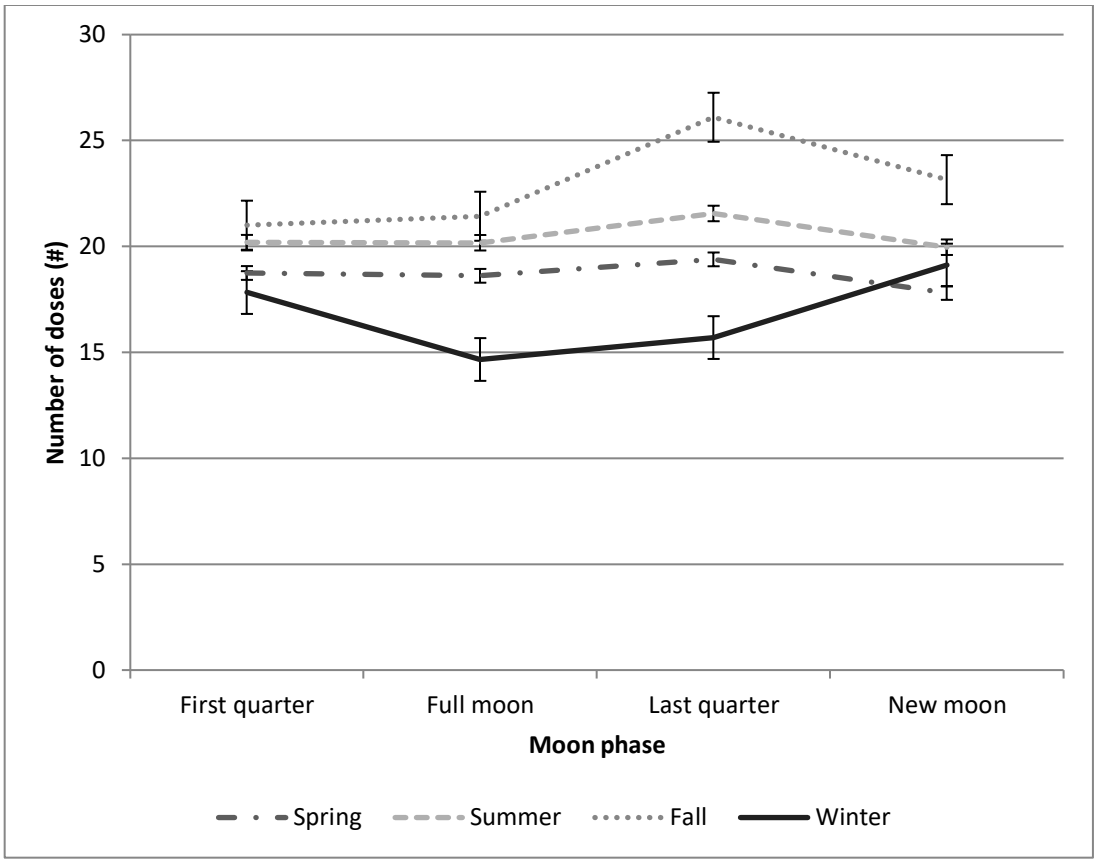

Figure 4.5. Interaction plot of season and moon phase at collection date for number of doses obtained per ejaculate (#). Non-overlapping error bars denote significance.

CHAPTER 5. PREDICTING LIVE WEIGHT OF RURAL AFRICAN GOATS USING BODY MEAUSREMENTS

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Abstract

The goal of the current study was to develop simple regression-based equations that allow small-scale producers to use simple body measurements to accurately predict live weight of typical African goats. The data used in this study were recorded in five African countries, and was composed of 814 individuals of 40 indigenous breeds or populations and crosses that included 158 males and 656 females. Records included the live weight measured with a hanging scale, linear body measurements, country, breed, owner, and age. Country, breed, age, chest girth, height at withers, body length, and shoulder width had large effects ($p<0.05$) on live weight. One linear model and two quadratic models were developed to predict weight from body measurements. The mean of the absolute value of the differences (mean absolute difference) between predicted and observed weights were compared to a standard body

measurement (BM) method live weight predictions. Based on the improved fit of the predictions, animals were divided into three chest girth classes. For the animals with chest girth of <55 cm the prediction model with linear terms for chest girth, body length, shoulder width and height at withers and chest girth and body length as a quadratic term was selected as the most accurate. For animals with chest girths of 56-75 cm and >76 cm, the prediction model selected that included linear terms for chest girth, body length, shoulder width and height at withers plus a quadratic term for chest girth was selected as the most accurate. When analyzed within country from Uganda and Zimbabwe, animals with chest girth < 55cm the linear model with additional quadratic terms for chest girth and body length was selected. For animals with chest girth 55-75cm the linear model with the added quadratic terms for chest girth and body length was selected for animals from Malawi and Zimbabwe while the linear model with a quadratic term for chest girth was selected for Mozambique, Tanzania and Uganda. For animals with chest girth of >76 cm the linear model with a quadratic term for chest girth was chosen for Tanzania, while for the other countries the linear model with quadratic terms for chest girth and body length was most accurate. In all cases, the models produced smaller mean prediction errors than the BM method.

Key Words: *Africa, body measurements, food security, goats, live weight, small ruminants*

Introduction

Livestock plays a key role in food security of smallholders in eastern and southern African countries. In these countries, the vast majority of people in rural areas, especially women, rely on investment in their livestock, with small ruminants serving as "current accounts" and larger species as "saving accounts" (Lebbie 2004). Of the small ruminants, goats represent an important livestock component across all agro-ecological zones in sub-Saharan Africa and goats exist in all production systems (Lebbie 2004). Native African breeds of livestock are known for their hardiness (Kouakou et al 2008), breeding capacity under harsh conditions (Simela and Merkel 2008), and most importantly their capacity to perform well under adverse conditions with minimal input of resources (Olivier et al 2002). While it is generally appreciated that they perform relatively well under harsh conditions, rural goat production in Africa faces challenges that include high disease and parasite prevalence, low levels of management, limited forage availability and poor marketing management (Gwaze et al 2009) that are responsible for poor overall productivity.

Of the 223 million goats in Sub Saharan Africa (SSA), about 64% are found in arid (38%) and semi-arid (26%) agro-ecological zones (Lebbie and Ramsay 1999) with more than 90% being owned by smallholder farmers (Gwaze et al 2009; Lebbie and Ramsay 1999). Marketing and trade of goats in Africa is based on subjective estimates of body weight because the access to livestock scales is very limited. Because of this, traders often underestimate body weight to lower the price of the animals, which adversely affects farmers because they receive less money for their animals than they are really worth (Walugembe et al 2014).

A key issue may arise when trying to use weight prediction methods developed for large breeds, or for animals managed in higher-input systems from the developed world. These weight equations may not be equivalent to smaller breed animals raised under poor management conditions. We hypothesized that African goats will rarely fit the growth curves used in developed countries to predict live weight and that these will prove inaccurate and often unreliable for conditions and breeds found in developing countries. Popular methods often used in developed countries like the BM method that uses the formula *body weight in pounds = ((chest girth inches^2 x body length inches)/300* (Horner 2013), or the caprine weight tape, which is a cloth measuring tape that is either designed for sewing or designed specifically as a goat weigh tape with predicted weights (based on chest girth) printed on the tape are likely to be inaccurate in developing countries. Conversion tables are available online for producers to predict body weights based on chest girth (Campbell 2014) and these will prove inaccurate and often unreliable for conditions and breeds found in developing countries. Based on this dilemma, the objective of this project was to develop a manual weight prediction method that can accurately be used on African goats.

Materials and Methods

The data used in this study were recorded in five African countries: Uganda, Zimbabwe, Tanzania, Malawi and Mozambique. The data set included 814 individuals of 40 indigenous breeds and crosses that consisted of 158 males and 656 females. Records included the live weight of the individual taken with portable, hanging (sling) scales, and body measurements were taken by cloth measuring tape. Chest girth was measured as body circumference at the heart, just behind the elbows. Height at the withers was measured at the highest point of back at the anterior thoracic spinal process between the lateral scapular

cartilage and measuring perpendicular from there to the ground)., body length was measured from point of shoulder (anterior point of humerus) to pin bone (ischiatic tuber) and pin width was the distance between left and right lateral ischiatic tuber bones. Shoulder width was the distance between the left and right lateral points of shoulder, (greater tubercles). Additionally, country, breed, owner and age were recorded. Age was determined by records where available, and otherwise was estimated. Sampling teams varied by country, or within country. All measurements were taken following approved animal care procedures using the AdaptMap Photo Protocol and Sampling Kit (USDA 2014).

After deleting incomplete observations, three additional observations considered statistical outliers were removed. Pearson correlation coefficients between the available body measurements and live weight were determined using Proc Corr (SAS 2013). A preliminary analysis of variance was performed using Proc GLM (SAS 2013) and the model for this initial analysis of variance (ANOVA) included the fixed effects of breed, country, age, and sex along with the five body measurements as covariates and the response variable was live weight.

Given that smallholders cannot use complicated models with fixed effects, an initial linear regression model utilizing the measurements for chest girth, height at withers, body length, and shoulder width were employed. Two additional models that included quadratic terms were also developed. The first one included the same linear terms as the basic model with the addition of chest girth as a quadratic term. The second quadratic model used the same terms as the basic linear model plus chest girth and body length as quadratic terms. All models are presented in Table 1.

Initially the entire data set was used as a whole, but as expected large prediction errors were obtained, and it was decided to divide the data in three categories according to chest girth. Chest girth was used because it showed the highest correlation to live weight, also, under field conditions chest girth is easier to measure, relative to the other manual measurements. Furthermore, under field conditions live weight of the animal to be weighed would be unknown. After trying different chest girth categories, the largest R^2 values were obtained when the categories used were chest girth under 55 cm, from 55 to 75 cm and over 76 cm.

Once the data were categorized, Proc Reg with the /p option (SAS, 2013) was used to calculate regression equations and the predicted live weight for each observation on each category. The absolute value of the residuals was averaged to calculate the average prediction error produced by each model for each category. Finally, the prediction errors produced by each model were compared to those produced by the BM method. Additionally, each category was separated by country and the data were analyzed in a similar manner with the objective of determining the model that was most appropriate for each country.

Results

Minimum, maximum range and average of live weight, age and the body measurements used in this studied are shown in Table 2. The results of the preliminary analysis of variance are shown in Table 3.

Country had an effect ($p<.0001$) on live weight along with breed and the covariates for chest girth, height at withers, body length and shoulder width. Age ($p= 0.008$) and the

covariate for pin bone width (p=0.016) were also associated with weight. Surprisingly, sex was not important (p=0.20).

The Pearson correlation coefficients are shown in Table 4. All the body measurements used in this analysis were found significantly correlated to live weight. Chest girth and body length were the body measurements that showed the highest correlation to live weight, with coefficients of 0.85 and 0.83, respectively. Pin bone width showed a correlation of 0.19, being the body measurement showing the lowest correlation to live weight. Pin bone width also showed the lowest correlations to other body measurements, having showed a correlation coefficient of 0.18 with both chest girth and height at withers. The highest correlation between body measurements was found for height at withers and body length with correlation coefficients of 0.78.

In Table 5 the R^2 and the mean difference between the recorded live weights and the predictions of each of the three models used for each chest girth category along with the differences for BM method are presented. The R^2 obtained for the linear models ranged for 0.61 to 0.74 and the category of chest girth under 55 cm showed the highest R^2 value. The model with the quadratic term for chest girth produced R^2 values ranging from 0.62 to 0.77, with the category of chest girth under 55 cm showing the highest value as well. The R^2 values obtained for the model that included the quadratic terms for chest girth and body length ranged from 0.63 to 0.80.

Interestingly, the model with the highest R^2 for each category was not the model with the smallest average absolute prediction error for each category. The live weight for observations with over 76 cm of chest girth was predicted most accurately by the model that included the quadric term for chest girth, this model produced a mean difference between

predicted and actual live weights of 3.14kg with an R^2 of 0.62 while the model that included quadratic terms for chest girth and body length produced a mean difference of 3.68 kg between predicted and actual live weights but produced a higher R^2 of 0.63. For the 55-75 cm of chest girth category, the highest R^2 was 0.73 and it was produced by both quadratic models. Also, both models produced a mean difference between predicted and actual live weight of 2.41kg. For the <55 cm of chest girth category, the model with quadratic terms for quadratic chest and body length had both the highest R^2 with 0.80 and the smallest mean difference between predicted and measured live weights. Finally, the BM method, originally developed for goats in the developed world, produced mean differences of 2.01, 2.63 and 3.7 for the categories of <55 cm, 55 to 75 cm and over 75 cm of chest girth, respectively

The R^2 and the mean difference between the recorded live weights and the predictions of each of the three models used for each chest girth category along with the BM method for each country are presented in Table 6. In all cases the mean absolute differences are smaller for the proposed prediction models than for the BM method. There were no observations from Malawi and Mozambique in the category of <55cm of chest girth while there was only one observation in this category for Tanzania, therefore these countries were excluded from this analysis. The linear model using additional quadratic terms for chest girth and body length showed the highest R^2 and the lowest mean average difference (MAD) for Uganda and Zimbabwe with R^2 values of 0.95 and 0.67 and MAD of 1.02 and 1.17, respectively. In the 55-75cm chest category, the model with additional quadratic chest and body length terms was the most accurate for Malawi and Zimbabwe producing R^2 of 0.86 and 0.91, respectively and MAD of 1.3kg for Malawi and 1.48kg for Zimbabwe. On contrary, the predictions for Mozambique were more accurate when the linear model included an additional quadratic

term only for chest girth and the R^2 for this model was 0.78 and the MAD was 2.00kg. For observations from Tanzania and Uganda, both models with additional quadratic terms were equally accurate, therefore the model with only one quadratic term was selected to simplify analyses for the producers with MADs of 2.00kg and 2.44kg, respectively. For the 76+cm category, the model with one quadratic term produced the best predictions for Tanzania with a MAD of 3.67kg. While the linear model with two additional quadratic term produced the lowest MAD for Malawi (2.22kg), Mozambique (1.94kg), Uganda (3.78kg) and Zimbabwe (1.97kg). . Overall, at least one of the models proposed in this study produced smaller mean differences between the predicted and recorded live weights than the BM method on a within country basis. The final overall models selected for each chest girth category with the coefficients for each measurement are shown in Table 7 and the models selected for each country are shown in Table 8.

Discussion

Goats represent an important livestock component across all agro-ecological zones in sub-Saharan Africa. Moreover, goats are found in all production systems ranging from pastoral and agro-pastoral systems through ranching range systems to small holder mixed-crop-livestock systems (Lebbie 2004). However, in countries like South Africa, 50% of the country's goat population are kept under small-scale conditions (Shabalala and Mosima 2002). Smallholder farming systems in developing countries are characterized by minimal resources in terms of land and capital, low income, poor food security, diversified agriculture and informal labor arrangements derived from family members (de Sherbinin et al2008).

Therefore, goats are an ideal vehicle for cash generation to improve food security and welfare among communal families (Gwaze et al 2009).

Three of the four fixed effects included in the preliminary analysis shown in Table 3 had significant effects on live weight of rural African goats. However, sex had no effect (p=0.20) and therefore it was decided that it was not needed to produce sex-specific models. Given that most comparisons of goats are within country and region when sold, it was decided to not correct the live weights recorded for the fixed effects of country, age and breed. Certainly, small holders could not make such adjustments so models including body measurements only were considered best.

The Pearson correlation coefficients shown in Table 4 are higher when compared to the ones obtained in a previous study that looked at the correlations between linear body measurements and body weight and how these changed with age and sex (Khan et al 2006). In that study, the body measurement with the highest overall correlation with body weight was body length for males older than 25 months old (r=0.82). This differs from the findings of the present study, where the highest correlation obtained was for chest girth (r=0.85). Our findings agree with those of a study on West African Dwarf (WAD) sheep (Sowande and Sobola 2008) that found chest girth had the highest correlation (r=0.94) with live weight. Furthermore, chest girth has been shown to be the body measurement with the highest correlation to live weight in other species like horses (Takaendengan et al 2012) and beef cattle (Ozkaya et al 2009). High correlations among body measurements as those found for body length and chest girth (r=0.76) and chest girth and height at withers (r =0.73) are similar to those obtained in a different study between chest girth and body length (r=0.74)

and chest girth and height at the withers (r=0.81) for animals 19-24 months old (Khan et al 2006).

The BM method mean differences (Tables 5 and 6) between predicted and measured live weight were compared to the models developed in here. The models developed for each specific country (Table 8) show higher R^2 and lower MADs than models shown in Table 7, probably because environmental and managemental conditions are different among each country and using only observations of the same country reduces the noise in the data due to breed and other factors. Even though we developed country-specific models (Table 8), the models shown in Table 7 are still useful and more accurate than the BM method and can provide a better estimation of live weight of the animals with chest girth under 55cm in Malawi, Mozambique and Tanzania, when environmental and managemental conditions are similar to those of Uganda and Zimbabwe.

Overall, the models developed in this study showed smaller mean differences between predicted and measured live weights than the BM method, which is widely used by dairy goat farmers in the United States as a simple way to predict live weight of animals when there is no access to a livestock scale. However, in rural Africa, poor access to scales to weigh animals at the time of sale may undervalue goats, and limit smallholder income due to the common practice of traders visually estimating body weight, and potentially underestimating it (Walugembe et al 2014). Moreover, another challenge faced by African producers is poor performance of animals due to poor nutrition (Lebbie 2004). In addition to poor nutrition and management, conformation and size differences between breeds from these diverse regions are also likely to exist. Therefore, the BM method may lose accuracy

when applied to goats from all over rural Africa where quadratic equations worked better at predicting live weights for these African goats.

Linear models using only the chest girth measurement have been used to predict weights of African goats (Alemu Yami et al 2009) and sheep (Sowande and Sobola 2008) previously. In the case of sheep, the linear models produced higher R^2 (0.91-0.94) than the quadratic models used in this study. However, other body measurements such as width of hindquarter were used. A valid option to improve the equations provided in this study may be to consider additional measurements that were not measured in this research such as head length, loin girth and width of hindquarters.

The full equations shown in Tables 7 and 8 could allow small producers in rural Africa to more accurately predict live weight of animals and potentially improve the income received at selling, as well as to potentially inform and improve management decisions about their animals that are based on body weight. It is important to note that the model selected for each category produced the smallest mean differences between live weight and predicted weight, independently of the R^2. However, for the animals in the category of chest girth measuring 55-75 cm, both quadratic models developed produced the same R^2 and the same mean differences, but the simpler quadratic model was chosen to simplify the usage for producers.

The goal of this research is to facilitate the accurate calculation of live weight of goats, as done in a similar study (Walugembe et al 2014). Such a calculation could be used in a cell phone application that will allow farmers to input their measurements and automatically apply these equations to predict the weights from simple body measurements. In case that there is no access to a calculator or a cellphone, another valid approach would be

to develop a measuring tape that uses the high correlation between chest girth and live weight found in this and other studies, to accurately predict chest girth to live weight and imprint the associated values directly on the cloth tape similar to current tools available and developed on large framed dairy breeds in developed countries.

Overall, this study confirms that using body measurements to predict live weight in goats is a valid strategy to improve the marketing management of rural African livestock producers.

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Tables

Table 5.1: Models used to predict live weights of live goats

Model name	Equation
The BM method*	LW = [(chest girth)^2 x length]/300
Linear model**	LW = b(chest girth)+b (body length)+b (shoulder width)+b (height)+e
Quadratic chest girth**	LW = b(chest girth)+b (body length)+b (shoulder width)+b (height) + b(chest girth)^2+ e
Quadratic chest girth, body length**	LW = b(chest girth)+b (body length)+b (shoulder width)+b (height)+e + b(chest girth)^2 +b(body length)^2 +e

LW = live weight
* English units – weights in pounds, lengths in inches
** Metric units – weights in kg, lengths in cm

Table 5.2 Descriptive statistics of biological parameters and body measurements

Measurement	Minimum	Maximum	Range	Average	S. D.*
Age (months)	3.60	180.00	176.40	38.80	25.49
Live weight (kg)	3.00	63.00	60.00	28.86	9.81
Pin bone width (cm)	2.00	18.50	16.50	7.77	4.03
Shoulder width (cm)	5.00	26.00	21.00	13.14	3.01
Chest girth (cm)	33.00	104.00	71.00	69.51	9.13
Body length (cm)	23.00	86.00	63.00	60.53	8.42
Height at withers (cm)	29.00	85.00	56.00	60.12	6.99

***Standard Deviation**

Table 5.3: Preliminary ANOVA[a] of several effects on live weight

Source	DF	Sum of Squares	Mean Squares	F Value	P Value
Country	4	952.59	238.15	20.77	<.0001
Age	21	462.00	22.00	1.92	0.008
Sex	1	19.03	19.03	1.66	0.20
Breed	38	2395.81	63.05	5.5	<.0001
Chest girth[b]	1	3050.13	3050.13	266.01	<.0001
Height at withers[b]	1	257.68	257.68	22.47	<.0001
Body length[b]	1	1325.81	1325.81	115.63	<.0001
Shoulder width[b]	1	375.74	375.74	32.77	<.0001
Pin bone width[b]	1	67.30	67.30	5.87	0.02
Error	742	8463.49	11.41		

[a]ANOVA = Analysis of Variance
[b]covariate

Table 5.4: Pearson correlation coefficients for live weight and body measurements of African goats all P<.0.0001

	Live weight	**Pin bone width**	**Shoulder width**	**Chest girth**	**Body length**	**Height at withers**
Live weight	1.00	0.19	0.54	0.85	0.83	0.77
Pin bone width	0.19	1.00	0.31	0.18	0.22	0.18
Shoulder width	0.54	0.31	1.00	0.55	0.50	0.45
Chest girth	0.85	0.18	0.55	1.00	0.76	0.73
Body length	0.83	0.22	0.50	0.76	1.00	0.78
Height at withers	0.77	0.18	0.45	0.73	0.78	1.00

Table 5.5: R^2 and mean absolute differences (MAD) between live and weight prediction models and The BM method

	Linear model*		Quadratic chest girth**		Quadratic chest girth and body length***		The BM method****	
Chest Girth (cm)	**R^2**	**MAD (kg)**	**R^2**	**MAD (kg)**	**R^2**	**MAD (kg)**	**R^2**	**MAD (kg)**
<55	0.74	1.54	0.77	1.45	**0.80**	**1.38**	0.21	2.01
55-75	0.72	2.46	**0.73**	**2.41**	0.73	2.41	0.59	2.63
76+	0.61	3.75	**0.67**	**3.14**	0.63	3.68	0.62	3.70

*Live Weight = b(chest girth)+b (body length)+b (shoulder width)+b (height)+e
** Live Weight = b(chest girth)+b (body length)+b (shoulder width)+b (height)+ b(chest girth)2 + e
*** Live Weight = b(chest girth)+b (body length)+b (shoulder width)+b (height)+e + b(chest girth)2+b(body length)2
**** Live Weight = [(chest girth)2 x length]/300

Table 5.6: Country-specific R^2 and mean absolute differences (MAD) between live and weight prediction models and the BM method.

Chest Girth (cm)	Country	N	Linear model*		Quadratic chest girth**		Quadratic chest girth and body length***		The BM method****	
			R^2	MAD (kg)	R^2	MAD (kg)	R^2	MAD (kg)	R^2	MAD (kg)
<55*****	**Uganda**	23	0.90	1.35	0.93	1.18	**0.95**	**1.02**	0.14	2.48
	Zimbabwe	23	0.65	1.20	0.65	1.18	**0.67**	**1.17**	0.42	1.59
55-75	**Malawi**	125	0.85	1.33	0.85	1.40	**0.86**	**1.31**	0.85	1.34
	Mozambique	85	0.77	2.08	**0.78**	**2.04**	0.78	2.06	0.68	2.07
	Tanzania	114	0.71	2.01	**0.71**	**2.00**	0.72	2.00	0.67	2.18
	Uganda	147	0.67	2.62	**0.69**	**2.44**	0.69	2.44	0.64	2.74
	Zimbabwe	83	0.90	1.50	0.91	1.50	**0.91**	**1.48**	0.83	1.99
76+	**Malawi**	55	0.68	2.28	0.70	2.23	**0.70**	**2.22**	0.67	2.27
	Mozambique	19	0.79	3.04	0.81	2.68	**0.90**	**1.94**	0.68	3.30
	Tanzania	34	0.81	3.74	**0.82**	**3.67**	0.82	3.73	0.71	4.53
	Uganda	93	0.64	3.88	0.66	3.79	**0.66**	**3.78**	0.49	4.60
	Zimbabwe	21	0.79	2.13	0.81	2.03	**0.82**	**1.97**	0.73	2.29

** Live Weight = b(chest girth)+b (body length)+b (shoulder width)+b (height)+ b(chest girth)^2 + e

*** Live Weight = b(chest girth)+b (body length)+b (shoulder width)+b (height)+ b(chest girth)^2+b(body length)^2 +e

**** Live Weight = [(chest girth)^2 x length]/300

***** Due to low numbers of observations for this category, only Uganda and Zimbabwe were analyzed

Table 5.7: Final models for live weight prediction of African goats for each category of chest girth

Chest Girth (cm)	Prediction Model
<55	LW = 16.3 - 0.17(chest girth) - 1.07(body length) - 0.139(shoulder width) + 0.3141(height)+ 0.0024(chest girth)^2 + 0.01671(body length)^2 +e
55-75	LW = 40.9 - 2.07(chest girth) + 0.286(body length) - 0.052(shoulder width) + 0.306(height)+ 0.020(chest girth)^2 + e
76+	LW = - 214 + 4.017(chest girth) + 0.69(body length) + 0.340(shoulder width) + 0.149(height) - 0.020(chest girth)^2 + e

Table 5.8: Final models for live weight prediction of African goats for each category of chest girth within country

Country	Chest Girth (cm)	Prediction Model
Malawi	**55-75**	LW = -2.97 + 0.108(chest girth) - 0.64(body length) + 0.007(shoulder width) + 0.17(height) + 0.004(chest girth)^2 + 0.008(body length)^2 + e
	76+	LW = -310.28 + 6.54 (chest girth) + 0.72(body length) + 0.11(shoulder width) + 0.21(height) - 0.036(chest girth)^2 - 0.002(shoulder width)^2 + e
Mozambique	**55-75**	LW = -14.69 - 36(chest girth) + 0.28(body length) + 0.004(shoulder width) + 0.072(height) + 0.008(chest girth)^2 + e
	76+	LW = 445.94 -10.04(chest girth) - 1.96(body length) + 0.32(shoulder width) + 0.64(height) + 0.04(chest girth)^2 + 0.02(body length)^2 + e
Tanzania	**55-75**	LW = -13.85 + 0.11(chest girth) + 0.26(body length) + 0.56(shoulder width) - 0.13(height) + 0.0047(chest girth)^2 + e
	76+	LW = -185.06 + 3.82(chest girth) + 0.66(body length) + 1.08(shoulder width) - 0.45(height) - 0.02(chest girth)^2 + e
Uganda	**<55**	LW = 15.00 - 0.49(chest girth) - 0.78(body length) - 0.05(shoulder width) + 0.35(height) + 0.005(chest girth)^2 + 0.014(body length)^2 + e
	55-75	LW = 103.81 - 3.96(chest girth) + 0.32(body length) + 0.15(shoulder width) + 0.24(height) + 0.03(chest girth)^2 + e
	76+	LW = -293.24 + 5.5(chest girth) + 1.08(body length) + 0.58(shoulder width) + 0.26(height) - 0.03(chest girth)^2 - 0.003(body length)^2 + e
Zimbabwe	**<55**	LW = 93.26 +2.9 (chest girth) - 7.83 (body length) + 0.57 (shoulder width) + 0.28 (height) - 0.02 (chest girth)^2 + 0.08(body length)^2 + e
	55-75	LW = 100.7 - 4.09(chest girth) + 0.59(body length) + 0.14(chest width) + 0.25(height) + 0.03(chest girth)^2 - 0.003(body length)^2 + e
	76+	LW = 135.15 - 9.05(chest girth) + 6.16(body length) + 0.30(shoulder width) - 0.004(height) + 0.06(chest girth)^2 - 0.04(body length)^2 + e

CHAPTER 6. GENERAL CONCLUSIONS

This thesis was undertaken to provide some insight into improving livestock production and food security through three manuscripts that describe an analysis of climatic effects such as heat stress on modern cattle reproduction, the exploration of new factors associated with maximizing the efficiency of intensive boar reproduction and the empowerment of small-stake holder farmers in developing countries to improve livestock marketing and food security.

The world, but especially developing countries, faces important challenges regarding food security in the 21st century. The global population is rapidly growing and becoming wealthier on average. One consequence of these changes is that the demand for land, and foods such as meat and dairy products is increasing (Rojas-Downing et al., 2017). While the demands for the planet's resources keep growing, the challenge of increasing production efficiency becomes more important.

The findings of the first manuscript suggest that climatic variables affect embryo production from the early activation period of ovarian follicular development and through the time of early embryonic development in cattle. When applied to a broader context, the influence of climatologic variables on livestock production should be carefully assessed to determine their importance and impacts on livestock production and reproduction. This will be more important as climatic pattern variability increases and efficient utilization of resources will be required. This is related to the findings of the second manuscript. In this project we studied the effects of climatic and lunar factors on boar ejaculate traits. The findings suggest that there may be an effect of the phase of the moon on livestock reproduction. The effect of season and moon phase on boar semen traits may be a remnant of

the seasonal reproductive behavior in earlier evolutionary development of swine and should be investigated further to ascertain whether understanding phase of the moon effects on reproduction could be important for maximizing semen production and quality in modern swine production systems. Following the example set by this research, new "out of the box" approaches should be taken to maximize productivity and efficiency of livestock production systems in the future.

The third and last manuscript aims to empower small-holder goat producers in rural Africa. Empowerment of small and rural producers in developing countries will grow in importance in the foreseeable future. Given the increasing pressure on natural resources and over population in developed countries; added to the fact that population is growing more rapidly in developing countries, it is noticeable that a transformation and improvement of livestock production systems in developing countries is needed. However, empowerment of producers is equally important to prevent their exploitation and even further impoverishment of the most vulnerable and food insecure sectors of society. This statement becomes more important when it is taken in consideration that women are often in charge of small ruminant systems in rural Africa and their input is key to the improvement of food security conditions.

Overall, this thesis provides insight on different alternatives to adapt and improve the efficiency and output of livestock production systems and empowering small livestock producers in rural Africa. With the aim of improving resilience in an era of rapid climatic changes, new phenotypes should be explored with the aim of finding possible phenotypes that can be selected for and improved. This being said, research should be made with the highly demanding environments and increasing demand for efficiency and sustainability that production systems will have to face in the near future.