PHENOTYPIC AND GENETIC RELATIONSHIPS BETWEEN DOCILITY AND REPRODUCTION IN ANGUS HEIFERS

by

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Abstract

This thesis includes two studies that assessed the relationships between docility and reproduction in Angus heifers, both from a phenotypic and genetic standpoint. The objective of the first study was to elucidate the phenotypic relationships between docility and first service AI conception rate in heifers. Data (n = 337) included exit velocity (EV), chute score (CS), fecal cortisol (FC), and blood serum cortisol (BC). Statistical analysis was done using logistic regression with 30 day pregnancy rate as the dependent variable. The model included the fixed effect of contemporary group, and the covariates FC, BC, EV, CS, weight, and age. Correlation coefficients were also calculated between all continuous traits. The power of our test could not detect any significant predictors of 30 d pregnancy for the combined data from all ranches. The objective of the second study was to determine the genetic control of docility and reproduction in heifers as measured by pregnancy rate. A subjective chute scoring system was used as the basis of their genetic evaluation for docility. Pedigree information was obtained on approximately 508,015 animals over 30 generations. Data included approximately 26,878 records on heifer pregnancy and 113,412 records on docility, with 7,849 animals having both docility and heifer pregnancy records. Contemporary groups were formed by the concatenation of weaning contemporary group, yearling contemporary group, and breeding contemporary group. Heritabilities were calculated from estimates of genetic and residual variance components computed using ASReml 3.0 (VSN International; Hemel Hempstead, UK). Heifer pregnancy variance components were estimated from a univariate, threshold model, with pregnancy outcome as the dependent variable. Animal and contemporary group were fit as a random effects, while age at first breeding was fit as a covariate. The heritability of heifer pregnancy was estimated to be 0.16 ± 0.02. Docility was fit as a univariate, linear animal model with docility score as the dependent variable. Animal and contemporary group were both modeled as random effects. The heritability for docility score was estimated to be 0.22 ± 0.03.

Fertility is a complex trait that is dependent on many factors; our data suggest that docility is one factor that warrants further investigation.

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Chapter 1 - Literature Review

Introduction

Reproductive success is an economically relevant trait in beef cattle operations, because the gross value of calves sold at weaning is influenced by the number of calves born. Income generated through the sale of weaned calves is often the source of a large portion of the operation's yearly income. For this reason, improvements in reproductive performance can be up to four times more important than improvements in end-product traits in a cow-calf operation selling market calves at weaning (Melton, 1995). It is often difficult, however, to select for fertility as it is a trait that is influenced by a variety of factors including species, breed, sex, and location (Martin et al., 1992; Patterson et al., 1992; Lopez et al., 2006).

The complex nature of fertility is not the only hindrance to genetic selection of the trait. Female fertility is not easily defined. There are many traits that are currently used to select for fertility in cattle operations, including conception rate, calving interval, number of insemination per conception, and many others. The binary nature of many of these traits, as well as the short controlled breeding season present in many cattle operations, complicate genetic analysis (Cammack et al., 2009). This is because binary reporting does not account for all the genetic variance between pregnant and open cows. Also, despite the fact that there are several measures of fertility, heritability estimates for most of these measures remain low, on average below 5 percent because of the influence of environmental and management effects (Tiezzi et al., 2011). There is evidence that heritability is higher if selection shifts to traits that are more representative of the cow physiology, such as selecting on days to first heat as opposed to days to first service. Correlations between fertility and production traits (such as the relationship between milk yield and fertility in dairy cattle) are also generally negative, which further complicates selection for reproductive efficiency (Tiezzi et al., 2011).

It has become increasingly evident that temperament is one of the factors affecting fertility that requires further investigation. Researchers report that physiological responses associated with temperament can influence the probability of cows becoming pregnant during the breeding season (Cooke et al., 2009). Stress hormones such as cortisol present in the

bloodstream can negatively affect the release of vital reproductive hormones (Cooke et al., 2009).

Presumably, human handling of cattle during typical management practices such as vaccination or artificial insemination is associated with short term changes in circulating concentrations of cortisol and other stress hormones. Blood serum sampling may provide insight into acute stressors, while fecal sampling may be reflective of longer-term or chronic stress (Huber et al., 2003).

The following review describes tools for genetic selection of fertility, specifically heifer pregnancy. It also describes temperament in beef cattle and specific ways to measure temperament in an individual. Temperament effects on circulating hormones will also be discussed. Finally, this review will discuss how differences in temperament and fluctuations of cortisol effect circulating reproductive hormones and subsequent pregnancy in cattle.

Heifer Pregnancy as a Tool for Genetic Selection of Fertility

There are several commonly utilized measures of female fertility, however, for the purposes of this review we will focus on measures of heifer fertility; specifically age at puberty, heifer pregnancy, and first service conception rate. Heifer pregnancy is economically relevant to a beef cow operation because replacement heifers require a great deal of time and resources. Therefore, having heifers bred and calved by two years of age contributes to the economic success of a cattle operation (Cammack et al., 2009).

Success of any replacement heifer program is largely dependent on inherent fertility and reproductive efficiency (Buskirk et al., 1995). Reproductively efficient heifers tend to reach puberty earlier, and therefore can potentially conceive earlier in the breeding season. Puberty in heifers is influenced by many factors, including birth weight, nutritional status, and breed (Martin et al., 1992). Most heifers have the potential to reach puberty and breed as a yearling if they are provided with good nutrition and management (Martin et al., 1992). Costs of developing heifers may vary among breed and as well as within a breed. Heifers with inherent ability to reach puberty at early ages may reach puberty and breed at a lesser cost than heifers with later inherent ages at puberty (Martin et al., 1992). Age at puberty has an estimated heritability of 0.10 to 0.67 (Cammack et al., 2009). It should be noted that overall, age at puberty has a heritability estimate much higher than the other female reproductive traits. Heifers

sired by breeds with large mature size (Charolais, Chianina) tend to be older at puberty than heifers sired by breeds with smaller mature size (Hereford, Angus) (Martin et al., 1992). Correlations between age at puberty and mature size are 0.57 in Bos Taurus and 0.25 in Bos Indicus cattle (Martin et al., 1992). Breeds selected for milk production reach puberty earlier than breeds of similar mature size and retail product than breeds not selected for milk (Simmental, Holstein, and Gelbvieh vs. Charolais) (Gregory et al., 1991). This is supported by the fact that the correlation between milk yield and age at pregnancy is -0.87 in Bos Taurus breeds (Martin et al., 1992). Breed differences in age at pregnancy and subsequent reproduction can be attributed to the additive effects of genes present at diverse frequencies between breeds. The reason this occurs is because when breeds are isolated from each other they tend to diverge in frequency for genes that affect the expression of certain traits (Martin et al., 1992).

Age at puberty is a robust measure of inherent fertility because it is immune from interactions with other traits that have a tendency to affect fertility later in life (Martin et al., 1992). Milking ability, for example, is unlikely to affect age at puberty because at the time of puberty the female has yet to lactate. A drawback to age at puberty as an indicator of fertility is that it can be difficult to observe in field populations. Puberty is typically defined as the time at which a heifer has exhibited 2 luteal phase progesterone values above 1 ng/mL when the samples have been collected three to four days apart (Day and Anderson, 1998: Lopez et al., 2006; Shirley et al., 2006). Determining these hormone levels requires frequent blood sampling and laboratory analyses, so this process is rarely done in an actual production setting. Age at puberty can influence subsequent reproductive trait performance (Cammack et al., 2009). Laster et al., in 1979 showed correlations among breed means for age at puberty with percentage calving in the first 25 days of the season of -0.75, and for age at puberty with pregnancy percentage of -0.42. The meaning of this research is that earlier age at puberty resulted in earlier and more numerous pregnancies.

In a study done by Gregory et al., (1992) the correlation between age at puberty and pregnancy rate was -0.79 among purebred cows (study included Angus, Braunvieh, Charloais, Gelbvieh, Hereford, Limousin, Pinzgauer, Red Poll, and Simmental purebreds) . Heifer pregnancy rate is a measure of reproduction indicative of sexual maturity and therefore is often included in the breeding objectives of cattle operations (Cammack et al., 2009). Heifer pregnancy can be defined as the probability of an exposed heifer being pregnant by the end of

the yearling breeding season (Eler et al., 2002) and remaining pregnant to palpation (approx. 120 days post breeding Evans et al., 1999). This means that a heifer became pubertal and pregnant at 12 to 15 months of age so as to calve by 24 months of age (Eler et al., 2002). Similar to cow pregnancy, heifer pregnancy is a binary trait with a score of one indicating a pregnant heifer and zero indicating an open heifer. Estimated heritability of this trait has been found to be 0.14 to 0.21 (Cammack et al., 2009). A later age at first calving is associated with a decrease in lifetime productivity of beef cows (Wiltbank et al., 1985, Nunez-Domingues et al., 1991, Guiterrez et al., 2002). This is supported by the genetic correlation between yearling pregnancy rate and lifetime pregnancy rate reported as 0.92 (Morris and Cullen, 1994) and 0.97 (Mwansa et al., 2000). A study by Minick Bormann et al., (2006) showed that estimated breeding values on percentage of daughters pregnant ranged from -0.02 to 0.05 for sires of beef heifers. This range of breeding values indicates that although heritability is low, genetic progress in fertility can be made by selection on heifer pregnancy rate.

First service conception rate is similar to pregnancy rate. It is defined as success or failure for becoming pregnant from the first AI (Minick Bormann et al., 2006). This trait has an advantage over pregnancy rate because it separates heifers that become pregnant on the first breeding from those that take many breedings to become pregnant. This is economically relevant for cattle in a production setting because of the cost of semen and labor involved in breeding for multiple artificial inseminations. Heifers that get pregnant on the first breeding will calve earlier, giving them better chances to breed back as 2 year olds. Heritability for first service conception rate in crossbred cattle has been estimated at 0.22 (Dearborn et al., 1973). Heifers that conceive early in their first breeding season have greater lifetime productivity than their counterparts that conceive later in their first breeding season (Lesmeister et al., 1973). A study by French et al., (2005) reported that females that conceived to AI as yearlings had greater lifetime weight weaned, calves weaned, calf weaning weight, and revenue than did females that conceived to natural service. Another study by Cushman et al., (2013) found that heifers that calved early in their first calving season had increased longevity and kilograms weaned than heifers that calved later in the calving season.

Temperament in Beef Cattle

Temperament in cattle has become an increasingly relevant topic for producers in the beef and dairy industries. An aggressive animal can cause problems during handling, which puts both the animal's and the stockperson's safety at risk (Grandin, 1989). Producers selecting cattle for more docile dispositions may decrease risk of accident for handlers, wear on facilities, as well as increase the animal's welfare. Studies show an unfavorable relationship between poor temperament and productivity, making docility an economically relevant trait (Beckman et al., 2005). Cattle in feedlots with calm temperaments were also found to have higher average daily gains when compared to cattle with excitable temperaments (Voisinet et al., 1997). Furthermore, more docile cattle are more likely to reach upper two thirds choice or a higher quality grade than nervous or aggressive steers (Busby et al., 2009). Conversely, nervous to aggressive steers were more likely than docile cattle to reach the lower quality grades of select and standard (Busby et al., 2009). Busby et al., (2009) studied the effect of disposition on feedlot performance and carcass quality grade and reported that overall, docile calves returned $62.19 per head more than aggressive calves. In another portion of industry, dairy cows with calmer temperaments had 25-30 percent increases in milk production (Drugociu et al., 1977).

Temperament in cattle has been defined as the reaction of cattle to handling by humans (Burrow et al., 1997). There are numerous measures of temperament, including flight speed (Burrow et al., 1988) docility test (LeNeindre et al., 1995; Grignard et al., 2001) crush test (Tulloh 1961; Grignard et al., 2001) and handling test (Bovin et al., 1994). Efficient temperament scoring systems must reflect typical handling practices on an operation and be simple as well as inexpensive to implement (Beckman et al., 2007).

Flight speed objectively measures the time taken in hundredths of a second for an animal to pass through two light beams separated by a distance of 1.7 m after leaving a weight crush or chute (Burrow et al., 1988). The system incorporates two light beams focused on infra-red reflectors which trigger an on/off mechanism as the light beams are broken. The correlation of flight speed and flight distance found by Burrow et. al., in 1988 was -0.45 ($P < 0.001$), which indicates that the fastest animals in terms of flight speed were also the least approachable in terms of distance. In other words, a faster flight speed reflects poorer temperaments in cattle and slower flight speeds indicate calmer temperaments (Burrow, 1997). For this reason, flight speed may be reflective of intrinsic fearfulness (Petherick et al., 2002). The heritability of flight speed

was found to be 0.40 in a tropical breed of beef cattle (Burrow, 2001). It should be noted that both data and pedigree files for this study were relatively small and included animals of two different but very similar composite breeds. Heritability of flight speed tends to vary with age, being high at weaning (0.54) and more moderate (0.26) at 18 months of age (Burrow et al., 1988). There was also no significant sex effect found at weaning, but became more prevalent ($P < 0.01$) at 18 months of age with bulls being found more temperamental than females (Burrow et al., 1988).

Other common methods to measure temperament include the docility test and the very similar handling test, which measure total time in locomotion and changes in mobility in an animal, along with their aggressiveness toward humans (Beckman et al., 2007). Using the handling test, Bovin et al., (1994) found significant sire effects that influenced aggressiveness toward humans in Limousin heifers ($P < 0.05$), indicating that genetic selection could promote improvement in the trait. Beef Improvement Federation (BIF) guidelines describe a temperament scoring system that has been adapted by breed associations for genetic evaluation of docility in cattle (Beckman, et al., 2007). Although subjective, BIF guidelines for docility include many aspects seen in other tests. These include general behavior in a chute (i. e. crush test, Tulloh, 1961), rate at which a calf exits the chute (slow vs. fast), vocalization (Watts et al., 2001) and aggressiveness toward humans (docility test, social separation test Muller and von Keyserlingk, 2005). The score is standardized to correct for subjectivity (Beckman et al., 2007). The chute scoring system ranges from one to six. An animal scored as a one will have a mild disposition, be gentle, and will handle quietly. They will exit the chute calmly. An animal scored as a two will be somewhat restless in the chute. They will be quieter than average, but may be stubborn during processing with some tail flicking and will exit the chute promptly. An animal scored as a three (average) will be manageable, but nervous and impatient. They will be in constant movement, continuously pushing and pulling on the head-gate, and will exit the chute briskly. A four will be flighty and somewhat wild. They will be jumpy, out of control and struggle violently in the chute with continuous tail flicking. When penned individually, they may frantically run the fence line and possibly jump. They will exhibit long flight distance and exit the chute wildly. A score five will be similar to a score four, but with added aggressive behavior. This includes extreme agitation, with continuous movement that may involve jumping and bellowing while in the chute. They will exit the chute frantically and may exhibit attack

behavior when handled alone. A score six will be extremely aggressive with pronounced attack behavior (Busby, 2009). A one or two score indicates highly acceptable behavior, with a three being average, and fours, fives and sixes deemed as unacceptable (Beckman et al., 2005).

Docility is analyzed as a threshold trait due to its categorical nature. A threshold analysis assumes that the trait of interest (observed categorical trait) is influenced by an underlying variable (not observed) that follows a normal distribution such that when the unobservable normal variable crosses a threshold it causes a change in the observable character (Gianola and Foulley, 1983). Heritability for docility by the North American Limousin Foundation (NALF) and the American Angus Association (AAA) are 0.40 and 0.37, respectively. It should be noted that pen scores, chute scores, and exit velocity are all positively correlated with each other and are all reliable measures of temperament (Curley, 2006). Australian work concluded temperament is highly repeatable, and changes little over time (Petherick, 2002).

Studies have shown that selection for cattle with a more favorable docility (chute) score would be effective in producing cattle with more acceptable dispositions (Beckman et al., 2005). Docility as measured by chute score has been found to be moderately heritable (Shrode and Hammack, 1971; Stricklin et al., 1980; Fordyce et al., 1988), with direct and maternal heritabilities being 0.37 ± 0.03 and 0.04 ± 0.01, respectively (Beckman et al., 2005). Some breeds have produced EPD rankings for docility. The docility EPD reflects the probability that the offspring will inherit genes for acceptable behavior, with a greater docility EPD associated with progeny exhibiting calmer behavior (Beckman et al., 2007). The first national genetic evaluation of docility in beef cattle was published by NALF in 1998 (NALF, 2004). In the spring of 2008 AAA released a docility EPD sire listing with their National Cattle Evaluation (Northcutt, 2007).

Temperament in cattle has been known to elicit changes in circulating hormone levels. Excitable Brahman heifers were shown to have significantly greater cortisol concentrations than calm heifers (Stahringer et al., 1990). Cattle with ‘calm’ temperaments have been shown to have lower cortisol and epinephrine serum concentrations (ng/mL respectively) compared to animals classified as ‘temperamental’ prior to shipment, at arrival and after 70 d on feed at a commercial feed yard (Curley et al., 2006). Temperament classification was based on observed exit velocity (EV, m/s) prior to shipment. Pen scores ($r = 0.29$, $P < 0.05$), exit velocity ($r = 0.26$, $P < 0.05$),

and chute scores, were all found to be positively correlated with cortisol levels (Curley, 2006; Cooke et al., 2009). Because of their corresponding with responsiveness to stress from a hormonal standpoint, these measures are a reliable tool for the assessment of cattle temperament and are a possible indicator of temperament through an animal's lifetime (Curley, 2006).

Stress can be defined as normal deviations from homeostasis, whereas distress pushes the body so far away from homeostasis that the body is more significantly taxed in its attempts to bring itself to a steady state (Lay et al., 2001). Exposure to stress in an animal activates the hypothalamic pituitary adrenal axis (HPA). This response to a stressor causes the release of corticotropic releasing hormone (CRH) which acts on the anterior pituitary to synthesize and release adrenocorticotropic hormone (ACTH) which in turn is released into the peripheral circulation to cause the release of glucocorticoids from the adrenal cortex. These act to increase the amount of glucose available in the body by breaking down glycogen, protein, and fat (Lay et al., 2001). This type of sympathoadrenal response, more commonly known as a "fight or flight" response, enables an animal to act immediately to a stressor. The activation of neurons in the hypothalamus causes the release of epinephrine from the adrenal medulla to increase heart rate, glucose availability, as well as blood pressure and volume. Blood is redirected away from non-essential organs (reproductive and gastrointestinal) toward the heart and striated muscles so that the animal may respond by fighting or fleeing the threat (Lay et al., 2001).

Temperament and Circulating Stress Hormone Effects on Reproductive Hormones

Stress is revealed by the inability of an animal to cope with its environment, a phenomenon that is often reflected in a failure to achieve genetic potential (Dobson et al., 2000). Researchers report that physiological responses associated with temperament can influence the probability of cows becoming pregnant during the breeding season (Cooke et al., 2009). Specifically, activation of both the HPA and the sympatho-adrenomedullary systems can be responsible for stress induced infertility in cattle (Goldstein 1987; Rivier and Rivest 1991). Studies in dairy cows show that stressors such as milk fever or lameness can increase the calving to conception interval by 13-14 days, and an extra 0.5 inseminations on average are required per conception (Dobson et al., 2000). This suggests that a variety of endocrine regulatory points exist were stress can limit the efficiency of reproduction. The specific effects of stress on

reproduction depend on the timing of stress in relation to the stage of the estrous cycle, the genetic predisposition for stress, and the type of stress (Madej et al., 2005).

Endocrine evidence shows that stressors can interfere with precise timings of reproductive hormone release within the follicular phase (Dobson et al., 2000). Luteinizing hormone (LH) acts to integrate the function of the hypothalamus and the function of the gonads (as reviewed in Chrousos et al., 1998). Activation of CRH secreting neurons in the paraventricular nucleus of the hypothalamus in response to a perceived distress can decrease LH secretion by directly inhibiting the release of GnRH from the ventro-medial nucleus of the hypothalamus (Chrousos, 1997). Reduction in LH and GnRH levels deprives the follicle of adequate gonadotropin support, leading to reduced estradiol production by follicles (Dobson et al., 2000). In some situations of chronic stress, the pulsatile GnRH/LH frequency will be so slow that initial follicular growth will occur but will be unable to continue into the later stages of growth that depend on faster pulse frequencies (Dobson et al., 2000). In less stressful situations, GnRH/LH frequency may be just fast enough to support follicular growth, but because frequency is still slow follicular growth will be susceptible to interruption (Dobson et al., 2000). Because of the change in hormone levels, the integrity of granulosa cells and the oocyte may be compromised so even if fertilization occurs, the conceptus will fail to develop into a pregnancy (Dobson et al., 2000). In other cases, slow LH frequency can get a follicle to later stages of growth but may not be enough to support ovulation, in which case the follicle becomes cystic (Dobson et al., 2000). Cortisol can also delay or block the preovulatory LH and FSH surges (Breen et al., 2005). Breen et al., (2005) found that cortisol significantly suppressed LH pulse frequency by as much as 35 percent, thus attenuating the high frequency LH pulses typical of the preovulatory period. Also, it has been found that in ovariectomized ewes, cortisol suppresses pulsatile LH secretion by inhibiting pituitary responsiveness to GnRH (Breen et al., 2004).

Other stress hormones can also have an effect on LH release, as it has been found that the cumulative LH response in intact heifers was reduced ($P < 0.05$) by ACTH treatment (Li et al., 1983). Giving ACTH during estrus can elevate concentrations of cortisol and progesterone as well as change the intraluminal environment, including exaggerated amounts of mucus in the utero-tubal-junction and isthmus (Einarsson et al., 2008). Treatment with ACTH after ovulation has also been shown to reduce numbers of spermatozoa at the zona pellucida and retard cleavage

rate of fertilized ova (Einarsson et al., 2008). Sows that were treated with ACTH also had fewer oocytes/embryos recovered than a control group (Einarsson et al., 2008).

Negative effects of cortisol on LH pulse frequency can also cause unfavorable effects on other reproductive hormones. At the level of the ovary, LH enhances estradiol secretion by stimulating thecal cell synthesis of androgens, which are then converted to estradiol by granulosa cells under the influence of FSH (Gore-Langton et al., 1994). Glucocorticoid receptors have been identified in granulosa cells of the rat (Schreiber et al., 1982) and glucocorticoids have been found to reduce the responsiveness of human and rat granulosa cells to gonadotropic hormones (Hsueh et al., 1978, Michael et al., 1993). Either impaired ovarian responsiveness to FSH or reduced FSH secretion would attenuate LH-stimulated estradiol synthesis and inhibit the preovulatory estradiol rise (Breen et al., 2005). Cortisol has also been found to interfere specifically with the timing of the follicular phase estradiol rise, either by preventing it or delaying the estradiol peak by as much as 20 hours (Breen et al., 2005).

Regardless of the specific mechanism of action, stress during proestrus has been shown to prolong estrus and disturb follicular growth and ovulation (Einarsson et al., 2008). Thus, measures of reproductive hormones and glucocorticoids can be employed to assess the degree of distress imposed by a set of environmental cues in an animal (Lay et al., 2001).

Cortisol Metabolites in Feces as an Indicator of Stress

Testing levels of stress hormones in animals may be difficult, as seasonal variation, sex differences, and invasive sample collection may confound glucocorticoid measures as indices of stress (Huber et al., 2003). Sample collection may itself induce corticosteroid secretion (in the blood) and interfere with the adrenocortical response that is under investigation (Reinhardt et al., 1990, Le Maho et al., 1992). Non-invasive methods such as fecal samples, however, can measure fluctuating blood concentrations of glucocorticoids during the previous one to two days (Monfort et al., 1998; Palme et al., 1999).

Fecal Sample Collection and Storage

Method of sample collection and storage is important to ensure reliable results with respect to quality of glucocorticoid measurements in feces (Keay et al., 2006). Improper handling and storage techniques can potentially artificially alter the samples and subsequent data

(Keay et al., 2006). Several different methods can be used to secure sample quality, although a significant number of studies do not indicate collection and storage methods.

Samples are generally taken from the animal with a nylon-covered hand and put into a plastic bag (Isobe et al., 2005) or plastic tubes (Huber et al., 2003). There is a possibility of uneven distribution of corticosteroid metabolites in the feces, so upon collection samples can be thoroughly mixed prior to collection of a subsample in order to retain unbiased results (Millspaugh et al., 2003). After collection, samples can be placed in a cooler with ice and transported to a lab to be frozen at -20˚C or can be immediately frozen at -20˚C (Huber et al., 2003, Millspaugh et al., 2002).

Without proper storage after collection, naturally occurring bacteria and their enzymes can degrade steroid hormone metabolites in the feces in a matter of hours (Mostl et al., 2002, Wasser et al., 1988). For this reason, care must be taken to prevent bacterial growth. This can be done by freezing the sample as discussed previously, or by adding preservatives such as sodium azide or ethanol. Immediately freezing samples in liquid nitrogen also works to prevent bacterial growth; however, availability of liquid nitrogen makes this technique impractical for field use (Creel et al., 1997, Wasser et al., 1988). Oven drying has also been used in some studies, but again availability of an oven makes this difficult for use in the field (Brockman et al., 1996).

Extraction of fecal samples in the field and storage in subzero temperatures can eliminate the need for a storage preservative, however this method does require procuring a freezer capable of maintaining a temperature of -20˚C at the location of collection (Lynch et al., 2003, Stavisky et al., 1995, Strier et al., 1999). The predominate method of storage used in the literature appears to be storing samples in a preservative of ethanol or methanol at -20˚C, although studies ranged in temperature from -10˚C to -20˚C (Beehner et al., 2004, Johnson et al., 1991).

Sample Preparation and Extraction

There are a wide variety of techniques used to prepare and extract steroid hormones from a fecal sample before quantifying the amount of glucocorticoid within the sample. Specific preparation for extraction depends largely on the type of preservation and storage of the samples.

Samples stored with preservatives such as ethanol are prepared for extraction by evaporating off the ethanol in a fume hood at room temperature either overnight or one to three days prior to extraction (Khan et al., 2006, Lynch et al., 2003, Wasser et al., 2004). Samples are

then freeze dried in a lyophilizer, which removes all the moisture from a sample while also keeping it constantly refrigerated (Goymann et al., 1999, Khan et al., 2006, Lynch et al., 2003, Wasser et al., 1997). Freeze-dried samples are then generally pulverized using a mortar or other similar techniques and then sifted to remove any vegetation and other debris (Goymann et al., 1999, Hunt et al., 2004, Lynch et al., 2003, Wasser et al., 2004).

Most extraction methods involve at least one step using a 90 percent methanol solution as the primary extraction agent, with many studies using a combination of 0.2g of feces and 2ml of methanol (Goymann et al., 1999, Hunt et al., 2004, Lynch et al., 2003, Wasser et al., 2004). Other studies used a combination of methanol and acetone (8:2, 100% methanol) (Beehner et al.,2004, Stavisky et al., 2001). Extractions using methanol involve vortexing the mixture of feces and methanol, usually for 30 minutes. Vortexing is followed by centrifugation from anywhere from 10 to 20 minutes (Goymann et al., 1999, Hunt et al., 2004, Lynch et al., 2003, Wasser et al., 2004). Some techniques exclude centrifugation after vortexing (Wasser et al., 2004). Some studies boiled the sample with ethanol prior to vortexing and centrifugation (Graham et al., 1996, Wasser et al., 2000).

Another method to store fecal samples is to freeze them without a preservative. The extraction method for these samples is also known as a "wet extraction", and uses a modified phosphate buffer (0.1 M, pH 7.0, 0.1% bovine serum albumin with 5% or 0.05% Tween-20 and 20% methanol) for extraction (Bardi et al., 2003, Barrett et al., 2002).

Field extractions are also plausible, but it is necessary to have a battery powered homogenizer that homogenizes samples of feces combined with methanol and acetone (8:2) at the time of collection. These samples are then capped and stored at ambient temperature for 10 hours or less. Following homogenization, samples are passed through a polytetrafluoroethylene syringeless filter. A methanol/acetone solution is used to wash the filtrate. Filtrate that has been diluted with distilled water is then loaded onto a cartridge that has been primed with 100% methanol and distilled water. To reduce steroid sample degradation, the cartridge is washed with sodium azide (0.1%) and placed in a bag with silica beads. All steps listed above can be performed in the field (with proper equipment) and the final product can be stored at ambient temperatures for up to 40 days until the sample can be shipped to a lab for assay (Beehner et al., 2004).

Fecal Glucocorticoid Assays

There are as many ways to quantify glucocorticoid metabolites in an extracted fecal sample as there are to store, prepare and extract the sample. High pressure liquid chromatography (HPLC) is a method that can be useful in separating fecal glucocorticoids (cortisol and corticosterone) and their metabolites in an accurate fashion (Turner et al., 2002). The metabolites can then be tested for immunoreactivity (Wasser et al., 2000). The results of the immunoreactivity tests can be used to select either a radioimmunoassay (RIA), enzyme immunoassay (EIA) or a fluoroimmunoassay test to measure for corticosteroids and their metabolites (Barrett et al., 2002, Whitten et al., 2008). Any immunoassay based tests using antibodies do not enable the researcher to specifically identify and quantify all the individual metabolites in a sample. The downside of using HPLC to identify metabolites is that it involves large and costly equipment, which often makes this type of identification very difficult in the field. Samples must therefore be properly preserved and shipped to a lab.

Variation exists between species in the type of glucocorticoid metabolites secreted in the feces. It is necessary, then, that emphasis is placed on the selection of proper antibody for use in immunoassay tests (Wasser et al., 2000). Many antibodies that can be chosen have cross-reactivity for other steroids or steroid metabolites in a sample (Wasser et al., 2000, Wasser et al., 1994, while others may be more specific for a steroid hormone and have little cross reactivity with other hormones (Goymann et al., 1999). Cross reactivity of an immunoassay should be considered when making conclusions about a specific glucocorticoid or metabolite evaluated in a species because may be measuring additional corticosteroids and metabolites.

Radioimmunoassay and enzyme immunoassay also require reagents that require storage in refrigerators, and both require centrifugation and vortexing, which can be difficult in the field without proper facilities. RIA also requires licensing for use. In spunoas0 1 seT4(il)-3(it)-3(i1T1 0ba)4(e)4(nks

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2.2%. Fertility is a complex trait that is dependent on many factors; our data suggest that docility is one factor that warrants further investigation.

Introduction

Reproductive success is relevant in beef cattle operations because the value of calves sold at weaning is influenced by the number of calves born. Income generated by the sale of calves is often a large portion of an operation's income. It is difficult to select for fertility as it is influenced by a variety of factors (Cammack et al., 2009). Temperament is a factor that requires further investigation. Physiological responses associated with temperament can influence the probability of cows becoming pregnant, as stress hormones in the bloodstream can negatively affect the release of reproductive hormones (Cooke et al., 2009). Differences in concentrations of circulating stress hormones have been associated with differences in cattle temperament (Curley et al., 2006). Cattle with 'calm' temperaments had lesser serum cortisol and epinephrine concentrations than animals classified as 'temperamental' at a commercial feed yard (Curley et al., 2006).

Methods have been developed to assess temperament in cattle. Exit velocity (EV) measures the time it takes for an animal to cover a predetermined distance after vacating the chute (Burrow et. al., 1988). Chute scores (CS) range from one (quiet) to six (aggressive) and are based on the animal's behavior when confined in a chute (Curley et. al., 2006). Positive correlations of CS and EV with cortisol indicate that both scores are reliable indicators of temperament (Cooke et al., 2009).

Handling of cattle is associated with changes in concentrations of stress hormones. Blood serum collection can provide insight into acute stressors (Curley et al., 2006). Fecal sampling can be reflective of chronic stress experienced 2-3 d before sampling (Huber et al., 2003).

The objective of this study is to elucidate relationships between docility and pregnancy rate. It is hypothesized that differences in temperament scores and associated cortisol levels of heifers are associated with differences in pregnancy rate, which was tested by the logistic regression of pregnancy rate on our various measures of temperament and stress hormone.

Materials and Methods

Data Collection

This research was conducted according to protocol number 3156 approved by the Institutional Animal Care and Use Committee at Kansas State University. Data for this project was collected from three different cooperator herds, two of which were affiliated with Kansas State University. Ranches 1 and 2 were located in the Flint Hills of north-eastern Kansas, with ranch 3 being located in central Kansas. A total of 337 yearling heifers were used in this study.

Ranch 1 (n = 117) heifers were synchronized using a combined melengestrol acetate(MGA)/prostaglandin (PG)/gonadotropin-releasing hormone (Cystorelin®, Merial, Duluth, GA) synchronization protocol. MGA was fed at 0.5 mg per head per d for 14 d. On d 33 (19 d following the final feeding of MGA) 5 ml of Lutalyse® (Zoetis, Florham Park, New Jersey) was injected, EV and CS were recorded, and fecal samples were collected for cortisol analysis. Heifers were then visually detected for standing estrous for 2 d and bred 10-14 h after observed standing estrous. On d three after Lutalyse® injection (d 36) all females not previously detected in heat were injected with 2 ml of Cystorelin®, and inseminated. Blood samples were collected for cortisol analysis following insemination. Females were exposed to natural service sires on d 37. Transrectal Ultrasoundography was used to determine pregnancy at 30 d. Ultrasonography was conducted by a trained technician.

Ranches 2 (n = 133) and 3 (n = 87) employed identical CoSynch-CIDR protocols to synchronize their heifers. EAZI-BREED™ CIDRs® (Zoetis, Florham Park, New Jersey) were inserted at d 0, in addition to a 2 ml injection of Cystorelin®. Exit velocity and CS were recorded at this time, and fecal samples were collected for cortisol analysis. EAZI-BREED™ CIDRs® were then removed on d 7, and a 2 ml injection of Lutalyse® was given. On d 9 the heifers were given a second 2 ml injection of Cystorelin®, and inseminated. Blood samples were also collected for cortisol analysis at this time. For an unrelated study, heifers were divided into three different groups for target insemination; right uterine horn, left uterine horn, and uterine body. Heifers were also divided into two different groups post-breeding, with the test group receiving a Banamine injection 14 d after breeding. These groups are accounted for by separate contemporary groups in the data. Transrectal-ultrasonography was used to determine pregnancy at 30d.

Exit velocity is an objective measure of temperament that records the time taken in hundredths of a second for an animal to pass through two light beams separated by a distance of 1.7 m after leaving a squeeze chute (Burrow et al., 1988). The system incorporates two light beams focused on infrared receivers which trigger an on/off mechanism as the light beams are broken.

Chute score is a subjective measure of temperament recommended by the Beef Improvement Federation (BIF) to aid in genetic improvement of docility (BIF, 2010). Chute scores range from one to six; a one representing calm, docile behavior, while a six represents aggressive, unacceptable behavior. An animal scored as a one will have a mild disposition, will handle quietly, and will exit the chute calmly. An animal scored as a two will be somewhat restless in the chute, but will be quieter than average. The animal may be stubborn during processing with some tail flicking and will exit the chute promptly. An animal scored as a three, which is average, will be manageable but impatient. They will continuously push and pull on the head-gate, and will exit the chute briskly. A four will be flighty and slightly wild. They will be jumpy and struggle violently in the chute with continuous tail flicking. They will exit the chute wildly. A score five will resemble a score four, but with increased aggressive behavior. This includes extreme agitation, and continuous movement that may involve jumping and bellowing while in the chute. They will exit the chute frantically and may exhibit attack behavior when handled alone. A score six will be extremely aggressive with pronounced attack behavior (Busby, 2009). A one or two score indicates highly acceptable behavior, with a three being average, and fours, fives and sixes deemed as unacceptable (Beckman et al., 2005). Chute scores for this study were taken by two separate evaluators and then averaged before data analysis.

Fecal samples were taken (d 0) while the animal was in the chute to avoid contamination. Samples were stored in individual containers on ice until they could be delivered to the lab and frozen at -20° C. Blood samples were collected via venipuncture into 15ml Vacutainer tubes with 18 gauge x 1.5 inch needles at breeding. Samples were immediately put on ice until they could be transported to the lab, where they were refrigerated for at least 8 h before centrifugation.

Laboratory analysis

Refrigerated blood samples were centrifuged at 2,400 x g for 20 min at 4°C. Plasma was stored at -20° C until assayed. Plasma concentrations of cortisol were determined using a radioimmunoassay kit specific to bovine serum (Coat-A-Count Cortisol, Siemens Medical

Solutions Diagnostics, Malvern, PA). The average intra and interassay CV were 12% and 3.5%, respectively.

Quantification of fecal corticosterone levels was modeled after protocols outlined by Huber et al., (2006). Concentrations of fecal corticosterone were determined using a commercial radioimmunoassay kit (MP Biomedicals, Solon, OH) validated for use on bovine samples July, 2012. For extraction, 0.5 g of thawed fecal matter was placed into a 15 ml centrifuge tubes. 4.5 ml of 80% methanol was added, and the tubes were placed in a lab rack vortexer for 40 min. Following vortexing, tubes were centrifuged at 3,000 x g for 15 min. The amount of corticosterone in the supernatant was determined by the I_{25}-Corticosterone RIA. The average intra and interassay CV were 3.5% and 5.5%, respectively.

Statistical Analysis

Statistical analysis for this study was performed with SAS (SAS Institute; Cary, NC). Logistic regression was used to determine the factors that influenced pregnancy rate. Contemporary group was fit as a fixed effect, while fecal cortisol (FC), blood cortisol (BC), EV, and CS, weight, and age were all included as covariates.

Contemporary group was based on ranch, horn/body target breeding treatment, and Banamine treatment. Correlation coefficients were also calculated between FC, BC, EV, CS, weight, and age using the MANOVA procedure. Data from all locations was analyzed jointly, as well as separated by ranch, due to the differences in ambient temperature and drought conditions at each location.

Results and Discussion

Summary Statistics

Table 1 contains means, standard deviations, minimums, and maximums for FC, BC, EV, CS, weight, and age for the data from all ranches combined.

Table 2 shows means, standard deviations, minimums, and maximums for FC, BC, EV, CS, weight, and age for the 117 heifers at ranch 1. Pregnancy percentage, defined here as the number of heifers pregnant at 30 d after breeding, was 60.87% for this ranch. Fecal glucocorticoid levels for ranch 1 were notably greater than the average of all ranches by 23.16 ng/0.5g fecal. Blood cortisol, alternatively, was lesser on average than the overall by 5.97 ng/ml.

Average EV was also lower than the group average by 0.32 m/s. The chute score average for ranch 1 was greater than the overall average by 0.32. Weight for the ranch 1 heifers was greater than average by 16.79 kg. Heifers on ranch 1 were also older than the overall average by 6.9 d.

Table 3 shows summary statistics for the 133 heifers at ranch 2. Pregnancy percentage at 30 d after insemination was 34.59%. Fecal corticosterone levels for ranch 2 were lesser than the group average by 27.32ng/0.5g fecal. Blood cortisol levels were also lesser than average by 1.85 ng/ml. The average EV of heifers at ranch 2 was higher than average, but not notably so with only a 0.05 m/s difference. Average chute score was less for ranch 2 by 0.37. Heifers at ranch 2 were lighter than average by 23.99 kg. Ranch 2 heifers were also 2.08 d older than the group average.

Summary statistics for the 87 heifers at ranch 3 can be found in Table 4. Pregnancy percentage 30 d after insemination was 50% for this group. Average FC levels for this location were greater than the group average by 6.36ng/0.5g fecal. Blood cortisol levels were less than average by 1.85 ng/ml. The average EV was greater than the group mean by 0.38 m/s. Chute score for this ranch was greater than average by 0.13. Heifers at this ranch were heavier by an average of 14.29 kg, and were 12.38 d younger than the group average.

Predictors of 30 day pregnancy

The power of our test could not detect any significant predictors of 30 d pregnancy with odds ratio confidence intervals different than 1 for ranches 2, 3, and the combined data (see Tables 5-8). However, CS ($P < 0.0348$) and weight ($P < 0.0082$) were both found to have odds ratio estimates different than 1 as significant predictors of 30 d pregnancy for ranch 1. The odds ratio estimate for CS has a significant interpretation, meaning that a 1 unit increase in average CS will reduce the probability of pregnancy at ranch 1 by 48.1%. Therefore, poorer temperament as indicated by increasing CS was associated with a decreased probability of becoming pregnant. This is consistent with the findings of Cooke et al., 2009 who reported that physiological responses associated with temperament can influence the probability of cows becoming pregnant during the breeding season. The odds ratio estimate for weight is somewhat more difficult to interpret, as a 1lb increase in weight will decrease the probability of pregnancy by 2.2%. In contrast to expectations, an increase in heifer weight at breeding was associated with a decrease in the probability in becoming pregnant.

Correlations

Tables 9-12 contain correlation coefficients between variables for the combined data as well as the individual ranches. A positive correlation between FC and age ($P < 0.0003$) was found for ranch 1, meaning that as age increased so did fecal corticosterone concentration. Fecal cortisol positively correlated with BC at ranch 3 ($P < 0.0109$), meaning that as FC concentrations increased so did BC concentrations.

Blood cortisol positively correlated with EV for the combined data ($P < 0.0001$), and for ranch 2 ($P < 0.0062$). This means that as BC increased, EV also increased. This is consistent with the findings of Curley 2006 and Cooke et al., 2009. Blood cortisol negatively correlated with age for the combined data ($P < 0.0369$) and for ranch 2 ($P < 0.0327$). In other words, as BC increased, age seemed to decrease, meaning younger animals tended to have higher BC concentrations.

Exit velocity positively correlated with CS for the combined data ($P < 0.0001$), ranch 1 ($P < 0.0302$), and for ranch 2 ($P < 0.0001$). This correlation is intuitive, meaning that as EV increased for an animal, average CS increased as well. This is consistent with a study done by Curley in 2006, who found that EV and CS were positively correlated. Exit velocity was negatively correlated with both weight and age for both the combined data ($P < 0.0084$) ($P < 0.0321$) and for ranch 2 ($P < 0.0001$) ($P < 0.0061$). This inverse relationship suggests that as EV went up, both weight and age decreased.

Average CS was found to be negatively correlated with age for the combined data ($P < 0.0127$). According to this result, older animals would have lower average CS than younger animals.

Weight positively correlated with age for the combined data ($P < 0.0001$), ranch 1 ($P < 0.0001$), ranch 2 ($P < 0.0001$), and ranch 3 ($P < 0.0002$). This result is obvious, meaning that weight increased steadily with age.

Conclusions

Although the results from our combined data were inconclusive for predictors of 30 d pregnancy, results from ranch 1 and the amount of variation in measures of temperament and reproductive status at all locations showed there is improvement to be made in these traits. It is

obvious that the interactions between temperament and reproductive success merit further investigation and could prove conclusive with a data set of ample size.

Figures and Tables

Table 2.1 Summary statistics for combined data of all ranches

Variable	N	Mean	SD	Minimum	Maximum
Fecal Cortisol, ng/0.5g	333	119.53	34.54	15.80	315.00
Blood Cortisol, ng/ml	336	40.96	21.85	4.45	113.50
Exit Velocity, m/s	329	1.89	0.77	0.23	7.32
Chute Score[1]	337	1.87	0.74	1.00	4.00
Weight, kg	336	763.64	77.84	510.00	964.00
Age, d	336	413.25	17.19	359.00	464.00

[1]Chute Scores (1-6) were assigned by trained observers using the standardized scoring method recommended by the Beef Improvement Federation.

Table 2.2 Summary statistics for ranch 1

Variable	N	Mean	SD	Minimum	Maximum
Fecal Cortisol, ng/0.5g	115	142.69	32.24	99.19	315.00
Blood Cortisol, ng/ml	117	34.99	20.59	4.45	94.12
Exit Velocity, m/s	116	1.57	0.38	0.23	3.04
Chute Score[1]	117	2.19	0.70	1.00	4.00
Weight, kg	116	780.43	62.94	584.00	922.00
Age, d	116	420.15	14.54	395.00	464.00

[1]Chute Scores (1-6) were assigned by trained observers using the standardized scoring method recommended by the Beef Improvement Federation.

Table 2.3 Summary statistics for ranch 2

Variable	N	Mean	SD	Minimum	Maximum
Fecal Cortisol, ng/0.5g	132	92.21	25.18	53.57	174.44
Blood Cortisol, ng/ml	132	39.11	21.71	5.88	107.89
Exit Velocity, m/s	130	1.94	0.77	0.36	4.85
Chute Score[1]	133	1.50	0.59	1.00	3.00
Weight, kg	133	739.65	84.46	510.00	960.00
Age, d	133	415.33	15.35	359.00	437.00

[1]Chute Scores (1-6) were assigned by trained observers using the standardized scoring method recommended by the Beef Improvement Federation.

Table 2.4 Summary statistics for ranch 3

Variable	N	Mean	SD	Minimum	Maximum
Fecal Cortisol, ng/0.5g	86	125.89	24.55	15.80	217.48
Blood Cortisol, ng/ml	87	51.79	19.94	11.55	113.50
Exit Velocity, m/s	83	2.27	0.89	0.77	7.32
Chute Score[1]	87	2.00	0.75	1.00	4.00
Weight, kg	87	777.93	76.69	632.00	964.00
Age, d	87	400.87	16.75	361.00	439.00

[1]Chute Scores (1-6) were assigned by trained observers using the standardized scoring method recommended by the Beef Improvement Federation.

Table 2.5 Odds ratio estimates (ORE), confidence limits, and P-value for the logistic regression of 30 d pregnancy on fecal cortisol, blood cortisol, exit velocity, average chute score, weight, and age for all data

Variable	ORE	Confidence Limits		P-value
Fecal Cortisol, ng/0.5g	1.006	0.998	1.015	0.1451
Blood Cortisol, ng/ml	1.007	0.995	1.018	0.2379
Exit Velocity, m/s	0.949	0.677	1.332	0.7639
Chute Score[1]	0.706	0.494	1.009	0.0560
Weight, kg	0.993	0.986	1.001	0.0724
Age, d	1.001	0.984	1.017	0.9316

[1]Chute Scores (1-6) were assigned by trained observers using the standardized scoring method recommended by the Beef Improvement Federation.

Table 2.6 Odds ratio estimates (ORE), confidence limits, and P-value for the logistic regression of 30 d pregnancy on fecal cortisol, blood cortisol, exit velocity, average chute score, weight, and age for ranch 1

Variable	ORE	Confidence Limits		P-value
Fecal Cortisol, ng/0.5g	1.009	0.995	1.023	0.2059
Blood Cortisol, ng/ml	1.005	0.985	1.025	0.6399
Exit Velocity, m/s	2.499	0.976	6.398	0.0562
Chute Score[1]	0.519	0.283	0.954	0.0348
Weight, kg	0.978	0.962	0.994	0.0082
Age, d	1.016	0.983	1.049	0.3471

[1]Chute Scores (1-6) were assigned by trained observers using the standardized scoring method recommended by the Beef Improvement Federation.

Table 2.7 Odds ratio estimates (ORE), confidence limits, and P-value for the logistic regression of 30 d pregnancy on fecal cortisol, blood cortisol, exit velocity, average chute score, weight, and age for ranch 2

Variable	ORE	Confidence Limits		P-value
Fecal Cortisol, ng/0.5g	1.002	0.987	1.018	0.7509
Blood Cortisol, ng/ml	1.005	0.987	1.023	0.6032
Exit Velocity, m/s	0.786	0.441	1.400	0.4131
Chute Score[1]	0.609	0.301	1.232	0.1675
Weight, kg	0.998	0.983	1.006	0.3874
Age, d	0.996	0.968	1.024	0.7681

[1]Chute Scores (1-6) were assigned by trained observers using the standardized scoring method recommended by the Beef Improvement Federation.

Table 2.8 Odds ratio estimates (ORE), confidence limits, and P-value for the logistic regression of 30 d pregnancy on fecal cortisol, blood cortisol, exit velocity, average chute score, weight, and age for ranch 3

Variable	ORE	Confidence Limits		P-value
Fecal Cortisol, ng/0.5g	1.005	0.985	1.024	0.6478
Blood Cortisol, ng/ml	1.010	0.986	1.034	0.4136
Exit Velocity, m/s	0.821	0.482	1.396	0.4660
Chute Score[1]	0.933	0.496	1.753	0.8286
Weight, kg	1.000	0.986	1.014	0.9950
Age, d	0.983	0.952	1.014	0.2727

[1]Chute Scores (1-6) were assigned by trained observers using the standardized scoring method recommended by the Beef Improvement Federation.

Table 2.9 Correlation Coefficients (with P-values below) between fecal cortisol (FC), blood cortisol (BC), exit velocity (EV), average chute score (CS), weight, and age for data from all ranches

	FC	BC	EV	CS	Weight	Age
FC, ng/0.5g	1.00	-0.01	0.01	-0.05	-0.04	0.09
		0.83	0.86	0.42	0.49	0.09
BC, ng/ml		1.00	0.22	0.09	-0.09	-0.12
			0.00	0.11	0.11	0.04
EV, m/s			1.00	0.24	-0.15	-0.12
				0.00	0.01	0.03
CS[1]				1.00	-0.08	-0.14
					0.16	0.01
Weight, kg					1.00	0.42
						0.00
Age, d						1.00

[1]Chute Scores (1-6) were assigned by trained observers using the standardized scoring method recommended by the Beef Improvement Federation.

Table 2.10 Correlation Coefficients (with P-values below) between fecal cortisol (FC), blood cortisol (BC), exit velocity (EV), average chute score (CS), weight, and age for data from ranch 1

	FC	BC	EV	CS	Weight	Age
FC, ng/0.5g	1.00	-0.12	-0.07	-0.02	0.01	0.34
		0.19	0.46	0.86	0.89	0.00
BC, ng/ml		1.00	0.26	0.03	0.04	-0.02
			0.01	0.78	0.65	0.79
EV, m/s			1.00	0.20	0.07	0.16
				0.03	0.46	0.09
CS[1]				1.00	-0.06	-0.08
					0.55	0.42
Weight, kg					1.00	0.36
						0.00
Age, d						1.00

[1]Chute Scores (1-6) were assigned by trained observers using the standardized scoring method recommended by the Beef Improvement Federation.

Table 2.11 Correlation Coefficients (with P-values below) between fecal cortisol (FC), blood cortisol (BC), exit velocity (EV), average chute score (CS), weight, and age for data from ranch 2

	FC	BC	EV	CS	Weight	Age
FC, ng/0.5g	1.00	-0.05	-0.02	-0.07	-0.09	0.01
		0.59	0.86	0.41	0.30	0.89
BC, ng/ml		1.00	0.24	0.09	-0.23	-0.19
			0.01	0.28	0.01	0.03
EV, m/s			1.00	0.41	-0.34	-0.24
				0.00	0.00	0.01
CS[1]				1.00	-0.11	-0.15
					0.23	0.10
Weight, kg					1.00	0.47
						0.00
Age, d						1.00

[1]Chute Scores (1-6) were assigned by trained observers using the standardized scoring method recommended by the Beef Improvement Federation.

Table 2.12 Correlation Coefficients (with P-values below) between fecal cortisol (FC), blood cortisol (BC), exit velocity (EV), average chute score (CS), weight, and age for data from ranch 3

	FC	BC	EV	CS	Weight	Age
FC, ng/0.5g	1.00	0.28	0.12	-0.10	-0.07	-0.17
		0.01	0.28	0.37	0.55	0.13
BC, ng/ml		1.00	0.17	0.18	-0.00	-0.12
			0.13	0.10	0.98	0.28
EV, m/s			1.00	0.11	0.00	-0.16
				0.33	0.99	0.14
CS[1]				1.00	-0.03	-0.19
					0.82	0.08
Weight, kg					1.00	0.39
						0.00
Age, d						1.00

[1]Chute Scores (1-6) were assigned by trained observers using the standardized scoring method recommended by the Beef Improvement Federation.

Figure 2.1 Histogram of fecal corticosterone (ng/0.5g) by frequency in number of head for ranch 1

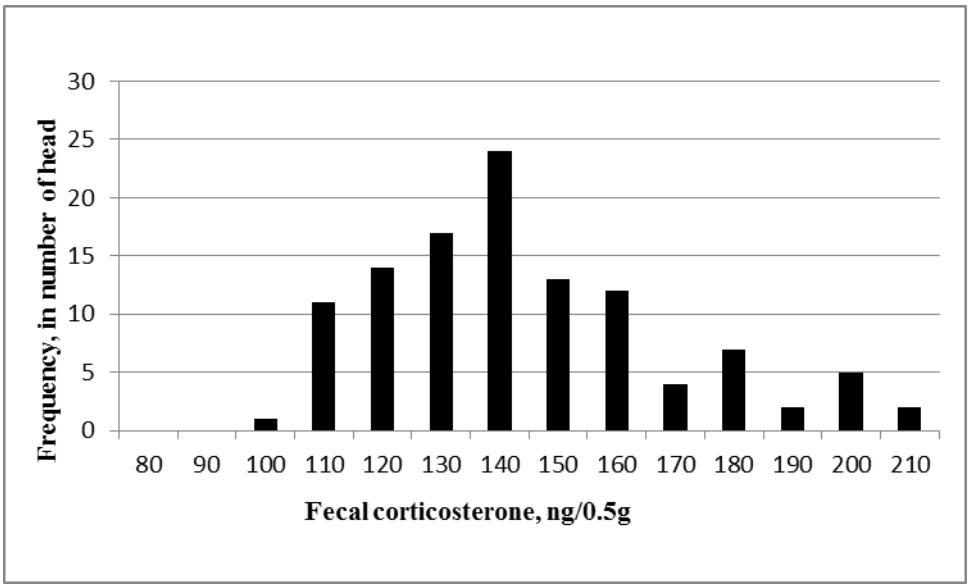


Figure 2.2 Histogram of blood cortisol (ng/ml) by frequency in number of head for ranch 1

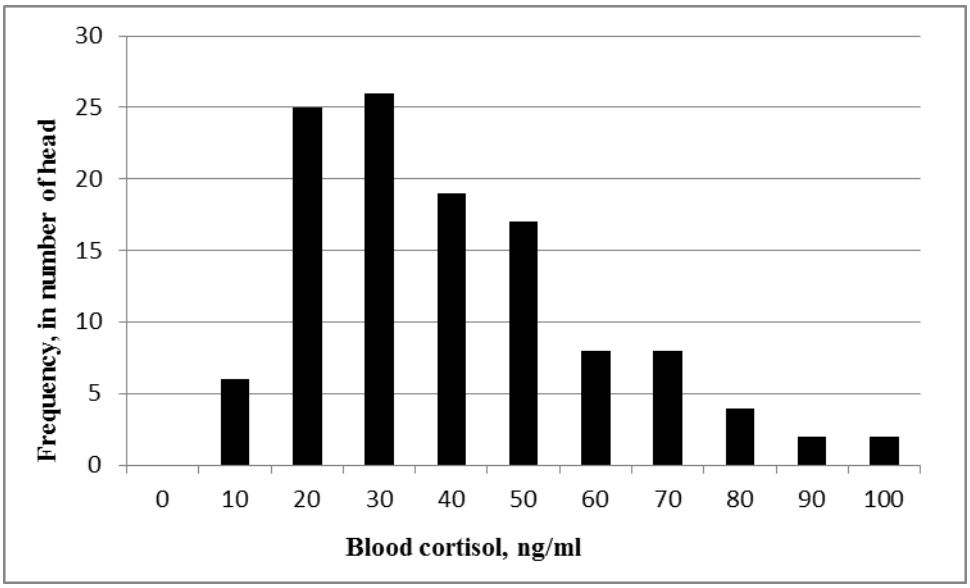


Figure 2.3 Histogram of exit velocity (m/s) by frequency in number of head for ranch 1

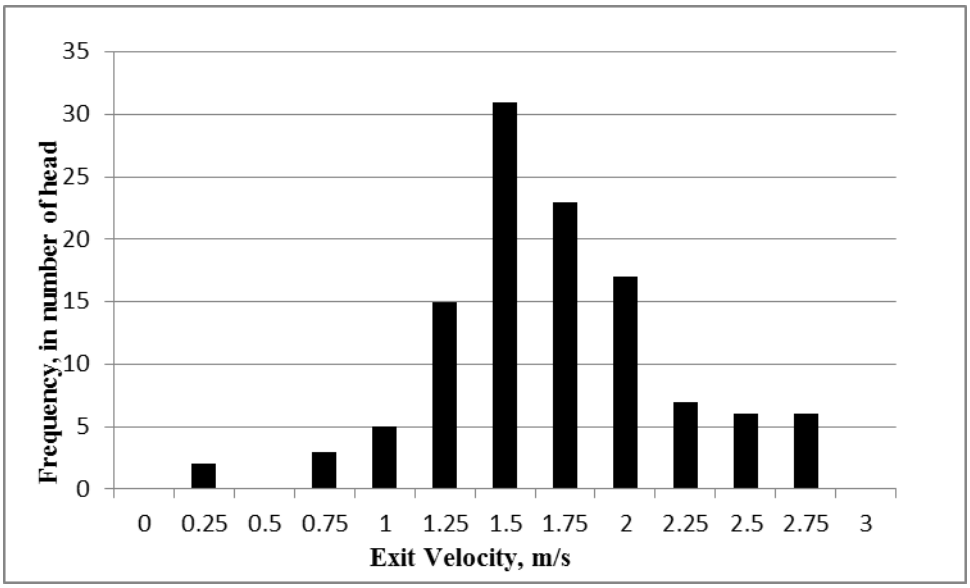


Figure 2.4 Histogram of chute score by frequency in number of head for ranch 1

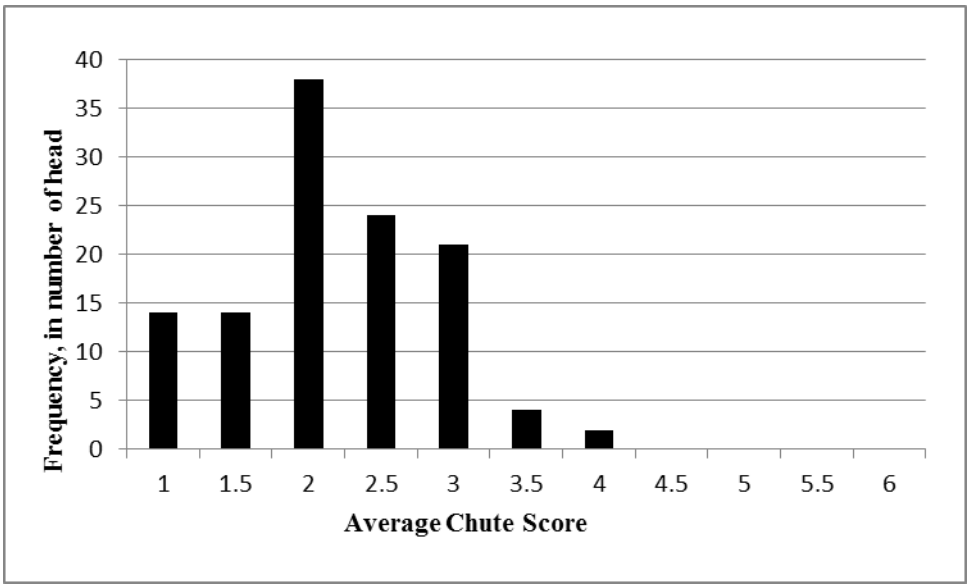


Figure 2.5 Histogram of weight (kg) by frequency in number of head for ranch 1

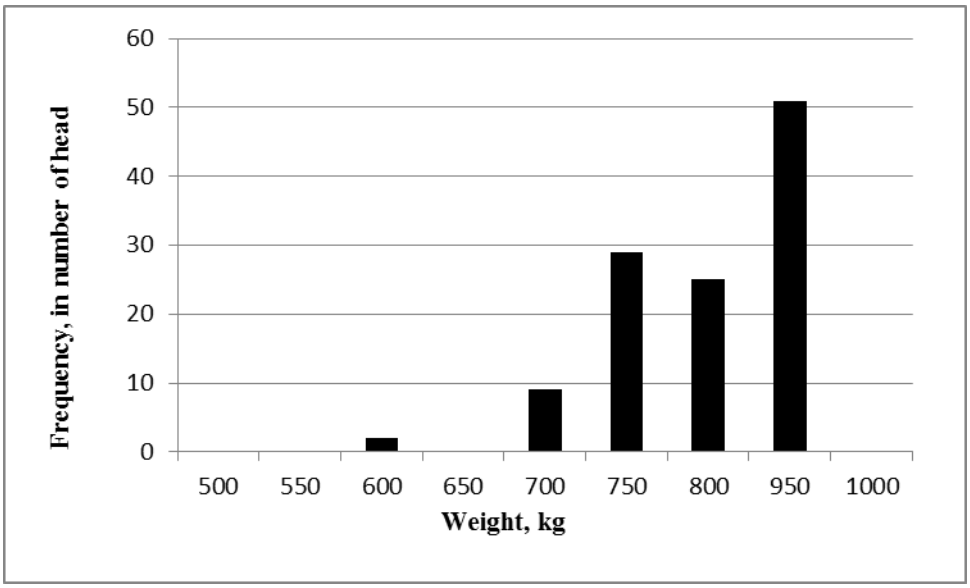


Figure 2.6 Histogram of age (d) by frequency in number of head for ranch 1

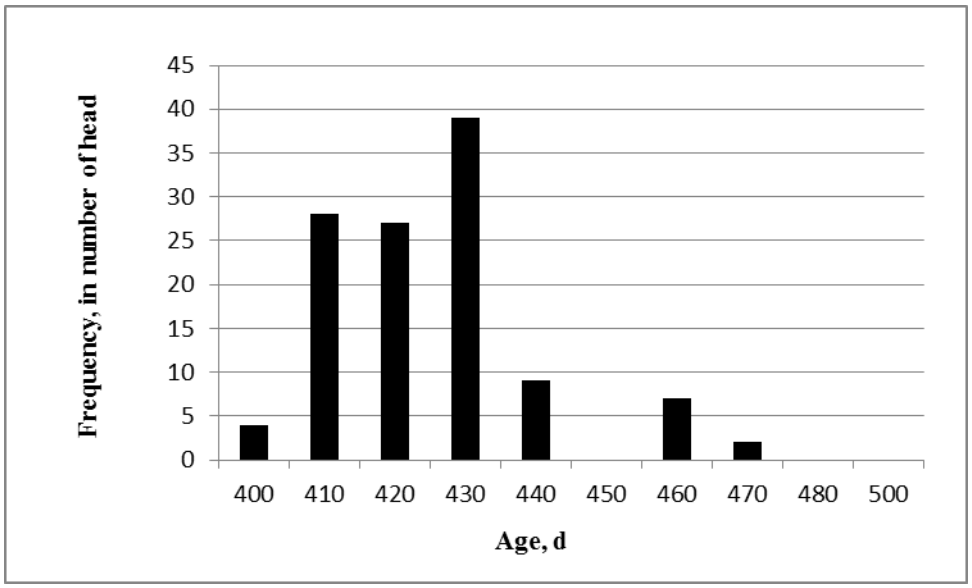


Figure 2.7 Histogram of pregnancy rate (0 = open, 1 = pregnant) by frequency in number of head for ranch 1

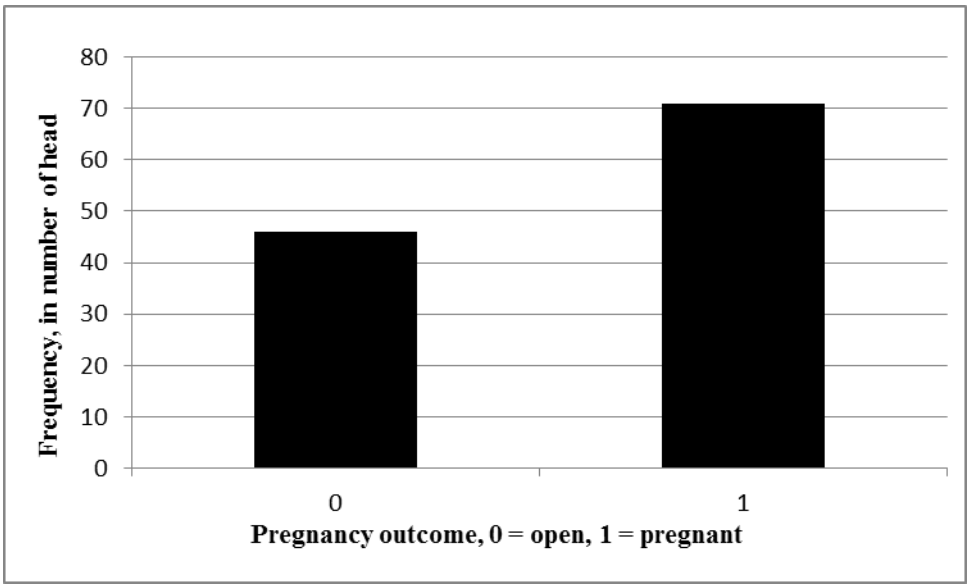


Figure 2.8 Histogram of fecal corticosterone (ng/0.5g) by frequency in number of head for ranch 2

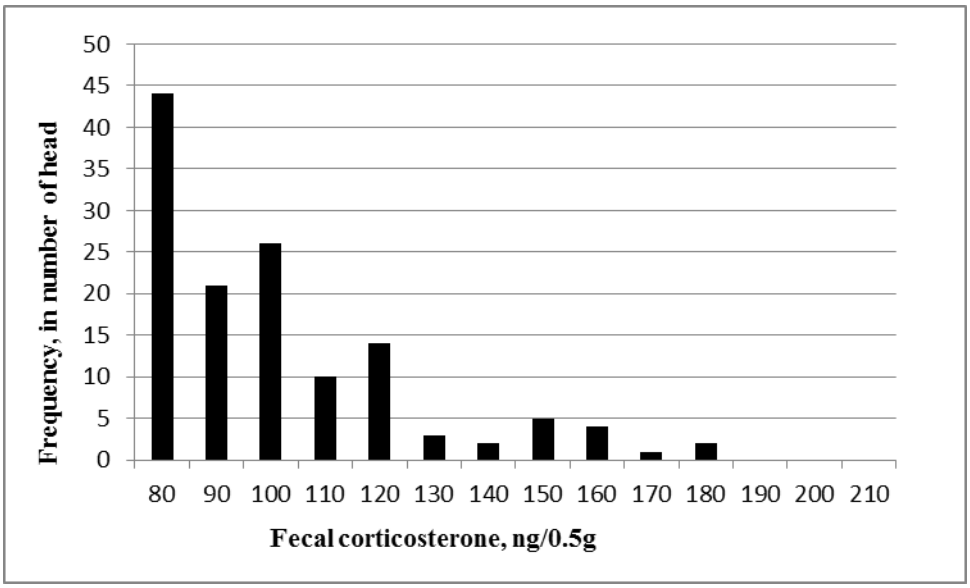


Figure 2.9 Histogram of blood cortisol (ng/ml) by frequency in number of head for ranch 2

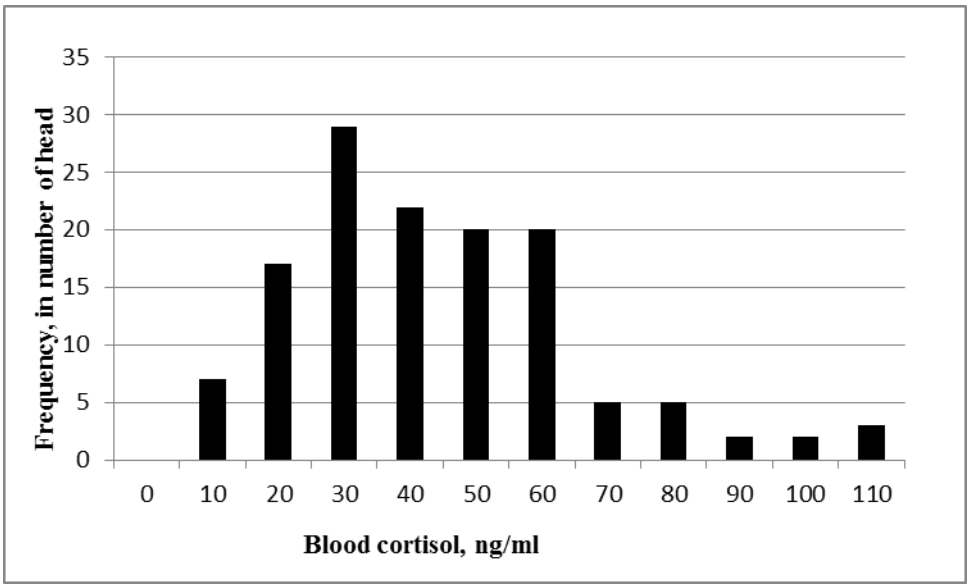


Figure 2.10 Histogram of exit velocity (m/s) by frequency in number of head for ranch 2

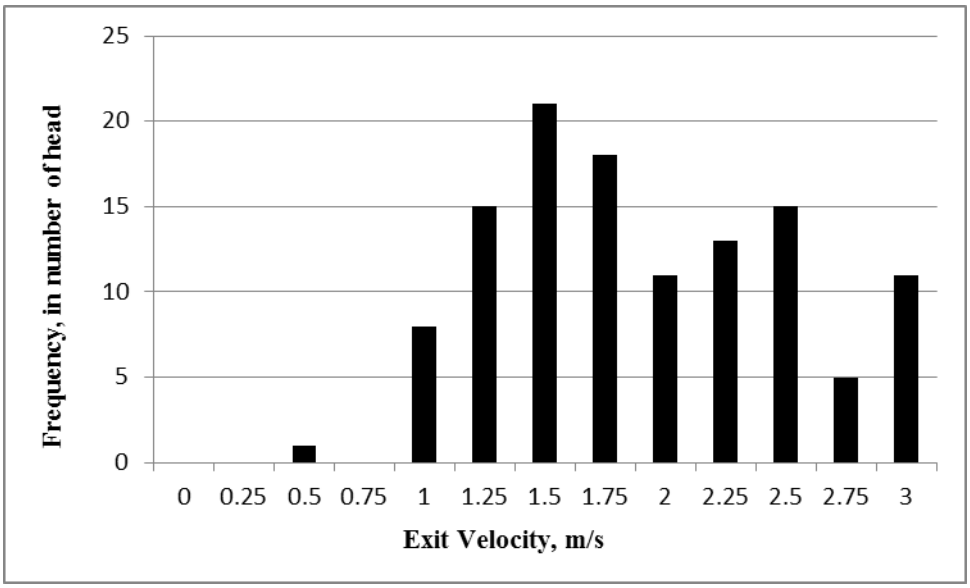


Figure 2.11 Histogram of chute score by frequency in number of head for ranch 2

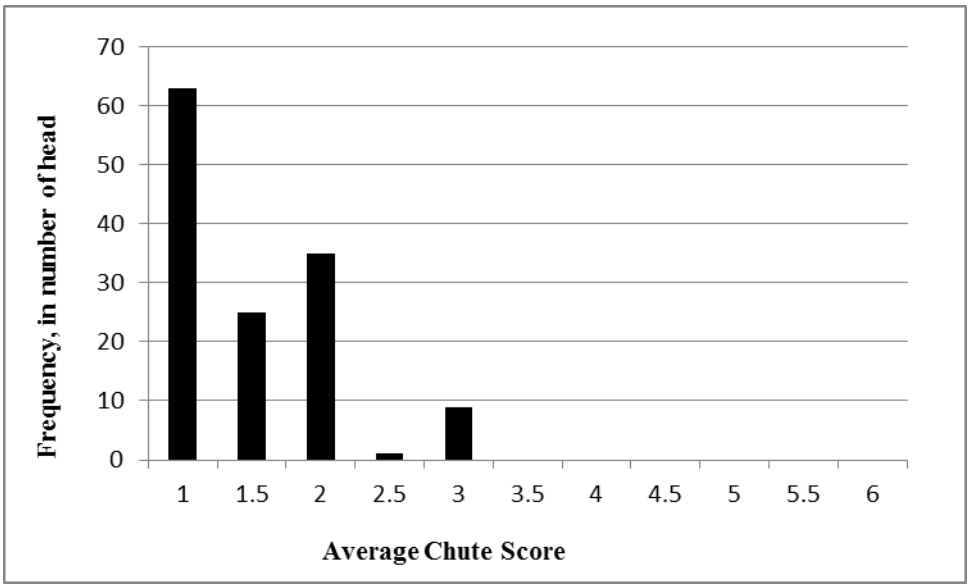


Figure 2.12 Histogram of weight (kg) by frequency in number of head for ranch 2

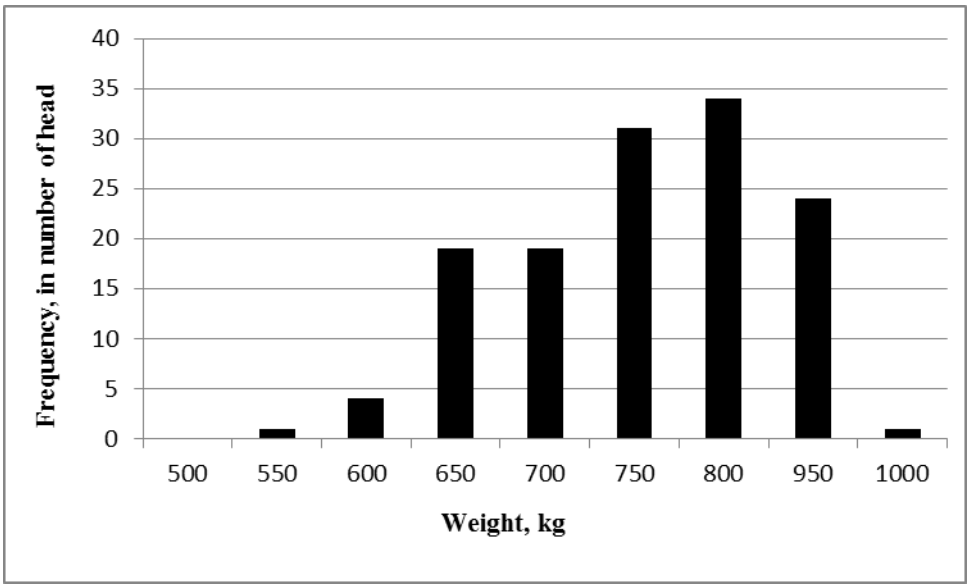


Figure 2.13 Histogram of age (d) by frequency in number of head for ranch 2

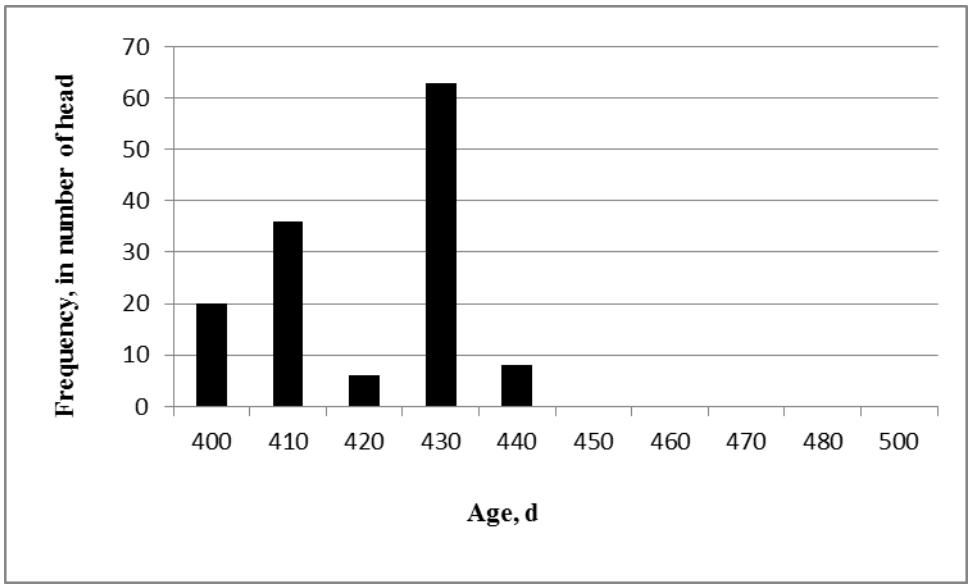


Figure 2.14 Histogram of pregnancy rate (0 = open, 1 = pregnant) by frequency in number of head for ranch 2

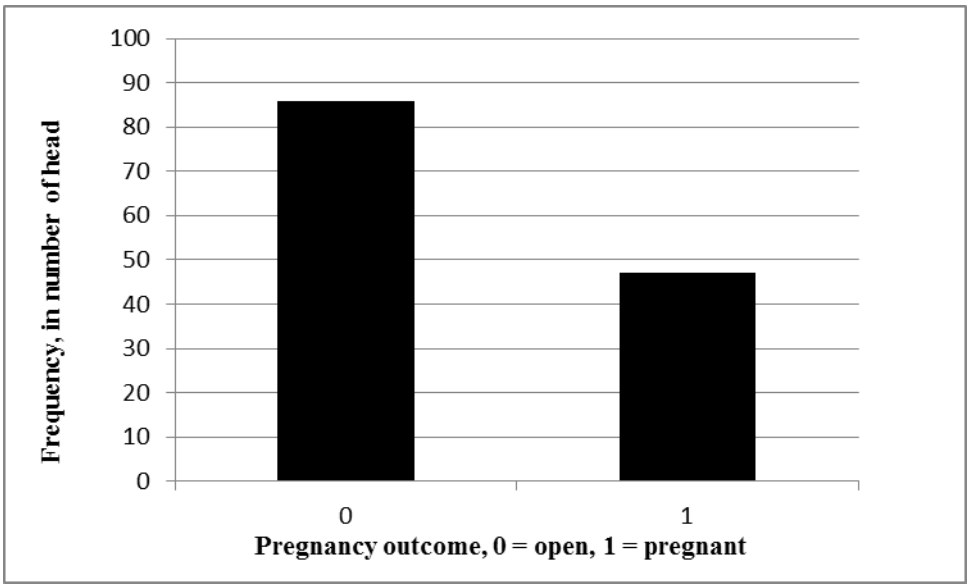


Figure 2.15 Histogram of fecal corticosterone (ng/0.5g) by frequency in number of head for ranch 3

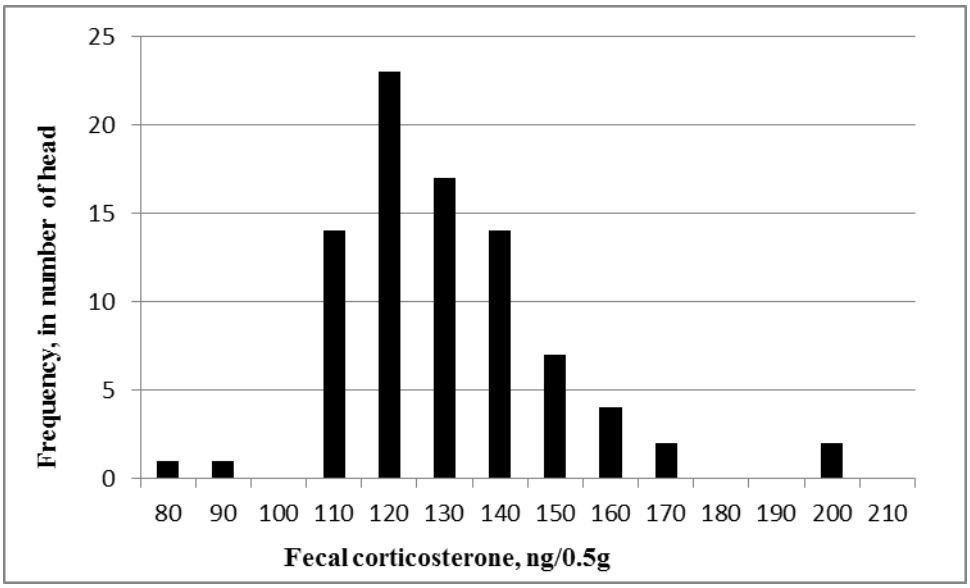


Figure 2.16 Histogram of blood cortisol (ng/ml) by frequency in number of head for ranch 3

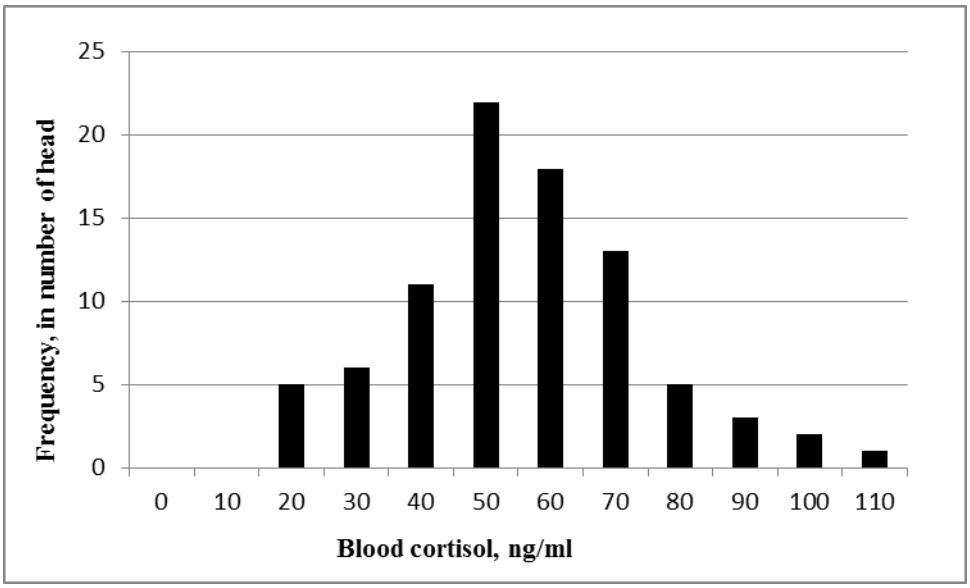


Figure 2.17 Histogram of exit velocity (m/s) by frequency in number of head for ranch 3

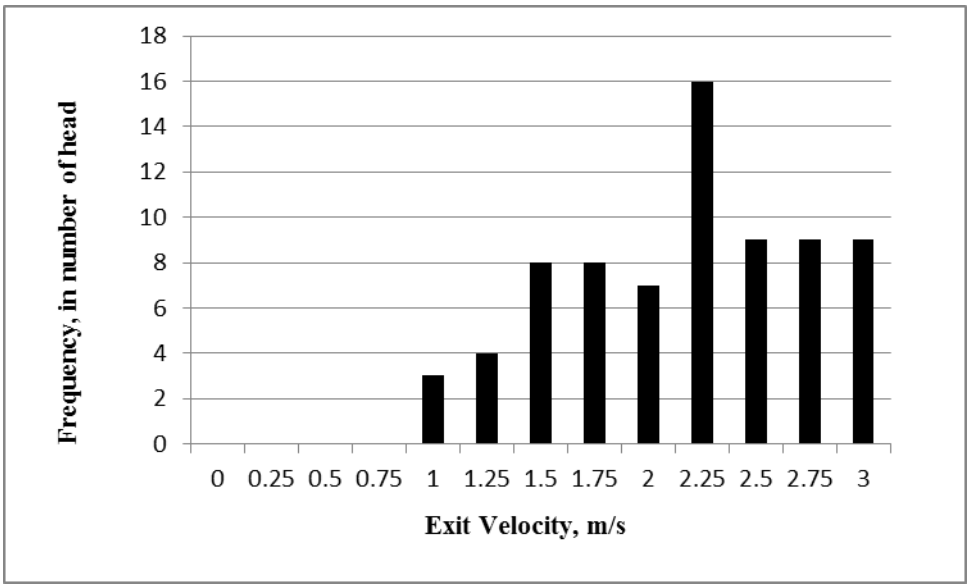


Figure 2.18 Histogram of chute score by frequency in number of head for ranch 3

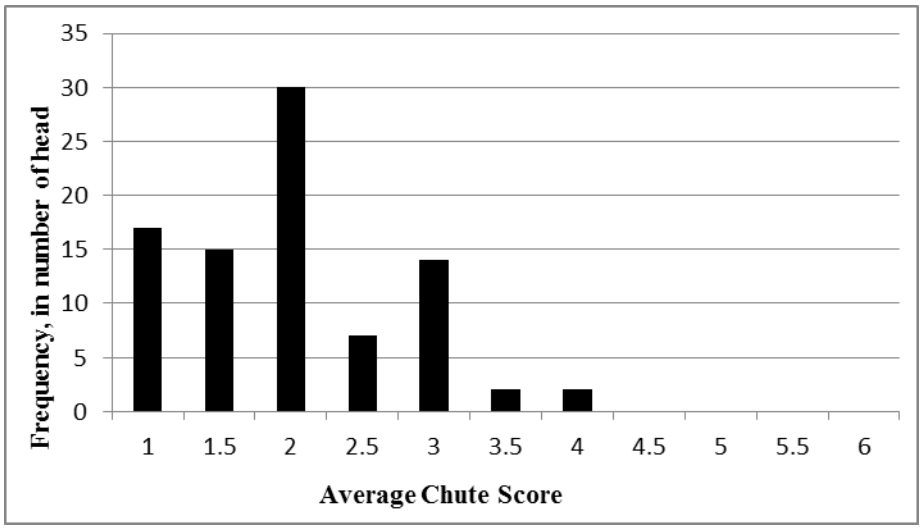


Figure 2.19 Histogram of weight (kg) by frequency in number of head for ranch 3

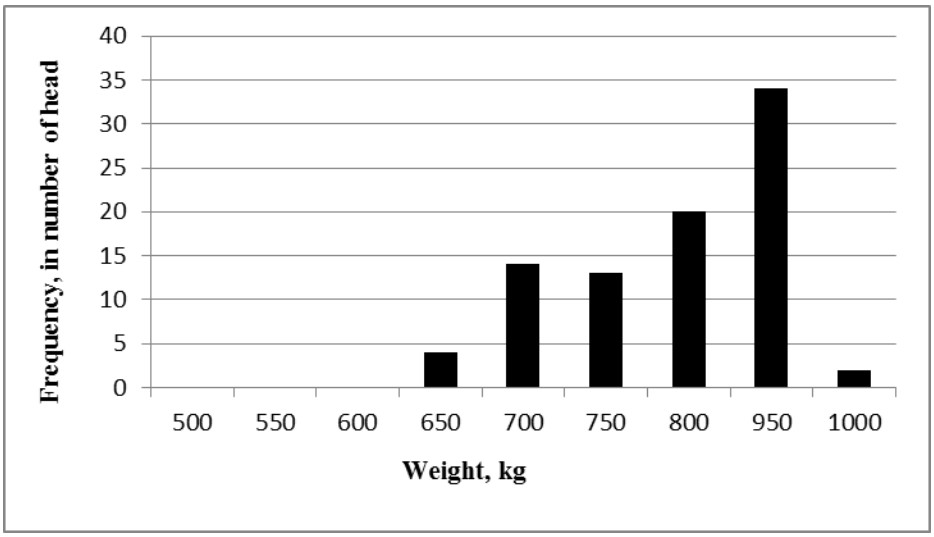


Figure 2.20 Histogram of age (d) by frequency in number of head for ranch 3

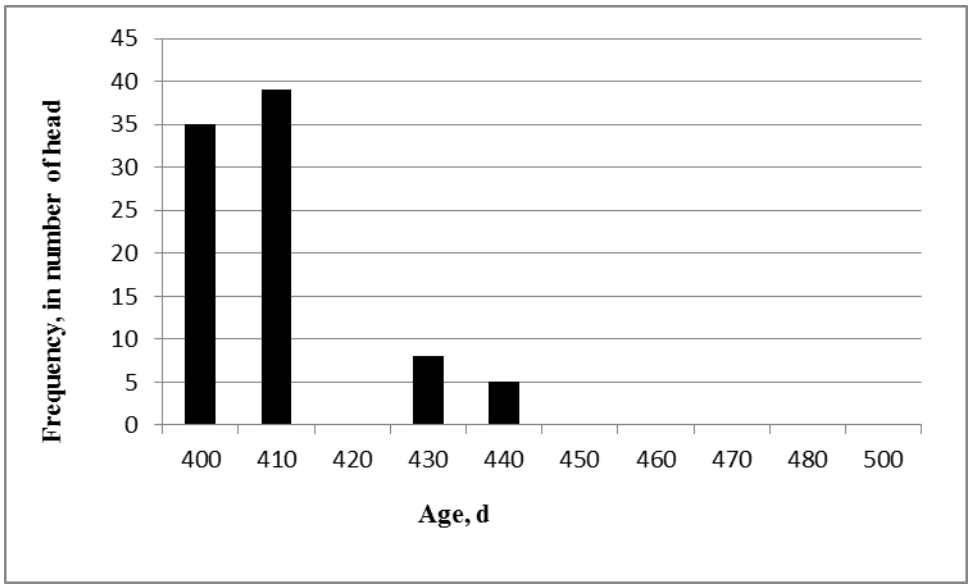
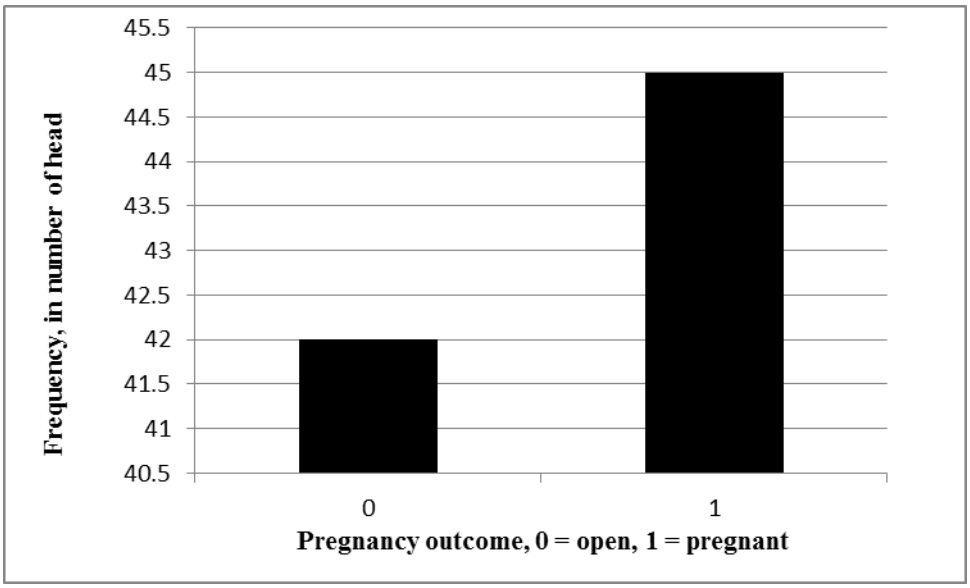


Figure 2.21 Histogram of pregnancy rate (0 = open, 1 = pregnant) by frequency in number of head for ranch 3



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Chapter 3 - Docility and heifer pregnancy estimates in Angus heifers

K.L. Otteman, J. M. Bormann, D. W. Moser, R. L. Weaber

Abstract

The objective of this study was to determine the genetic control of docility and reproduction in heifers as measured by pregnancy rate. Data included weaning contemporary group information, yearling contemporary group information, gender, docility score, yearling weigh date, age of dam, breeding contemporary group data including age at first breeding, pregnancy check results, and first service sire. A subjective chute scoring system was used as the basis of their genetic evaluation for docility. Pedigree information was also obtained on approximately 508,015 animals over 30 generations. Data included approximately 26,878 records only heifer pregnancy and 113,412 records only docility, with 7,849 animals having both docility and heifer pregnancy records. Contemporary groups were formed by the concatenation of weaning contemporary group, yearling contemporary group, and breeding contemporary group. Heritabilities were calculated from estimates of genetic and residual variance components computed using ASReml 3.0 (VSN International; Hemel Hempstead, UK). Heifer pregnancy variance components were estimated from a univariate, threshold model, with pregnancy outcome as the dependent variable. Animal and contemporary group were fit as a random effects, while age at first breeding was fit as a covariate. The heritability of heifer pregnancy was estimated to be 0.16 ± 0.02. Docility was fit in a univariate, linear animal model with docility score as the dependent variable. Animal and contemporary group were both modeled as random effects. The heritability for docility score was estimated to be 0.22 ± 0.03. Low to moderate heritability on these traits indicates that slow but definite genetic improvement can be made by selection on heifer pregnancy and docility.

Introduction

Reproductive success is economically relevant in beef cattle operations, because the gross value of calves sold at weaning is influenced by the number of calves born. Improvements in reproductive performance can be up to 4 times more important than improvements in end-product traits in an operation selling calves at weaning (Melton, 1995). It is difficult to select for fertility as it influenced by a variety of factors. (Martin et al., 1992).

It has become evident that temperament is one of the factors affecting fertility that requires further investigation. Researchers report that physiological responses associated with temperament can influence the probability of cows becoming pregnant during the breeding season (Cooke et al., 2009). Stress hormones such as cortisol present in the bloodstream can negatively affect the release of vital reproductive hormones (Cooke et al., 2009).

Methods have been developed to assess temperament in cattle. Beef Improvement Federation (BIF) guidelines describe a temperament scoring system that has been adapted by breed associations for genetic evaluation of docility in cattle (Beckman, 2008). The chute scoring system ranges from one to six. A one or two score indicates highly acceptable behavior, a three being average, and fours, fives and sixes deemed as unacceptable (Beckman et al., 2005). Studies have shown selection for cattle with a more favorable docility (chute) score would be effective in producing cattle with more acceptable dispositions (Beckman et al., 2005). Docility is generally analyzed as a threshold trait due to its categorical nature. Some breeds have produced EPD rankings for docility. The docility EPD reflects the probability that offspring will inherit genes for acceptable behavior, a greater docility EPD associated with progeny exhibiting calmer behavior (Beckman et al., 2007). Docility measured by chute score has been found to be moderately heritable (Shrode and Hammack 1971; Stricklin et al., 1980; Fordyce et al., 1988).

Materials and Methods

Statistical analysis for this study was computed using ASReml 3.0 (VSN International; Hemel Hempstead, UK). Data included approximately 26,878 records only heifer pregnancy and 113,412 records only docility, with 7,849 animals having both docility and heifer pregnancy records. Pedigree information was also obtained on approximately 508,015 animals over 30 generations, which included 49,091 sires, 292,715 dams, 9,802 paternal grand sires, and 35,068 maternal grand sires. For animals with performance records, 10,137 sires and 92,471dams were represented. Contemporary groups were formed by the concatenation of weaning contemporary group, yearling contemporary group, and breeding contemporary group. There were 12,782 contemporary groups for heifer pregnancy with an average of 24.33 records per contemporary group, and 12,954 contemporary groups for docility with an average of 10.59 records per contemporary group. Convergence was achieved when the REML log-likelihood changed less than 0.002* from the previous iteration and the variance parameter estimates changed less than

1% (Gilmour et al.., 2009). Heifer pregnancy variance components were estimated from a univariate, threshold model, with pregnancy outcome as the dependent variable. Animal and contemporary group were fit as a random effects, while age at first breeding was fit as a covariate. Docility was fit as a univariate, linear animal model with docility score as the dependent variable. Animal and contemporary group were both modeled as random effects. Genetic variance components were estimated for heifer pregnancy using the following model (Mrode, 2005):

$$y = Xb + Zu + Wcg + e$$

were

y = a vector of phenotypic heifer pregnancy observations,

X = an incidence matrix relating animal records to fixed effects which in this case was a covariate of age (d) at breeding,

b = a vector of fixed effects,

Z = an incidence matrix relating animal records to animal effects

u = a vector of random animal effects, and

e = a vector of residual effects.

The assumed model variance was:

$$var(y) = ZGZ' + R$$

where

$G = \{g_{ij}\}$ is the additive genetic variance and covariance matrix for animal effects,

Z = previously defined

$R = \{r_{ij}\}$ is the variance and covariance matrix

The mixed model equation in this analysis is as follows:

$$\begin{bmatrix} X'X & X'Z \\ Z'X & Z'Z + \lambda A^{-1} \end{bmatrix} \begin{bmatrix} \hat{b} \\ \hat{u} \end{bmatrix} = \begin{bmatrix} X'y \\ Z'y \end{bmatrix} \qquad \text{where } \lambda = \sigma^2_e / \sigma^2_a$$

Here, *X*, *Z*, and *y* are as previously described, $\hat{b}$ is a vector of fixed effect solutions, $\hat{u}$ is a vector of random effect solutions, *G* is the additive genetic variance matrix, and A^{-1} is the inverse of the numerator relationship matrix.

Genetic variance components were estimated for docility using the following model (Mrode, 2005):

$$y = Zu + Wcg + e$$

where

y = a vector of docility phenotypic observations

Z = an incidence matrix relating animals to performance records

u = a vector of random animal effects

W = an incidence matrix relating contemporary group to animal records

cg = a vector of random contemporary group effects

e = a vector of residual random effects

The assumed model variance was:

$$\text{var}(y) = ZAZ'\sigma^2_a + WI\sigma^2_{cg}W' + R$$

where

A = is the numerator relationship matrix

R = is the residual variance matrix

I = identity matrix

y, *Z*, *W* = defined above

The mixed model equation for this analysis is as follows:

$$\begin{bmatrix} Z'Z + A^{-1}\alpha_1 & Z'W \\ W'Z & W'W + I\alpha_2 \end{bmatrix} \begin{bmatrix} \hat{u} \\ \widehat{cg} \end{bmatrix} = \begin{bmatrix} Z'y \\ W'y \end{bmatrix}$$

where

$\alpha_1 = \sigma^2_e / \sigma^2_a$ and $\alpha_2 = \sigma^2_e / \sigma^2_{cg}$

Heritabilities were computed by dividing the additive genetic variance by the sum of the additive, contemporary group, and residual variance estimates ..

Results

The heritability of heifer pregnancy was estimated as 0.16±0.02. These findings are within the range reported by Cammack and others (2009), who found that heifer pregnancy has an estimated heritability between 0.14 and 0.21. The heritability of docility was estimated to be 0.22±0.03 which is lower than the heritabilities reported by the North American Limousin Foundation and the American Angus Association for docility, which are 0.40 and 0.37, respectively. The heritability estimate for docility obtained from this data is also low compared to the findings of Beckman et al., (2005), who reported a direct heritability of 0.37 for docility as measured by chute score. The heritability estimate from this study does, however, fall within the moderately heritable range reported by Shrode and Hammack, (1971), Sticklin et al., (1980) and Fordyce et al., (1988).

Conclusions

In conclusion, this study has shown low to moderate heritabilities estimates of the traits of heifer pregnancy and docility. This indicates that while progress may be slow, genetic improvement through selection can still be made on these traits. Further investigation on the relationships between docility and reproduction would be valid and useful research for the beef industry, due to the economic importance of both traits.

Figures and Tables

Table 3.1 Heifer pregnancy phenotypic summary statistics

n	26,878.00
$\bar{x}$	0.15
$\hat{\sigma}$	0.36

Table 3.2 Docility phenotypic summary statistics

n	113412.00
$\bar{x}$	1.45
$\hat{\sigma}$	0.68

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