8-2011

Effects of Lactate Dehydrogenase Haplotypes and Body Condition on Beef Cow Production

Olfat Taleb Alaamri
University of Arkansas, Fayetteville

EFFECTS OF LACTATE DEHYDROGENASE HAPLOTYPES AND BODY CONDITION ON BEEF COW PRODUCTION

EFFECTS OF LACTATE DEHYDROGENASE HAPLOTYPES AND BODY CONDITION ON BEEF COW PRODUCTION

A thesis submitted in partial fulfillment
of the requirements for the degree of
Master of Science in Cell and Molecular Biology

By

Olfat Taleb Alaamri
King Abdulaziz University
Bachelor of Science in Biochemistry, 2002

August 2011
University of Arkansas

ABSTRACT

Lactate dehydrogenase (LDH) catalyzes the conversion of the pyruvate to lactate (forward) or lactate to pyruvate (reverse) in the last step of glycolysis. Objectives were to document the effects of LDH haplotypes and its SNPs, found in the promoter and coding sequence site, and body condition on beef cow production. Four single nucleotide polymorphisms (SNP) of LDH-B and Five single nucleotide polymorphisms of LDH-A were detected. Eight haplotypes of LDH-B were assigned with the same order of SNPs: G-348A, A-261G, N-222D, and C541A and four haplotypes of LDH-A were assigned with the same order of SNPs: T-327G, D-263C, G390A, A406G, and T530C. Specific primers were designed for polymerase chain reaction and amplification of 507-base pair (bp) fragment and 555-base pair (bp) fragment and 452-base pair fragment and 457-base pair fragments of the bovine LDH-A and LDH-B coding sequence and promoter, respectively. Brahman-influenced cows (n = 109) were managed to achieve either low (BCS = 4.3± 0.1) or moderate (BCS = 6.4± 0.1) body condition. Cows grazed stockpiled and spring growth, endophyte-infected tall fescue pastures prior to 60-d breeding period to obtain desired BC; serum samples collected on d -35. The results of LDH-B gene showed that cows that were heterozygous (GA) had a lower calving rate than homozygous with the major allele (53.3 vs. 79.1 %, respectively) at base position G-348A. In addition, IGF-I was affected (P < 0.05) by body condition and G-348A. A-261G had an effect (P < 0.05) on LDHf and follicle size. Deletion of six nucleotides (GGCCGC) was detected at base N-222D. LDHf of fifty-five cows that were either hetero or homozygous for the deletion was affected (P < 0.02) by the N-222D. Interaction between BC x C541A had an effect (P < 0.05) on LDHr. NEFA (non essential fatty acid) was affected (P < 0.02) by base position C541A. the results of LDH-A showed that NEFA were affected (P < 0.02) by T-327G. Deletion of six nucleotides (GGCCGC)

was detected at base D-263C with an insertion of cytosine. LDHf and LDHr of LDH-A were not affected (P >0.10) neither by the genotypes nor by BCS. However, the interaction between BCS and D-263C affected (P< 0.05) LDHf. Haplotype 1 had the same sequence as that published at GenBank "No SNP". Haplotypes of LDH-A had no effect on both LDHf and LDHr (P >0.10), while the interaction between BCS X haplotypes had an effect on LDHf. Calving date was not affected by genotypes nor by haplotypes. In contrast, haplotypes of LDH-B had an effect on LDHf (P < 0.05). IGF-I, PRL, T3, and T4 were significantly affected by BCS. Our results demonstrated that the polymorphism of the coding sequence and promoter region can be used as a genetic marker for the selection of cows with high fertility.

This thesis is approved for recommendation
to the Graduate Council

Thesis Director:

_____

Dr. Charles Rosenkrans, Jr.

Thesis Committee:

_____

Dr. David L. Kreider

_____

Dr. Young Min Kown

_____

Dr. Dan Lessner

THESIS DUPLICATION RELEASE

I hereby authorize the University of Arkansas Libraries to duplicate this thesis when needed for research and/or scholarship.

Agreed   _____
    Olfat Alaamri

Refused _____
    Olfat Alaamri

TABLE OF CONTENTS

ACKNOWLEDGEMENTS

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF TABLES

LIST OF FIGURES

ARTICLE I

ARTICLE II

1.0 LITERATURE REVIEW

1.1 Body condition

Just like human body shape implies wellness and fitness of the body, body condition is an indication of overall health and normal performance in animals. Human body shape can be built up by exercises for different reasons either for fitness enhancement, as in sports performance preparation, or maintaining health. Muscle mass can be influenced by exercises, and fat distribution can be influenced by hormone fluctuations as well as genetics which play an important role in the development of body shape. While many people all over the world are working on their own body shape and physical condition to improve their health and reduce medical cost. The application of the same concept could be applied to other aspects of life such as economic situation. Body condition scoring (BCS) is one important and effective management tool to be put in consideration. By modifying body condition, producers can reduce herd expenses and increase profits by increasing fertility during postpartum period (Short et al., 1990). Animal's body condition estimation can be made by eye, so as the old saying says "beauty is in the eye of the beholder. What is the appropriate body condition of cows?

A BCS has been developed to evaluate normal performance for growth, reproduction, and nutritional status. This system has been used to determine the average body condition (BC) of cows in a herd. Body condition scores (BCS) are numbers used to assess the relationship of fatness to skeletal features of the cow (Long et al., 1979; Kress et al., 1969; Thompson et al., 1983; Klosterman et al., 1968). Body condition can be assigned visually or palpated manually depending on circumstances (Long et al., 1979; Wiltbank et al., 1962; Nelsen et al., 1985; Kress et al., 1969; Klosterman et al., 1968). Pregnancy, shrink, and length of cattle, can alter the appearance and feel of the cattle around one BC score (Kunkle et al., 1994). Moreover, BCS is a

more reliable marker of nutritional status than body weight BW (Berry et al., 2006; Thompson et al., 1983; Neslen et al., 1985). BW does not determine BC and fat reserves because BW could vary through the seasons. A full gut, pregnancy, and forage availability can affect BW, so cows with the same body condition can have a different body weight (Grainger et al., 1982; Enevoldsen and Kristensen, 1997). The correlation between BCS and live weight (LW) were relatively constant. Mean variation in BCS explains approximately 30% of the variation in LW (Berry et al., 2006). Variation in regression of LW on BCS was present within stage calving interval and parity subclasses (Berry et al., 2006).

The most commonly used body condition scoring system ranges from 1 (very thin and carry no fat) to 9 (extreme fatness) (Wagner et al., 1988). Brisket, spine, ribs, hooks, pins, and tail-head are body areas evaluated. A thin cow looks very weak and skinny with easily visible vertebrae while fat cow vertebra cannot be seen and it has smooth and rounded flesh (Wagner et al., 1988). To achieve high reproductive efficiency and performance, Body condition should be determined and cows should be managed to have a score of 5 at calving (Wagner et al., 1988; Dunn and Kaltenbach, 1980; Wiltbank et al., 1964; Spitzer et al., 1995). Body condition scores below 5 or greater than 6 in mature cows before calving, can negatively affect rebreeding and productivity. It may lead to dystocia (Bellows et al., 1971; Bellows and short, 1978; Selk et al., 1988; Spitzer et al., 1995; Short et al., 1990). In contrast, BCS at calving for a two-year-old, first-calf heifers should be a score of 6.Heifer need additional BCS because are prone to fail to rebreed rather than mature cows and need a good nutrition for their growth (Bellows et al., 1982; Lalman et al., 1997; Bellows and short, 1978; Fleck et al., 1980; Patterson et al., 1991; DeRouen et al., 1994). Mature cows below 5 should be separated and fed with heifers that are provided

with a higher nutrition diet than mature cows (Thompson et al., 1983; Klosterman et al., 1968; Patterson et al., 1991).

Body condition scoring system is applied on horses as well. The system was developed by Dr Henneke in the early 1980s (Henneke et al, 1984). Since that time people who are involved with evaluating animal welfare, have incorporated the BCS system into their businesses (Stephenson and Freeman, 2011). Horse's body condition determines the balance between intake and consumption of energy. The horse's body condition can be influenced by several factors including: food availability, reproductive activities, dental problems, and performance or work activities (Hintz and Cymbaluk, 1994; Nagy et al., 1998). Equine BCS can impact reproduction, performance ability, work function, health condition, and endocrine status (Stephenson and Freeman, 2011; Hintz and Cymbaluk, 1994; Garlinghouse and Burrill, 1999; Nagy et al., 1998)). Unlike cows, the required horses' body condition is different between horses. Horses used in athletic performance need lower BC than non- performance horses (Lawrence et al 1992; Scott et al., 1992; Hintz and Cymbaluk, 1994; Cavimder et al., 2009).

1.1.1 Managing and feeding beef cows using body condition

Research and studies have demonstrated that dividing cows into groups according to their body condition and nutritional needs can reduce feeding cost (Wagner et al., 1988; Klosterman et al., 1968). Thin cows which are below the target body condition (below score of 5) could be sorted and additional nutritional diet should be supplied to improve their BC and their chances of pregnancy in early breeding season (Wiltbank et al., 1964; Selk et al., 1988; Spitzer et al., 1995; Patterson et al., 1991; Lalman et al., 1997; Dunn and Kaltenbach, 1980). Producers should follow a strategy to manage nutrition to achieve the reproductive performance goal. By understanding the production of the cow, the strategic feeding to acquire the target BCS can be

3

achieved. The period at which cattleman can adjust the nutrition to increase or decrease BC is shortly after weaning when cows are in mid-gestation (Spitzer et al., 1995; Fox et al., 1988). At this time, cows require fewer amounts of nutrition in order to maintain their metabolism (Ferrell and Jenkins 1984; Fox et al., 1988). Thus, the most economical time to increase BC for thin cows is immediately following weaning because cow's energy needs are at the minimum immediately after weaning (Peterson et al., 1987; Fox et al., 1988). During the second stage of the cows' production cycle, the fetus starts to grow very fast. In addition, cows need to be prepared for lactation; therefore, manipulating cow's BC needs additional feed since this stage occurs usually during the winter when nutrition becomes expensive due to forage availability (Fox et al., 1988; Houghon et al., 1990; Klosterman et al 1968; Thompson et al., 1983; Murrieta et al., 2010; DeRouen et al., 1994). Shortly after calving, cows require the maximum needs of nutrition to produce milk for growing calves. They must regain lost weight after calving in order to be able to become pregnant and decrease the time between calving and rebreeding (within three months after calving; Houghton et al., 1990; Dunn and Kaltenbach, 1980; Richards et al., 1986; Wiltbank et al., 1964; Randel., 1990; Ferrel and Jenkins, 1984). Yet, adjusting BC at this time is very hard and costly because the grass will have a higher percentage of moisture which can lead to a deficiency in energy (Fox et al., 1988). In stage four of lactation cow, a higher nutrient level is required, but BC could be adjusted (Ferrel and Jenkins, 1984; Lelman et al., 1997; Fox et al., 1988). Lack of success to maintain BCS in target score prior to production period can be potentially destructive to cowherd productivity because waiting too long to increase BC can be cost prohibitive and sometimes impossible (Kress et al., 1969; Wiltbank et al., 1964; Richards et al., 1986; Selk et al., 1988; Wagner et al., 1988; Houghton et al., 1990). According to Peterson et al. (1987) 70% of cow costs are linked with feed. Knowing when to

feed cows , what kind of supplement that cows need at certain times, and nutrition availability, will allow producers to decrease the cost of the supplements and avoid reproductive failure in the future. Moreover, understanding the factors which increase forage consumption such as BW, pregnancy, lactation, and environmental condition also might be economically important and might increase productivity (Wagner et al., 1988, Peterson et al., 1987; Kock and Algeo, 1983; Sawyer et al., 2004).

1.1.2 Body condition and reproduction

Reproduction performance of cows is closely related to the body condition (body energy reserves) at certain time during reproduction cycle (Selk et al., 1988; Wiltbank et al., 1962; Lelman et al., 1997; Dunn et al., 1969; Dunn and Kaltenbach, 1980). Both very thin and very fat cows at calving can experience birth problems or reproductive deficiency such as dystocia and long postpartum interval (time from calving to rebreeding). Thin cows and those with low nutrition prior to calving will have a weak calf that are more susceptible to disease due to low milk production during lactation (Bartle et al., 1984; Spitzer et al., 1995; Clutter and Nielsen, 1987; Kress et al., 1969; Bellows and Short, 1978; Richards et al., 1986; Bellows et al., 1982; Patterson et al., 1991; Patterson et al., 1992; Corah et al., 1975). As a result, reduction of weaning weight of calves from thin cows will occur. By increasing the reproduction of beef cows in herds, producers can increase the profits and decrease investment expense and cost. The main constraint to efficient reproduction in a cowherd is the duration between calving and rebreeding PPI (Selk et al., 1988; Lalman et al., 1997; Dunn and Kaltenbach , 1980). So, the one way to enhance reproduction is to reduce the postpartum interval (PPI) in order to maintain an annual production cycle (Selk et al., 1988; Dunn et al., 1969; Dunn and Kaltenbach, 1980; Houghton et al., 1990). Since reproductive performance is associated with body condition and since the body

5

condition is the significant factor affecting PPI and pregnancy rate, managing BC is important to be taken into consideration. The body condition of the beef herd is variable throughout the year. In mid to late summer, body condition will be at the highest and then decrease in the fall or winter. BC is the lowest in late winter or early spring (Kunkle et al., 1994). The BC in fall for spring calving cows influences the amount and type of winter nutrition supplement requirement (Klosterman et a;., 1968; Thompson et al., 1983). Thus, thin cows need large amount of nutrition, while cows in a proper BC need small quantities of nutrition in winter (Thompson et al., 1983; Wagner et al., 1988; Klosterman et a;., 1968; Thompson et al., 1983). In the late spring freeze and the hot dry summer of the Midwest, producers experience reduction in the stored forage availability for the winter months. Added to that, most of the stored forage is low quality and high price including other forage alternative such as corn stalks will be expensive (Kunkle et al., 1994). For that reason, maintaining BC prior to calving will reduce feeding cost and increase pregnancy rate. The degree of loss of body condition in winter should be steady and not severe as much as possible (Kunkle et al., 1994). Since the body condition will change during the year, it is important to estimate the BCS of cowherds frequently to detect any changes in BC. Furthermore, the repeated evaluation of BCS in the winter is significant because this time is correlated with the third trimester of pregnancy for most spring calving herds (Kunkle et al., 1994; Ferrel et al., 1976). During pregnancy, nutrition requirements are increased to help fetal growth (Ferrel et al., 1976; Barker and Clark, 1997). The fetal development processes, calf birth, milk production, and reproductive tract recovery are all physiological stresses (Selk, 2004). For that reason, the availability and utilization of large amount of energy is required in order to allow cows to be rebred in short PPI. Environmental stresses including wet weather, extreme cold, and wind chill, all adding to the physiological stresses. If the energy intake is below the cow's needs

for physiological or environmental stresses, the cows will compensate by mobilizing stored energy (adipose tissue) and thus will lose body condition after a period of time (Selk, 2004). So, it is important to ensure that sufficient nutrition is provided to monitor and adjust body condition prior to calving and that cows are in a good condition by calving. Cows that are in a thin body condition take longer time to return to estrus compared to those with a good body condition. So, as BCS at calving decline, the postpartum interval extends. Supplementation is necessary for cows that are in thin body condition prior to calving in order to increase postpartum body condition and modify PPI. However, modifying PPI and postpartum condition prior to calving to achieve optimal reproductive performance by increasing the quality and quantity diet, can be very costly (Selk, 2004). Failing to attain a required BCS at calving, could negatively affect different feature of reproductive performance. Many factors affect PPI such as the cow's age and BC, suckling of the calf, and birth difficulty (Wiltbank et al., 1962; Selk et al., 1988; Lalman et al., 1997; Bellows et al., 1982; Wiltbank et al., 1964; Kress et al., 1969; Peterson et al., 1987; Sawyer et al., 2004; Perry et al., 1991). To keep a 12-month calving interval, cows must rebreed within 80 to 85 days of calving (Herd and Sprott, 1986). If the PPI is extended to more than 85 days, the calving interval will be extended (Kunkle et al., 1994). Cows with a BCS below 5 at calving and during rebreeding do not conceive at satisfactory level. Thin cows might take up to 200 days to rebreed, so they will not have 12-month calving interval (Herd and Sprott, 1986). Therefore, controlling of nutritional status and BC may have an impact on cow's reproductive efficiency since most reproductive failures correspond with nutritional status and BC. Studies in Texas illustrated that maintenance and reproduction cost per calf for cows with a 12-month calving interval is less compared to the cows with longer calving interval (Herd and Sprott, 1986). Cows with a good BCS tend to have healthier calves compared to the cows with poor

7

BCS. Cows with pre-calving BCS below 5 tend to have long production, more than a year, while cows with pre-calving BCS of 5 or 6 will have shorter calving interval. Therefore, cows with pre-calving BCS below 5 have low reproductive efficiency due to long PPI. In addition to pre-calving BC, post calving BC influences reproductive performance as well. Cows with moderate BCS at calving need to be provided with a required nutrition to keep their BCS in order to achieve reproductive goal. In addition, the cyclic activity in thin cows can be increased by providing them with high energy intake that leads to increased BW and BC. The percentage of thin cows was provided with high energy intake started estrus cycle by greater percentage than cows that were provided with low energy intake (Richards et al., 1986). However, it is better to feed cows and maintain their BC after calving than for moderate condition cow to lose weight after calving (Rutter and Randel, 1984).

The relationship between body condition and reproductive performance occurs in other animals such as horses. Several studies and field trial demonstrated that reproductive efficiency in mares is related to BC. The time of first ovulation will be delayed for mares with BCS of 4 or less, and this delay can be three to four weeks compared to mares with BCS of 5 or greater (Cavinder et al., 2009). Therefore, mares with BCS of 4 or less will be deficient breeders and more likely to have pregnancy losses than mares with moderate BCS.

Not only animals but also human reproduction can be affected by body composition and body shape. Professor Rose Frich, (2002), who works at Harvard University indicated that "under nutrition and intense physical activity can have a limiting effect on female fertility. Women who have too little body fat, because of injudicious dieting and/or intensive physical activity, also have disruption or impairment of their reproductive ability; they are infertile due to hypothalamic dysfunction which is correlated with weight loss or excessive leanness."

Obesity also can affect reproduction. Very fat cows with body condition above 6 prior to calving in first calf heifers might result in increased case of birth difficulty (Abeni et al., 2004). Excess energy intake during the dry period may lead to obesity in cows prior to calving. These obese cows are more likely to have metabolic problems such as depression, anorexia, ketonuria, marked decrease in production, progressive debilitation, weakness, nervous signs, metritis, elevated body temperature due to infectious diseases, cystic ovarian disease, reproductive problems, and increased chance of mortality and morbidity (Morrow, 1976; Gearhart et al.,1990). Obesity can be a factor of development of polycystic ovary syndrome (Pasquali et al., 1997). Mares maintained in a fleshy body condition (BCS 7 to 8) tended not experience reproductive dysfunction or lowered levels of fertility compared to mares with moderate BCS (Cavinder et al., 2009). Mare should be at body condition of at least 6 to avoid the effects of losing body fat after foaling and in early lactation on reproduction (Cavinder et al., 2009). Obesity has been related to reproduction in human and animals. Unlike animals, long estrus cycle is one of the significant problems associated with obesity in human (Hartz et al., 1979).

1.1.3 The relationship between body condition and hormones

Small amounts of hormones can alter the metabolism of the cell. Improvement of human health in developed countries can be reduced due to unhealthy life style factors such as obesity and stress. The energy homeostasis regulation is achieved by a combination of several factors, including neural afferents and nutritional signals. These factors send information to the brain to regulate energy intake and expenditure. The association of energy homeostasis and stress can affect nutrition intake and energy expenditure. Metabolism affects the majority of biological functions, including growth and reproduction. Metabolic status reflects the general health. So, maintaining normal metabolic status is very important for health improvement. One of the most

important biological functions which are regulated by hormones is reproduction. Obesity is a common health problem associated with high rate of mortality and morbidity due to several diseases such as heart disease, hypoglycemia, and hyperglycemia. Moreover, obesity increases the risk of complication in pregnant women (Hollmann et al., 1996). Obesity is associated with infertility and menstrual irregularity in some women due to hormonal changes such as distorted LH (luteinizing hormone): FSH (follicle-stimulating hormone), low GH (growth hormone) concentration (Hollmann et al., 1996). Furthermore, polycystic ovary syndrome (PCOS) may develop in obese women. Pasquali et al., (1997) determined that a large number of women with (PCOS) are obese or overweight. Weight loss improved menstrual function by 80% and increased the pregnancy rate of 29%, and was associated with decreased plasma insulin (Hollmann et al., 1996).

Both humans and cattle reproduction is affected by hormones and body shape. One study suggested that cows which maintained their body condition after parturition were able to release endogenous luteinizing hormone (LH) in response to exogenous GnRH. Additionally, maintained body condition cows have a higher LH concentration and shorter PPI compared to cows that lost body conditions after calving. Another study determined that the alteration in LH secretion may be independent of ovarian control in low energy reserves cows. There was no increase in LH and IGF-1(Insulin- like Growth factor 1) in lost body condition ovariectomized (OVX) cows after treated with estradiol while LH and IGF-1 increased in maintained body condition OVX cows after estradiol treatment. Furthermore, cows with low energy reserves have lower concentrations of LH and IGF-1 when compared with high energy reserve cows after OVX (Richards et al., 1991). Several studies determined the influence of body condition on and alteration of reproductive function and alteration of hormones. Anestrous happened when body

10

condition reduced to approximately 3.5 accompany with a decrease in LH secretion. In contrast, increased nutritional intake and increased body condition to 4.6 restarted the estrous cycle and normal pregnancy rates in nutritionally anestrous beef cows by increasing in LH secretion. Therefore, the nutritional intake and BCS are important factors to maintain normal ovarian activity estrus in beef cows (Richards, Wettemann, and Schoenemann, 1989). This study supported the concept that nutritional intake and body energy reserves influence the hypothalamic-pituitary function and consequently ovarian activity in beef cows (Rutter and Randel 1984). Furthermore, other research determined the influence of BC at calving and postpartum on endocrine function and reproduction of beef cows. The study demonstrated that high energy intake and increase in BCS after calving increased the concentration of IGF-1, leptin, insulin, glucose, and thyroxin in plasma and shorten PPI (Ciccioli et al., 2003). In contrast, cows that had a low energy intake after calving and lost BCS had lower concentrations of IGF-1, leptin, insulin, glucose, and thyroxin in plasma and longer PPI. So, increased energy intake after parturition induced the secretion of anabolic hormones and increased fat deposition and milk production of cows that calved with moderate or thin BC (Ciccioli et al., 2003).

The concept of the influence of body condition on hormones and reproductive performance is also applicable to horses. Insulin resistance (normal concentration of insulin is not able to cause a normal response) is a danger factor in obesity that can cause metabolic syndrome in horses (Hoffman et al., 2003). High sugar and starch intake will decrease insulin sensitivity of horses. Therefore, maintenance of BC and low sugar intake can decrease the risk of insulin resistance development (Hoffman et al., 2003). In addition, low energy intake decreases BCS in mares resulting in intense seasonal anovulatory period that is associated with low leptin,

IGF-1, and prolactin concentrations. Mares with high body condition continue to ovulate all through the winter and had high ovarian activity (Gentry et al., 2002).

In conclusion, body condition scoring is an important tool that can be simply used to adjust nutritional program in cowherd in order to reduce feeding cost. In addition, producers can use body condition scoring to improve reproductive performance, minimize replacement expenses, and increase income. Body condition scoring at calving affects calf survival because thin cows may have a higher degree of calving difficulty and weak calves at birth, thus they will be more sensitive to disease (Richards et al., 1986; Bellows et al., 1982; Patterson et al., 1991; Patterson et al., 1992). Several factors are associated with changing body condition through the year, so it is important to evaluate body condition all through the year. At calving time, cows should have body condition score of 5 to 6, and first calf heifers should have BCS because it is very hard and very costly to increase body condition after calving to obtain acceptable rebreeding rate.

1.2 Forage, Body Condition, and Reproductive Performance Relationship

Research and economic studies showed that management of forage and nutritional intake is very important in livestock operations. Monitoring nutritional intake throughout the year influences the BCS of beef cow. Since reproductive performance can be affected by nutritional intake and BCS (Selk at el., 1988; Lalman et al., 1997), management of forage and body condition must be taken into consideration. The most important and effective way to achieve acceptable cow/calf profitability is to be a low cost producer (have low breakeven point; high long term profitability) (Taylor and Field, 1995). Feed cost form for approximately 60% of the annual cow costs in many cow/calf businesses, so this part must be a primary issue in evaluating production cost (Taylor and Field, 1995). According to several studies, forage, body condition,

and reproduction are the most important factors that affect profitability regardless of environmental factors such as weather and disease which can be associated. These three factors form a loop in which one feeds the other. Failure to maintain the balance of this loop leads to a failure to achieve the desired profit thus producer exits the cattle business as a result of increase cost and decrease production (Taylor and Field, 1995).

Since nutritional intake has important effects on cyclic ovarian activity and estrus in beef cows (Richards et al., 1989), cows should be supplied with forage that contains nutrients that meet their need to maintain their reproductive performance. Seasonal variation can affect forage quality, and cows may gain the highest weight during the season of high quality forage (Halls, 1964). Forage in spring and summer becomes most nutritious with lower nutritious value during fall and winter (Halls, 1964). The variation of forage helps the balance of cattle diet. Furthermore, the diversity of cool-season (C3- the first organic product during carbon fixation is three- carbon 3-phosphoglysrades) and warm-season (C4- the first organic product is four-carbon oxaloacetate) forage (Lambers et al., 1998) prolong the availability of green feed. Cool season (C3) forage is higher quality than warm season grasses (Barbehenn et al., 2004) because it is higher in nonstructural protein, carbohydrates, and water and digest faster due to lower concentration of cell walls and leaf tissue than warm season (C4) forage, but lower levels of fiber and toughness, and lower total carbohydrate: protein than C4 grasses (Barbehenn et al., 2004; Wilson et al., 1983; Barbehenn & Bernays, 1992; Van Soest, 1993; Jung and Alle, 1995). Although warm season forage contains less protein, the utilization of this protein can be more efficient since a portion or fraction of the protein may evade digestion in the rumen (remain undegraded for longer periods) where microbes would utilize some of the protein (Redfearn et al., 1995; Van Soest, 1982; Mathison and Milligan, 1971). In addition, warm season grasses have

a thick-walled bundle sheath cells which are indigestible (Barbehenn et al., 2004). According to several researchers, warm season forages such as common bermudagrass (CB) and cool season forages such as tall fescue (E) can influence body condition score of beef cow, hormones, and reproductive performance.

1.2.1 Effects on Beef Cows Grazing Common Bermudagrass or Infected Tall Fescue, and the interaction of heat stress and toxic endophyte tall fescue

Poisonous plants are significant elements of many grazing lands (Taylor and Ralphs, 1992). All plants have toxins, and the quantity of the ingested toxins based on the type and amount of toxins and nutrients in the available forage (Provenza et al., 2003). Selection of nutritious diet from varied range of plants that differ in type and concentration of nutrients and toxins to meet ruminants' needs of nutrition depend on physiological and environmental conditions (Provenza, 1995). In addition, low body condition animals have different needs of nutrition for their growth and maintenance when compared to animals with adequate body condition (DeRouen et al, 1994). Therefore, to replace body fat reserves, thin body condition animals may try to ingest more available forage, including toxic plants (Sibbald and Kerr, 1994; Sibbald 1997, and Hayirli et al., 2002). Consequently, animals with low body condition may change their grazing behavior until they meet their nutritional needs (Lopez-Ortiz et al., 2007)

Endophyte-infected tall fescue is a significant kind of poisonous and nutritious plant, and it is available and abundant source of forage for grazing ruminants in the United States of America (Jacson et al., 1984). Endophyte tall fescue is prevalent cool season perennial grown forage in the United States for over 8.5 million cattle (Lyons, et al., 1986; Hoveland, 1993), particularly found across the eastern half of USA (Steen et al., 1979; Pendulum et al., 1980). Unfortunately, these plants are commonly toxic to cattle (Lyons et al., 1986). The toxic

14

syndrome that occurs due to the ingestion of infected tall fescue has been associated with grass endophytic infection caused by *Clavicipitaceae* (sexual fungus; Lyons et al., 1986; Tsai et al., 1994; Yates et al., 1985). The association of tall fescue with endophytes (seed-borne fungal symbiots) is necessary for the fitness of the host grass, and the association is important for its survival under environmental stress (Tasi et al., 1994). Consumption and grazing of the infected tall fescue can frequently cause and develop symptoms of fescue toxicosis (Stuedemann and Hoveland, 1988). The symptoms are characterized by decreased milk production, weight gains, reproductive efficiency, serum concentrations of prolactin and melatonin, increased rectal temperature and respiration rate, and altered neurotransmitter metabolism in the hypothalamus (Paterson et al., 1995; Hemken et al., 1981; Porter and Thompson, 1992). It has been suggested that ergot alkaloids produced by endophytes is the primary causative agent of fescue toxicosis (Porter and Thompson, 1992). Reduction of prolactin, elevation of body temperatures, and vasoconstrictive effects is caused by ergot alkaloid compounds (Porter and Thompson, 1992). This compound is commonly found and present in above ground parts of all infected tall fescue plants, and its synthesis in the grass does not require any special environment or host condition (Lyons et al., 1986). In addition, the concentration of ergot alkaloids can be affected by growth condition and other factors, and the concentration of these compounds increases with high nitrogen fertilization rate (Lyons et al., 1986). The ruminants can extract these compounds from plants tissue very efficiently, and long term consumption of small amounts of ergot alkaloids causes less acute symptoms (Lyons et al., 1986). On other hand, bermudagrass is a warm season and widely spread grass in the hot climates and it is adapted to high-temperature and high-light environments (Teeri and Stowe, 1976; Barbehenn et al., 2004). Furthermore, the anatomical and biochemical features that have produced this adaptation influence their nutritional quality for

herbivores (Barbehenn et al., 2004). Cattle grazing on common and costal bermudagrass developed a toxic syndrome in several southern states (Porter et al., 1974). This syndrome, commonly known as bermudagrass tremors, is characterized by a general nervousness in cattle, this symptom differs in degree from minor twitching or palsy of shoulder muscles and flank region to an inability to walk or stand due to posterior paralysis (Porter et al., 1974). An alkaloid fraction that consists of more than 45% ergot type alkaloid produced by isolated *Claviceps* from toxic *Cynodon dactylon* (common bermudagrass; Porter et al., 1974). Since the nervous or convulsive ergotism is a syndrome analogous to the syndrome of bermudagrass tremors, *Claviceps* might be a causative agent of bermudagrass tremors (nervous disorder; Porter et al., 1974).

Several researchers have demonstrated the interaction between infected tall fescue and environmental conditions such as temperature. fescue toxicosis increased during the months of July and August when temperature exceeded 32C (Hemken et al., 1981). This condition was associated with poor performance, changed with environmental temperature (Hemken et al., 1981; Jacobson et al., 1970). Since vasoconstriction can be caused by ergot alkaloids produced by endophytes (Porter and Thompson, 1992), as well as decrease blood flow to the skin (Solomon et al., 1989; Rhodes et al., 199; Oliver, 1997), an increase in body temperature due to inability to evaporate excess body heat through skin surface has been reported at elevated environmental temperature (Aldrich et al., 1993). Unlike body temperature, the reduction in the plasma prolactin as a result of the consumption of infected tall fescue did not interact with the environment temperature (Aldrich et al., 1993). The environmental temperature interacts with the consumption of infected tall fescue, and high temperature that exceed 32C lead to decrease forage intake in cattle grazing infected tall fescue (Paterson et al., 1995). In addition, it has been

16

reported that steers grazing infected tall fescue showed a modification in their physiology and body composition and reduction in daily weight gain (Nihsen, et al., 2004). Steers grazing infected tall fescue had long and rough hair coats, and cattle maintained their winter long hair coats until the summer (Nihsen et al., 2004; Stuedemann and Hoveland, 1988). Paterson et al. (1985) reported the interaction effects of E+ and environmental temperature on rats. Consumption of E+ at 32C leaded to slower body fat gain and obvious nutrient digestibility and lower PRL concentration compared with rats on non toxic forage. Additionally, rats on infected tall fescue seed at high environmental temperature increased body fat metabolism and reduced nutrient digestion (Paterson et al., 1985).

Heat stress and consumption of infected tall fescue association has been documented to have effects on the reproductive performance and endocrine status of cows. Cows grazing endophyte infected tall fescue lost body weight, low reproductive performance and milk production, and weaned weak calves (Paterson et al., 1995). Postpartum nutrition is important to ensure future reproduction, and heifers with BCS of 4 and 5 have less pregnancy rate and reproductive performance than heifers with BCS of 6 and 7 (DeRouen et al., 1994). Drewnoski et al. (2009) demonstrated that beef heifers grazing infected tall fescue (E+) forage in the spring reduced the average daily weight gain, but did not negatively affect the reproductive performance due to the cool temperature during breeding. Since grazing E+ did not affect the conception rate and since it reduced the gain thus failure to reach the target weight essential for reproductive efficiency, the authors suggested that E+ can be used as a source of winter forage in production system but alternative forage is required in the spring for heifer development (Drewnoski et al., 2009). Another study has shown the effects of the interaction of E+ and heat stress on ovarian function in beef heifers (Burke et al., 2001). Burke et al. (2001) determined that

the association of E+ and heat is necessary to cause a negative effect on the diameter of the preovulatory follicle, but neither E+ nor heat stress alone can impair follicle function. Moreover, this combination of E+ and heat stress reduced serum progesterone, estradiol, and total cholesterol compared with thermoneutral conditions. Therefore, their results indicated the severity of fescue toxicosis signs is less when heifers are not heat stressed (Burke et al., 2001). In agreement with the previous study, another study reported embryonic loss early in pregnancy after environmental temperatures increased above 37.8C, and pregnancy rate and embryonic loss were not affected under management conditions in cows grazing E+ (Burke et al., 2001). Looper et al. (2009) indicated a decrease in semen quality of bulls grazing toxic tall fescue associated with the increase of the surrounding temperature. Furthermore, endophytes have indirect effects on serum LH level and secretion. Consumption of E+ reduced body condition of beef cows and cycling heifers resulting in the decrease of LH and prolactin level (Mizinga et al., 1992). This result supported the result of Lishman et al. (1979) that indicated the decrease of serum LH concentration due to the reduction of energy intake in postpartum cows. According to Nihsen et al. (2004), suppression of serum prolactin, alkaline phosphatase (ALP), lactate dehydrogenase (LDH), cholesterol, and triglycerides was detected in steers grazing toxic endophyte.

Healthy productive cows are important to increase and manage profitability in livestock operations. Since cows' low BCS can negatively affect the cows/calves reproduction and weaning calves weight and health according to several studies indicated earlier, efficient forage/beef cattle system and condition management is required (Brown et al., 1993; Burke et al., 2001). Evidence from several studies reported a differential response in beef cattle reproductive performance to two forage systems [common bermudagrass (CB) and endophyte-infected tall fescue (E+)]. Brown et al. (1993) reported that the advantage of maternal weaning weight and

hip height effects were larger on bermudagrass than toxic tall fescue. Forage environment and body condition interaction has been reported; thin body condition cows grazing E+ had the least BCS, reduced calving rate and IGF-1 and prolactin (PRL) concentrations. Whereas thin BC cows grazing CB had increased in BC and body weight and prolactin, IGF-, and cortesol concentrations were greater than in cows grazing E+ (Looper et al., 2010). On the other hand, cows in a good body condition grazing E+ had decreased PRL and had increased PRL on CB; there was no change in IGF-1 concentration in good body cows grazing either CB or E+. Good-BC cows grazing either CB or E+ lost body weight during breeding season, as well as the level of calving rate (Looper et al., 2010). However, cows in a good body condition could be more tolerant to E+ negative effects (Looper et al., 2008). Moving cows and calves from E+ to CB in summer may reduce the negative effects of E+ and supplementation alternative non toxic forage with E+ may reduce E+ intake (Brown et al., 2000; Stokes et al., 1988).

It has been reported that not only cows but also horses can be negatively affected by consumption of E+ (Porter and Thompson, 1992). According to a Missouri survey, 26% of horses and mare farms on E+ had indicated reproductive problems (53% agalactia, 18% abortion, 16% foal losses, and 9% thickened placentas; Garrett et al., 1980). Agalactia and foal mortalities have been reported in mares on E+ (Taylor et al., 1985). Similar reproductive abnormalities such as agalactia, placental thickening, and high foal mortality due to consumption of E+ have been described (Poppenga et al., 1984). The decreased blood flow to the placenta may result in placental changes consistent with hypoxia (Poppenga et al., 1984). Therefore, pregnant mares on E+ have more critical consequences than sheep or cattle (Porter and Thompson, 1992).

The decreased pregnancy rate and cows/calf weight gains due to consumption of toxic tall fescue have been estimated to cause economic losses in the United States more than $600

million annually (Hoveland, 1993). Therefore, to elucidate the causative agent and to reduce the symptoms of tall fescue toxicosis to minimize the economic losses many considerable research has been done.

1.3 Lactate dehydrogenase

Several investigators demonstrated that stage of growth and lactation, breed, age, and reproductive status are variables which influence many blood parameters (Doornenbal et al., 1988). These blood parameters such as hormones and metabolite concentrations, in prepartum cows, and their relationship with calves birth and postpartum evaluation have been investigated to be used as an indicator for reproductive performance, body energy reserve, calf birth weight, and carcass quality (Guedon et al., 1999; Abeni et al., 2004; Flores et al., 2005). In addition, enzymes such as lactate dehydrogenase, in prepartum cows, have been suggested to have a relationship with prepartum cow's activity and calf performance and then be used as a marker to predict some disease, carcass composition, exercise performance, and nutritional status (Looper et al., 2008; Flores et al., 2005; Doornenbal et al., 1985; Akerstedt et al., 2010).

Lactate dehydrogenase can be found in a wide variety of organisms such as mammals, birds, and plants. It is found in the liver, skeletal and heart muscles, lung, spleen, pancreas and kidney, and each of these organs have different enzymatic activity (Wroblewski, 1958). Furthermore, lactate dehydrogenase (LDH) has been found in extracellular fluid, including, serum, urine, cerebrospinal and synovial fluid in both humans and animals (Wroblewski, 1958; Rejno, 1976). Therefore, LDH is widely distributed in body tissues, but the activity of the enzyme is not organ specific. However, LDH isozymes activity and regulation can be organ specific (Markert et al., 1975; Goldberg, 1977)

Lactate dehydrogenase consists of at least two structural-function domains (Li et al., 1985). One domain binds to coenzyme and another binds to the substrate (Li et al., 1985). It is a zinc-containing metalloenzyme (Valle and Wacker, 1956; Gallagher, 1964). In mammals and birds, lactate dehydrogenase is present in five somatic isozymes, which can be separated by electrophoresis (Li et al 1985; Cardinet, 1997). These five somatic isozymes are tetrameric combinations of two 35-kDa subunits, including M (muscles) and H (heart); whereas C subunit is found only in mature testes and sperm (Markert et al., 1975; Goldberg, 1963; Jungmann et al., 1998). The five somatic isoenzyme types with different combinations of two subunits are LDH-1 (B4), LDH-2 (AB3), LDH-3 (A2B2), LDH-4 (A3B), and LDH-5 (A4) (Jungmann et a., 1998; Goldberg, 1963). In skeletal muscles, LDH-5 is the main and common form of LDH, and it has a plasma half life of less than six hours (Stockham and Scott, 2002). LDH-2 and LDH-3 concentration is high in case of lung damage. LDH-1 and LDH-2 are dominant in ruminants' liver while LDH-4 and LDH-5 are the most common in dog and cat' liver (Washizu et al., 2002, Arai et al., 2003). In human liver, LDH-5 is prevalent; whereas, LDH-1 and LDH-2 are common in chicken liver (Cahn et al., 1962).

As a cytoplasmic enzyme LDH catalyzes the conversion of the pyruvate to lactate (forward; LDHf) or lactate to pyruvate (reveres; LDHr) in the last step of glycolysis (Hinchcliff et al., 2004, Looper et al., 2008). Pyruvate is reduced in the presence of the reduced form of nicotinamide adenine dinucleotide (NADH) to produce lactate and oxidized nicotinamide adenine dinucleotide (NAD+; Katz and Sahlin, 2002). During normal metabolism and exercise in animals, LDH catalyzes the reaction of conversion of pyruvate to lactate as an energy source to fuel the muscles (Chapman, 1989).

Lactate is the product of glycolysis during exercise in working muscle due to consumption of oxygen (O2) and insufficient delivery of O2 to the muscles resulting in anaerobic metabolism to provide anaerobic energy (Wasserman et al., 1973). Since inadequate delivery to the muscles produces lactate, accumulation of lactate during exercises in working muscles and blood requires about 1/2 to 2/3 (50- 70%) of the maximal O2 uptake (VO2 max) (Katz and Sahlin, 1988; Gladden, 2004). Additionally, lactate is the last product in glycolysis as a result of hypoxia (insufficient O2 supply to the organ following exercises) which is the main cause of muscle fatigue and acidosis triggered tissue damage (Gladden, 2004). Katas et al., (1988) suggested that low supplementation of O2 to mitochondria resulted in an impairment of mitochondrial respiration increase NADH in muscles, which increases lactate production. Therefore, this result demonstrated that the major inducement for the conversion of pyruvate to lactate during exercise is an increased cytosolic redox state (balance of NADH/NAD+ and NAD+/NADH in the biological system). The redox state depends on the availability of the O2. Therefore, this result supports the hypothesis that lactate production during submuscular exercise depends on O2 availability in contracting muscles (Katz and Sahlin, 1988). LDH activity should be applicable as an accurate marker of the cytosolic redox state, and has been used in several tissues such as liver, heart, skeletal muscles, and erythrocytes (Akerboom et al., 1979).

Lactate concentration along with LDH activity in response to exercise and stress can be used as a marker for health and fitness in humans and animals. In addition, several studies done on animals and humans shown the relationship between lactate and LDH, It has been reported that the increase of lactate is accompanied by an increase in LDH activity in order to utilize lactate or to convert pyruvate to lactate. The increase of lactate concentration in the skeletal muscles due to submaximal exercise is responsible for development of muscle fatigue in animals

22

and humans (Pilegaard et al., 1999). Since the accumulation of lactate in skeletal muscles during intense exercise as a result of low O2 supplementation, several studies have been done to examine whether training can increase the maximum O2 uptake capacity (VO2max) thus reducing in LDH activity consequently decrease in lactate production.

The elevation in serum LDH was marked in untrained rat after endurance exercise. This increase in not exclusively derived from muscle tissue (Garbus et al., 1963). The authors suggested that a significant portion contributed by heart and probably some other tissue might be responsible for the increase of LDH during prolonged exercise. In contrast, exercise trained rats were protected against the increase of LDH concentration and other abnormal isoenzymes in their serum after endurance exercise. Moreover, the authors have shown that trained rats have fewer amounts of fat than untrained rats due to rapid metabolism of fat to meet their energy requirement after utilization of stored glycogen maintaining normal cellular permeability and decrease lactate concentration and LDH activity. An increase in muscle vascularity in trained individuals resulted in an increase in blood flow; therefore, reducing in tissue hypoxia which is one of the factors of the increase of lactate production (Garbus et al., 1963).

Similar results have been demonstrated on exercise trained late pregnant and non-pregnant dairy cows (Davidson and Beede, 2008). The authors reported that exercise training of late-pregnant and non-pregnant dairy cows improved their fitness. Improvement of fitness in trained late-pregnant and non-pregnant dairy cows is due to more increase in muscle oxidative capacity resulting in lower production and LDH concentration when compared to non-exercised cows. Moreover, trained cows experienced less metabolic stress related to anaerobic metabolism and metabolic acidosis than non-exercised cows. Improvement of overall energy status of cows might be attributed to the acceleration in muscle oxidative capacity. Trained animals may show

improvement in their response to stressors associated with parturition or environmental changes (Davidson and Beede, 2008). In addition, reduction in muscle fatigue after parturition might cause an increase in postpartum feed intake (Davidson and Beede, 2008). A study done on Pirenaica calves reported that Pirenaica calves' muscles enzyme activities such as LDH and creatine kinase were always higher than cows of the same breed under basal conditions and after exercise (Belenguer et al., 1996). These results reflect the calves' sensitivity to muscular work, and often show skeletal and cardio-muscular problems when they walk to mountain pasture resulting in muscular fatigue. In addition, the emotional stress also may be higher in calves than cows of the same breed. So, these results suggested that age plays a significant role in muscle metabolism development due to muscle training or to muscle maturation (Belenguer et al., 1996). Similarly, horse training resulted in minor changes in blood lactate levels and lactate to pyruvate ratio due to either lower production of lactate or due to an increase in VO2max in working muscle causing an increase in aerobic pathway or rapid release of lactate in order to reconvert lactate to glucose in liver (Anderson, 1975).

The previous results that demonstrated the effect of training on blood lactate and LDH levels are similar to the results from several investigations with humans. The reduction in muscle force during muscle fatigue development in humans as a result of intense exercise is associated with accumulation of lactate and accompanies low pH in the muscle (Pilegaard et al., 1999). Additionally, the working muscles becomes hypoxic during intense exercise in non-exercised individuals while hypoxia will decrease in trained individuals exposed to the same type of exercise due to increase of VO2max in response to training (Holloszy et al., 1984). Moreover, the depletion of the stored glycogen in muscles is less rapid in trained than untrained persons. Endurance training changes the glycolytic enzyme activities resulting in reduction in skeletal

24

muscles exercise and LDH isozymes (Holloszy et al., 1984). This result is similar to the results

of trained and untrained rats. Another result indicated that intense exercise training can elevate

transportation capacity of lactate/H+ in human skeletal muscle, and improve release of lactate

and H+ in muscles during contractions (Pilegaard et al., 1999). Therefore, the adaptation of

working muscles to prolonged exercise increases VO2max thus decreasing the development of

hypoxia that leads to muscle fatigue and decreases lactate accumulation as well as LDH activity

in humans and animals. However, the exposure to two stresses such as exercise and epinephrine

can increase LDH activity in exercise trained individuals; therefore, endurance training will not

protect them against cardiac stress stimulated by epinephrine. Yet, trained individuals will be

more able to cope with two stresses than untrained individuals (Garbus et al., 1963).

Stresses other than exercise and cardiac stress that are induced by epinephrine such as

weaning stress in calves and fasting have been reported to have effects on LDH activity. Feeder

cattle may respond to weaning, transit, and different periods of fasting and refeeding (Galyean et

al., 1981; Crookshank et al., 1979). The level of LDH activity was increased in fasting and

fasting transit steers (Galyean et al., 1981). Ruppanner et al. (1978) observed that lightweight

transit calves showed elevated LDH, and increased concentrations last up to twenty one days

after arrival in the feedlot. Transport and handling process of beef cattle during normal

marketing could be an important stressor (Schaefer et al., 1997). These kinds of stressors can

reduce body weight and carcass yield, decrease meat quality, and lower animal well-being

(Schaefer et al., 1997). Likewise, most athletes during time of competition are susceptible to loss

of their body weight due to competition stress, so the nutritional supplement to prevent

dehydration, electrolyte loss, and hypoglycemia is very important during such times (Schaefer et

al., 1997). Electrolyte therapy has been reported to also reduce stress in transported cattle

(Schaefer et al., 1997). Averós et al. (2008) indicated that young bulls showed stress response prior the transportation. So it is essential to improve pre-transport conditions to avoid the effect of the handling and transportation stress such as dehydration and physical exhaustion sign. The LDH level was decreased at the end of the transportation due to low physical demand of young bulls in conventional conditions during transportation in response to long transport stress (Averós et al., 2008).

Pre-slaughter handling stress showed a significant increase in LDH activities in pasture-fed steers, and it also showed glycogen depletion in pasture-fed steers due to the inducement of nervous system as a result of excitation and stress (Daly et al., 1999). Therefore, excitation and stress elevated secretion of adrenaline and noradrenaline in ruminants which is the main cause of muscle glycogen depletion resulting in an increase in lactate concentration (Daly et al., 1999). Since this experiment showed a low physical activity by cattle, the concentration of enzyme level was high due to membrane permeability in response to stress.

Lactate usually present in the digestive tract only at low concentrations of ruminants (Owens et al., 1998). Acidosis is digestive disturbance of rumen and intestines, and it has two forms: acute and chronic (Owens et al., 1998). Acute acidosis occurred after a large amount of starch or other rapidly fermented carbohydrate intake (Owens et al., 1998). Lactate accumulation in the rumen due to a quick change from high forage to high-concentrate diet can lead to acute acidosis which is a marked illness (Goad et al., 1998; Co et al., 1999; Owens et al., 1998). Acidosis can decrease performance and feed intake (Owens et al., 1998; Koers et al., 1976). Bevans et al. (2005) reported that lactate concentration increased in rapid adaptation (high-concentrate diet) heifers, as well as LDH activity. Lactate dehydrogenase has a tendency to increase during acidosis in order to metabolize lactic acid, and it can be used as a marker of

metabolic acidosis (Owens et al., 1998; Bevans et al., 2005). Additionally, LDH activity can be used as a marker for some diseases in human and animals. A two-fold increase in serum ALP, AST and LDH concentrations was determined in calves with foot and mouth disease while other haematological and biochemical findings in calves were normal (Gunes et al., 2011). LDH is increased in cows with mastitis (Viguier et al., 2009). LDH, N-acetyl-β-D-glucosaminidase (NAGase) and alkaline phosphatase (AP), parameters have been suggested as markers for mastitis, and LDH was the biomarker with the lowest variation (Åkerstedt et al., 2010). In humans LDH activity in patients (adults and children) with acquired immune deficiency syndrome (AIDS) or AIDS-related complex may be a useful indicator of pulmonary interstitial inflammation (Silverman and Rubinstein, 1984).

Age, nutrition, stage, and sex affect LDH activity in cattle LDH activity can be used as marker for energy and nutritional intake. It has been shown that LDH activity increases during lactation although LDH is low in the early lactation and increases with age in cows; whereas, LDH decreases with age in steers, and bulls have higher LDH activity than steers (Doornenbal et al., 1985). Grain diet increases stored muscle glycogen concentration which can decrease lactate and LDH activity due to increases in aerobic pathway, but pasture-fed steers had higher LDH activity than grain-fed steers (Daly et al., 1999). The interaction between physiological stage and feeding management system was important for LDH activity (Petreson and Waldern, 1981). LDH activity of lactating cows in winter is higher than during summer while non-lactating cows showed the opposite affect (Paterson and Waldern, 1981). In addition, LDHr activity was less in calves with choice carcasses than calves with select carcasses at time of feedlot entry thus LDH was an indicator for carcass composition and quality (Flores et al., 2005). It has been suggested that LDH activity was constant in mature cows although Angus-sired cows had greater

27

prepartum LDHr than Charolais-sired cows, and Angus –sired calves had high LDHf activity and low LDHr when compared with Charolais-sired calves (Looper et al., 2008). Moreover, LDHr was low in Angus heifers with high reproductive performance (Looper et al., 2002). This data suggested that the increase in reproduction in cattle is related to decrease LDHr activity in heifers.

1.4 Literature Cited

Abeni, F., G. Bergoglio, G. Masoero, G. M. Terzano, and S. Allegrini. 2004. Plasma hormones and metabolites in piedmontese cows during late pregnancy: Relationships with calf birth weight. J. Anim. Sci. 82:438-444.

Åkerstedt, M., L. Forsbäck, T. Larsen, and K. Svennersten-Sjaunja. 2011. Natural variation in biomarkers indicating mastitis in healthy cows. J. Dairy Res. 78: 88.

Aldrich, C. G., J. A. Paterson, J. L. Tate, and M. S. Kerley. 1993. The effects of endophyte-infected tall fescue consumption on diet utilization and thermal regulation in cattle. J. Anim. Sci. 71:164-170.

Anderson, M. G. 1975. The effect of exercise on blood metabolite levels in the horse. Equine Vet. J. 7:27-33.

Averós, X., S. Martín, M. Riu, J. Serratosa, and L. F. Gosálvez. 2008. Stress response of extensively reared young bulls being transported to growing-finishing farms under spanish summer commercial conditions. Livest Sci. 119:174-182.

Barbehenn, R. V., and E. A. Bemays. 1992. Relative nutritional quality of C3 and C4 grasses for a graminivorous lepidopteran, Paratrytone rnelane (hesperiidae). Oecol. 92:97- 103.

Barbehenn, R. V., Z. Chen, D. N. Karowe, and A. Spickard. 2004. C3 grasses have higher nutritional quality than C4 grasses under ambient and elevated atmospheric CO2. Global Change Biol. 10:1565-1575.

Barker, D., and P. Clark. 1997. Fetal undernutrition and disease in later life. Rev. Reprod. 2:105-112.

Bartle, S. J., J. R. Males, and R. L. Preston. 1984. Effect of energy intake on the postpartum interval in beef cows and the adequacy of the cow's milk production for calf growth. J. Anim. Sci. 58:1068-1074.

Bellows, R. A., and R. E. Short. 1978. Effects of precalving feed level on birth weight, calving difficulty and subsequent fertility. J. Anim. Sci. 46:1522-1528.

Bellows, R. A., R. E. Short, D. C. Anderson, B. W. Knapp, and O. F. Pahnish. 1971. Cause and effect relationships associated with calving difficulty and calf birth weight. J. Anim. Sci. 33:407-415.

Bellows, R. A., R. E. Short, and G. V. Richardson. 1982. Effects of sire, age of dam and gestation feed level on dystocia and postpartum reproduction. J. Anim. Sci. 55:18-27.

Berry D.P., K.A. Macdonald, J.W. Penno, J.R. Roche. Association between body condition score and live weight in pasture-based Holstein-Friesian dairy cows. 2006. J Dairy Res. 73:487-91.

Bevans, D. W., K. A. Beauchemin, K. S. Schwartzkopf-Genswein, J. J. McKinnon, and T. A. McAllister. 2005. Effect of rapid or gradual grain adaptation on subacute acidosis and feed intake by feedlot cattle. J. Anim. Sci. 83:1116-1132.

Brown, M. A., A. H. Brown Jr, W. G. Jackson, and J. R. Miesner. 2000. Genotype x environment interactions in angus, brahman, and reciprocal-cross cows and their calves grazing common bermudagrass, endophyte-infected tall fescue pastures, or both forages. J. Anim. Sci. 78:546-551.

Brown, M. A., L. M. Tharel, A. H. Brown Jr, W. G. Jackson, and J. R. Miesner. 1993. Genotype x environment interactions in preweaning traits of purebred and reciprocal cross angus and brahman calves on common bermudagrass and endophyte-infected tall fescue pastures. J. Anim. Sci. 71:326-333.

Burke, J. M., R. W. Rorie, E. L. Piper, and W. G. Jackson. 2001. Reproductive responses to grazing endophyte-infected tall fescue by postpartum beef cows. Theriogenology. 56:357-369.

Burke, J. M., D. E. Spiers, F. N. Kojima, G. A. Perry, B. E. Salfen, S. L. Wood et al. 2001. Interaction of endophyte-infected fescue and heat stress on ovarian function in the beef heifer. Biology of Reproduction. 65:260-268.

Cavinder C. A., M. M. Vogelsang, P. G. Gibbs, D. W. Forrest, and D. G. Schmitz. 2009. Variances in Reproductive Efficiency of Mares in Fat and Moderate Body Conditions Following Parturition. Prof. Anim. Sci. 25:250-255.

Cardinet, G.H. 1997. Skeletal muscle function. In: Kaneko, J.J, J.W. Harvey, M.C. Bruss, eds. Clinical biochemistry of domestic animal. 5th ed. Academic Press; p407-440. San Diego, CA

Chapman, J.F. 1989. Lactate dehydrogenase isoenzymes. In: Clinical chemistry: theory, analysis, and correlation. 2nd Ed. edited by Kaplan, and A.J. Pesce. St. Louis: C.V. Mosby Co. P928-933.

Ciccioli, N. H., R. P. Wettemann, L. J. Spicer, C. A. Lents, F. J. White, and D. H. Keisler. 2003. Influence of body condition at calving and postpartum nutrition on endocrine function and reproductive performance of primiparous beef cows. J. Anim. Sci. 81:3107-3120.

Clutter, A. C., and M. K. Nielsen. 1987. Effect of level of beef cow milk production on pre- and postweaning calf growth. J. Anim. Sci. 64:1313-1322.

Coe, M. L., T. G. Nagaraja, Y. D. Sun, N. Wallace, E. G. Towne, K. E. Kemp et al. 1999. Effect of virginiamycin on ruminal fermentation in cattle during adaptation to a high concentrate diet and during an induced acidosis. J. Anim. Sci. 77:2259-2268.

Corah, L. R., T. G. Dunn, and C. C. Kaltenbach. 1975. Influence of prepartum nutrition on the reproductive performance of beef females and the performance of their progeny. J. Anim. Sci. 41:819-824.

Crookshank, H. R., M. H. Elissalde, R. G. White, D. C. Clanton, and H. E. Smalley. 1979. Effect of transportation and handling of calves upon blood serum composition. J. Anim. Sci. 48:430-435.

Daly, C. C., O. A. Young, A. E. Graafhuis, S. M. Moorhead, and H. S. Easton. 1999. Some effects of diet on beef meat and fat attributes. N. Z. J. Agric. Res.. 42:279-287.

Davidson, J. A., and D. K. Beede. 2009. Exercise training of late-pregnant and nonpregnant dairy cows affects physical fitness and acid-base homeostasis. J. Dairy Sci. 92:548-562.

DeRouen, S. M., D. E. Franke, D. G. Morrison, W. E. Wyatt, D. F. Coombs, T. W. White et al. 1994. Prepartum body condition and weight influences on reproductive performance of first-calf beef cows. J. Anim. Sci. 72:1119-1125.

Doornenbal, H., A. K. Tong, and N. L. Murray. 1988. Reference values of blood parameters in beef cattle of different ages and stages of lactation. Can. J. Vet. Res. 52: 99-105.

Drewnoski, M. E., E. J. Oliphant, M. H. Poore, J. T. Green, and M. E. Hockett. 2009. Growth and reproductive performance of beef heifers grazing endophyte-free, endophyte-infected and novel endophyte-infected tall fescue. Livestock Science. 125: 254-260.

Dunn, T. G., J. E. Ingalls, D. R. Zimmerman, and J. N.Wiltbank. 1969. Reproductive performance of 2-year-old Hereford and Angus heifers as induced by pre- and postcalving energy intake. J. Anim. Sci. 29:719-726.

Dunn, T. G., and C. C. Kaltenbach. 1980. Nutrition and the postpartum interval of the ewe, sow and cow. J. Anim. Sci. 51: 29-39.

Ferrell, C. L., W. N. Garrett, N. Hinman, and G. Grichting. 1976. Energy utilization by pregnant and non-pregnant heifers. J. Anim. Sci. 42:937-950.

Ferrell, C. L., and T. G. Jenkins. 1984. Energy utilization by mature, nonpregnant, nonlactating cows of different types. J. Anim. Sci. 58:234-243.

Fleck, A. T., R. R. Schalles, and G. H. Kiracofe. 1980. Effect of growth rate through 30 months on reproductive performance of beef heifers. J. Anim. Sci. 51:816-821.

Flores, R. 2005. Relationship between calf serum metabolites at feedlot entry and subsequent carcass charactreristics i brangus-crossbred calves. Proceeding, Western Section, American Society Animal Science. 56: 240-242.

Flores, R., M. L. Looper, R. W. Rorie, D. M. Hallford, and C. F. Rosenkrans. 2008. Endocrine factors and ovarian follicles are influenced by body condition and somatotropin in postpartum beef cows. J. Anim. Sci. 86:1335-1344.

Fox, D. G., C. J. Sniffen, and J. D. O'Connor. 1988. Adjusting nutrient requirements of beef cattle for animal and environmental variations. J. Anim. Sci. 66:1475-1495.

Frisch, RE. 2002. Female Fertility and the Body Fat Connection. paperback Ed 2004. With a Foreword by Robert L. Barbieri, MD. Univ. of Chicago Press.

G. Selk. 2004. Body condition scoring of beef cows. Oklahoma State University. Oklahoma Cooperative Extension Service Publication ANSI-3283.

Gallagher, C.H. 1964. In nutrition factors and enzymological disturbances in animals. Cosby Lockwood and Son Ltd., London.

Galyean, M. L., R. W. Lee, and M. E. Hubbert. 1981. Influence of fasting and transit on ruminal and blood metabolites in beef steers. J. Anim. Sci. 53:7-18.

Garbus, J., B. Highman, and P. D. Altland. 1964. Serum enzymes and lactic dehydrogenase isoenzymes after exercise and training in rats. Am. J. Physiol. 207:467-472.

Gearhart, M. A., C. R. Curtis, H. N. Erb, R. D. Smith, C. J. Sniffen, L. E. Chase et al. 1990. Relationship of changes in condition score to cow health in holsteins. J. Dairy Sci. 73:3132-3140.

Gentry, L. R., D. L. Thompson, G. T. Gentry, K. A. Davis, R. A. Godke, and J. A. Cartmill. 2002. The relationship between body condition, leptin, and reproductive and hormonal characteristics of mares during the seasonal anovulatory period. J. Anim. Sci. 80:2695-2703.

Gladden, L. B. 2004. Lactate metabolism: A new paradigm for the third millennium. The Journal of Physiology. 558:5-30.

Goad, D. W., C. L. Goad, and T. G. Nagaraja. 1998. Ruminal microbial and fermentative changes associated with experimentally induced subacute acidosis in steers. J. Anim. Sci. 76:234-241.

Goldberg, E. 1963. Lactic and malic dehydrogenase in human spermatozoa. Science. 139: 602-603.

Grainger C., G.D. Wilhelms, A.A McGowan. 1982. Effect of body condition at calving and level of feeding in early lactation on milk production of dairy cows. Aust. J. Exp. Agri. and Anim. Husbandry. 22:9–17.

Guedon, L., J. Saumande, and B. Desbals. 1999. Relationships between calf birth weight, prepartum concentrations of plasma energy metabolites and resumption of ovulation postpartum in limousine suckled beef cows. Theriogenology. 52:779-789.

Gunes, V., H. M. Erdogan, M. Citil, and K. Ozcan. 2005. Assay of cardiac troponins in the diagnosis of myocardial degeneration due to foot-and-mouth disease in a calf. Vet. Rec. 156:714-715.

Halls, L. K., R. H. Hughes, R. S. Rumell, and B. L. Southwell.1964. Forage and cattle management in longleaf-slash pine forests. Washington, D.C.: united states department of agriculture.

Hartz A. J., P. N. Barboriak, A. Wong, K. P. Katayama, and A. A. Rimm. 1979. The association of obesity with infertility and related menstrual abnormalities in women. Int. J. Obes. 3:57.

Hayirli, A., R. R. Grummer, E. V. Nordheim, and P. M. Crump. 2002. Animal and dietary factors affecting feed intake during the prefresh transition period in Holsteins. J. Dairy Sci. 85:3430

Hemken, R. W., J. A. Boling, L. S. Bull, R. H. Hatton, R. C. Buckner, and L. P. Bush. 1981. Interaction of environmental temperature and anti-quality factors on the severity of summer fescue toxicosis. J. Anim. Sci. 52:710-714.

Henneke, D.R., G.D. Potter, and J.L. Kreider. 1984. Body condition during pregnancy and lactation and reproductive efficiency of mares. Theriogenology. 21:897-909

Herd, D.B. and L.R. Sprott. 1986. "Body condition, nutrition, and reproduction of beef cows."Texas Agric. Ext. Ser. Bull. No.B-1526.

Hinchcliff, KW., A. Kaneps, and R, Geor. 2004. Equine Sports Medicine and Surgery. Basic and clinical science of the Equine Athlete. First edition. Saunders, imprint of Elsevier.

Hintz, H. F., and N. F. Cymbaluk. 1994. Nutrition of the horse. Annu. Rev. Nutr. 14:243-267.

Hoffman, R. M., R. C. Boston, D. Stefanovski, D. S. Kronfeld, and P. A. Harris. 2003. Obesity and diet affect glucose dynamics and insulin sensitivity in thoroughbred geldings. J. Anim. Sci. 81:2333-2342.

Hollmann, M., B. Runnebaum, and I. Gerhard. 1996. Infertility: Effects of weight loss on the hormonal profile in obese, infertile women. Human Reproduction. 11:1884-1891.

Holloszy, J. O., and E. F. Coyle. 1984. Adaptations of skeletal muscle to endurance exercise and their metabolic consequences. J. App. Physiol. 56:831-838.

Houghton, P. L., R. P. Lemenager, L. A. Horstman, K. S. Hendrix, and G. E. Moss. 1990. Effects of body composition, pre- and postpartum energy level and early weaning on reproductive performance of beef cows and preweaning calf gain. J. Anim. Sci. 68:1438-1446.

Hoveland, C. S. 1993. Importance and economic significance of the acremonium endophytes to performance of animals and grass plant. Agric. Ecosyst. Environ. 44:3-12.

Jung, H. G., and M. S. Allen. 1995. Characteristics of plant cell walls affecting intake and digestibility of forages by ruminants. J. Anim. Sci. 73:2774-2790.

Jungmann, R.A., D. Hurry, and Tran. 1998. Regulation of LDH-A gene expression by transcriptional and post transcriptional signal transduction mechanisms. J. Exp. Zool. 28L: 188-195.

Kang S.K., C.Y. Ha, K.H. Cho, S.K. Park, U.H. Kim. Changes of lactate dehydrogenase and its isoenzyme activity in renal diseases. 1991. Nephron. 57:55-9.

Katz, A., and K. Sahlin. 1988. Regulation of lactic acid production during exercise. J. App. Physiol. 65:509-518.

Klosterman, E. W., L. G. Sanford, and C. F. Parker. 1968. Effect of cow size and condition and ration protein content upon maintenance requirements of mature beef cows. J. Anim. Sci. 27: 242-246.

Koers, W. C., R. Britton, T. J. Klopfenstein, and W. R. Woods. 1976. Ruminal histamine, lactate and animal performance. J. Anim. Sci. 43:684-691.

Kress, D. D., E. R. Hauser, and A. B. Chapman. 1969. Efficiency of production and cow size in beef cattle. J. Anim. Sci. 29:373-383.

Kunkle, W.E., R.S. Sand and D.O. Rae. 1994. Effect of Body Condition on Productivity in Beef Cattle. In: Factors Affecting Calf Crop. Eds. Michael J. Fields and Robert S. Sand. CRC Press. Boca Raton.

Lalman, D. L., D. H. Keisler, J. E. Williams, E. J. Scholljegerdes, and D. M. Mallett. 1997. Influence of postpartum weight and body condition change on duration of anestrus by undernourished suckled beef heifers. J. Anim. Sci. 75:2003-2008.

Lambers H., F. S. Chapin, III, and T. L. Pons. 1998. Pages 10-72 in Plant Physiological Ecology. Springer-Verlag. New York, Inc.

Lawrence, L., S. Jackson, K. Kline, L. Moser, D. Powell, and M. Biel. 1992. Observations on body weight and condition of horses in a 150-mile endurance ride. J. Equine Vet. Sci. 12:320-324.

LI, S. S. -., H. F. TIANO, K. M. FUKASAWA, K. YAGI, M. SHIMIZU, F. S. SHARIEF et al. 1985. Protein structure and gene organization of mouse lactate dehydrogenase-A isozyme. Europ. J. of Bioch.: 149:215-225.

Long, C. R., T. S. Stewart, T. C. Cartwright, and T. G. Jenkins. 1979. Characterization of cattle of a five breed diallel: I. measures of size, condition and growth in bulls. J. Anim. Sci. 49:418-431.

Looper, M. L., S. T. Reiter, B. C. Williamson, M. A. Sales, D. M. Hallford, and C. F. Rosenkrans. 2010. Effects of body condition on measures of intramuscular and rump fat, endocrine factors, and calving rate of beef cows grazing common bermudagrass or endophyte-infected tall fescue. J. Anim. Sci. 88:4133-4141.

Looper, M. L., S. T. Reiter, B. C. Williamson, M. A. Sales, D. M. Hallford, and C. F. Rosenkrans Jr. 2010. Effects of body condition on measures of intramuscular and rump fat, endocrine factors, and calving rate of beef cows grazing common bermudagrass or endophyte-infected tall fescue. J. Anim. Sci. 88:4133-4141.

Looper, M. L., R. W. Rorie, C. N. Person, T. D. Lester, D. M. Hallford, G. E. Aiken et al. 2009. Influence of toxic endophyte-infected fescue on sperm characteristics and endocrine factors of yearling brahman-influenced bulls. J. Anim. Sci. 87:1184-1191.

Looper, M.L., Neidecker, T.P.,Wall, C.W., Reiter5, S.T., Flores, R., Brown, A.H., Jr., Johnson, Z.B., and Rosenkrans, C.F., Jr. 2002. Relationship of Lactate Dehydrogenase Activity with Body Measurements of Angus x Charolais Cows and Calves. Prof. Anim. Sci. 18:120.

Lopez-Ortiz, S., K. E. Panter, J. A . Pfister{ddagger}, and K. L. Launchbaugh. 2004. The effect of body condition on disposition of alkaloids from silvery lupine (lupinus argenteus pursh) in sheep. J. Anim. Sci. 82:2798-2805.

Lyons, P. C., R. D. Plattner, and C. W. Bacon. 1986. Occurrence of peptide and clavine ergot alkaloids in tall fescue grass. Science. 232:487-489.

Mathison, G. W., and L. P. Milligan. 1971. Nitrogen metabolism in sheep. Br. J. Nutr. 25:351.

Mizinga, K. M., F. N. Thompson, J. A. Stuedemann, and T. E. Kiser. 1992. Effects of feeding diets containing endophyte-infected fescue seed on luteinizing hormone secretion in postpartum beef cows and in cyclic heifers and cows. J. Anim. Sci. 70:3483-3489.

Morrow, D. A. 1976. Fat cow syndrome. J. Dairy Sci. 59:1625-1629.

Nagy, P., G. Huszenicza, J. Juhász, M. Kulcsár, L. Solti, J. Reiczigel et al. 1998. Factors influencing ovarian activity and sexual behavior of postpartum mares under farm conditions. Theriogenology. 50:1109-1119.

Nelsen, T. C., R. E. Short, W. L. Reynolds, and J. J. Urick. 1985. Palpated and visually assigned condition scores compared with weight, height and heart girth in hereford and crossbred cows. J. Anim. Sci. 60:363-368.

Nihsen, M. E., E. L. Piper, C. P. West, R. J. Crawford, T. M. Denard, Z. B. Johnson et al. 2004. Growth rate and physiology of steers grazing tall fescue inoculated with novel endophytes. J. Anim. Sci. 82:878-883.

Nihsen, M. E., E. L. Piper, C. P. West, R. J. Crawford, T. M. Denard, Z. B. Johnson et al. 2004. Growth rate and physiology of steers grazing tall fescue inoculated with novel endophytes. J. Anim. Sci. 82:878-883.

Oliver, J.W. 1997. Physiological manifestation of endophyte toxicosis in ruminant and laboratory species. In: C.W. Bacon and N.S. Hill (ed.) Neotyphodium/Grass Interaction. pp 311-346. Plenum Press, New York.

Owens, F. N., D. S. Secrist, W. J. Hill, and D. R. Gill. 1998. Acidosis in cattle: A review. J. Anim. Sci. 76:275-286.

Pasquali, R., F. Casimirri, and V. Vicennati. 1997. Weight control and its beneficial effect on fertility in women with obesity and polycystic ovary syndrome. Human Reproduction. 12(suppl 1):82-87.

Paterson, J., C. Forcherio, B. Larson, M. Samford, and M. Kerley. 1995. The effects of fescue toxicosis on beef cattle productivity. J. Anim. Sci. 73:889-898.

Patterson, D. J., L. R. Corah, J. R. Brethour, M. F. Spire, J. J. Higgins, G. H. Kiracofe et al. 1991. Evaluation of reproductive traits in bos taurus and bos indicus crossbred heifers: Effects of postweaning energy manipulation. J. Anim. Sci. 69:2349-2361.

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

Pendlum, L. C., J. A. Boling, L. P. Bush, and R. C. Buckner. 1980. Digestibility and metabolism of kenhy tall fescue harvested at three stages of physiological maturity. J. Anim. Sci. 51:704-711.

Perry, R. C., L. R. Corah, R. C. Cochran, W. E. Beal, J. S. Stevenson, J. E. Minton et al. 1991. Influence of dietary energy on follicular development, serum gonadotropins, and first postpartum ovulation in suckled beef cows. J. Anim. Sci. 69:3762-3773.

Peterson, G. A., T. B. Turner, K. M. Irvin, M. E. Davis, H. W. Newland, and W. R. Harvey. 1987. Cow and calf performance and economic considerations of early weaning of fall-born beef calves. J. Anim. Sci. 64:15-22.

Peterson, R. G., and D. E. Waldern. 1981. Repeatabilities of serum constituents in holstein-friesians affected by feeding, age, lactation, and pregnancy. J. Dairy Sci. 64:822-831.

Pilegaard, H., K. Domino, T. Noland, C. Juel, Y. Hellsten, A. P. Halestrap et al. 1999. Effect of high-intensity exercise training on lactate/H+ transport capacity in human skeletal muscle. Am. J. Physiol - Endocrinol Metab. 276:E255-E261.

Porter, J. K. W. B. Charles, D. R. Joe. 1974. Major alkaloids of a Claviceps isolated from toxic Bermuda grass. J. Agric. Food Chem. 22:838–841.

Porter, J. K., and F. N. Thompson Jr. 1992. Effects of fescue toxicosis on reproduction in livestock. J. Anim. Sci. 70:1594-1603.

Provenza, F. D. 1995. Postingestive feedback as an elementary determinant of food preference and intake in ruminants. J. Range Manage.48:2.

Provenza, F. D., J. J. Villalba, L. E. Dziba, S. B. Atwood, and R. E. Banner. 2003. Linking herbivore experience, varied diets, and plant biochemical diversity. Small Ruminant Research. 49:257-274.

Randel, R. D. 1990. Nutrition and postpartum rebreeding in cattle. J. Anim. Sci. 68:853-862.

Redfearn, D. D., L. E. Moser, S. S. Waller, and T. J. Klopfenstein. 1995. Ruminal degradation of switchgrass, big bluestem, and smooth bromegrass leaf proteins. J. Anim. Sci. 73:598-605.

Rejnö S. LDH and LDH isoenzymes of synovial fluid in the horse. Acta. 1976. Vet. Scand. 17:178-89.

Rhodes, M. T., J. A. Paterson, M. S. Kerley, H. E. Garner, and M. H. Laughlin. 1991. Reduced blood flow to peripheral and core body tissues in sheep and cattle induced by endophyte-infected tall fescue. J. Anim. Sci. 69:2033-2043.

Richards, A. H., and R. Hilf. 1972. Influence of pregnancy, lactation and involution on glucose-6-phosphate dehydrogenase and lactate dehydrogenase isoenzymes in the rat mammary gland. Endocrinology. 9:287-295.

Richards, M. W., R. P. Wettemann, L. J. Spicer, and G. L. Morgan. 1991. Nutritional anestrus in beef cows: Effects of body condition and ovariectomy on serum luteinizing hormone and insulin-like growth factor-I. Biol. Reprod. 44:961-966.

Richards, M. W., J. C. Spitzer, and M. B. Warner. 1986. Effect of varying levels of postpartum nutrition and body condition at calving on subsequent reproductive performance in beef cattle. J. Anim. Sci. 62:300-306.

Richards, M. W., R. P. Wettemann, and H. M. Schoenemann. 1989. Nutritional anestrus in beef cows: Body weight change, body condition, luteinizing hormone in serum and ovarian activity. J. Anim. Sci. 67:1520-1526.

Ruppanner R., B.B. Norman, C.J. Adams, D.G. Addis, G.P. Lofgreen, J.G. Clark, and J.R. Dunbar. 1978. Metabolic and cellular profile testing in calves underer feed lot conditions: minerals, electrolytes, and biochemical components- changes over time in feed lot. Am. J. Vet. Res.39:841-844.

Rutter, L. M., and R. D. Randel. 1984. Postpartum nutrient intake and body condition: Effect on pituitary function and onset of estrus in beef cattle. J. Anim. Sci. 58:265-274.

Sawyer, J. E., C. P. Mathis, and B. Davis. 2004. Effects of feeding strategy and age on live animal performance, carcass characteristics, and economics of short-term feeding programs for culled beef cows. J. Anim. Sci. 82:3646-3653.

Schaefer, A. L., S. D. Jones, and R. W. Stanley. 1997. The use of electrolyte solutions for reducing transport stress. J. Anim. Sci. 75:258-265.

Scott, B. D., G. D. Potter, L. W. Greene, E. Hargis, and J. G. Anderson. 1992. Efficacy of a fat-supplemented diet on muscle glycogen concentrations in exercising thoroughbred horses maintained in varying body conditions. J. Equine Vet. Sci. 12:109-113.

Selk, G. E., R. P. Wettemann, K. S. Lusby, J. W. Oltjen, S. L. Mobley, R. J. Rasby et al. 1988. Relationships among weight change, body condition and reproductive performance of range beef cows. J. Anim. Sci. 66:3153-3159.

Short, R. E., R. A. Bellows, R. B. Staigmiller, J. G. Berardinelli, and E. E. Custer. 1990. Physiological mechanisms controlling anestrus and infertility in postpartum beef cattle. J. Anim. Sci. 68:799-816.

Sibbald, A. M., and W. G. Kerr. 1994. The effect of body condition and previous nutrition on the herbage intake of ewes grazing autumn pasture at two sward heights. Anim. Prod. 58:231.

Sibbald, A. M. 1997. The effect of body condition on the feeding behaviour of sheep with different times of access to food. Anim. Sci. 64:239.

Solomons, R. N., R. D. Linnabary, and J. W. Oliver. 1989. Reactivity of dorsal pedal vein of cattle to selected alkaloids associated with acremonium coenophialum-infected fescue grass. Am. J. Vet. Res. 50:235-238.

Spitzer, J. C., D. G. Morrison, R. P. Wettemann, and L. C. Faulkner. 1995. Reproductive responses and calf birth and weaning weights as affected by body condition at parturition and postpartum weight gain in primiparous beef cows. J. Anim. Sci. 73:1251-1257.

Steen, W. W., N. Gay, J. A. Boling, G. Lacefield, L. P. Bush, and R. C. Buckner. 1979. Evaluation of kentucky 31, G1-306, G1-306 and kenhy tall fescue as pasture for yearling steers I. forage composition. J. Anim. Sci. 48:607-617.

Stokes, S. R., A. L. Goetsch, H. H. Nejad, G. Murphy, A. L. Jones, S. Mashburn et al. 1988. Effects of supplemental bermuda grass hay or corn on intake, digestion and performance of cattle consuming endophyte-infected fescue. J. Anim. Sci. 66:204-212.

Stuedemann, J.A., and C.S. Hoveland. 1988. Fescue endophyte: history and impact on animal agriculture. J. prod. Agri. 1:39-44

Taylor, C. A., R.J, and M. H. Ralphs. 1992. Reducing livestock losses from poisonous plants through grazing management. J. Range Manage. 45:9.

Taylor, R. E., and T. G. Field.1995. Achieving cow/ calf profitability through low- cost production . the range beef cow symposium XIV, Dec 5, 6 and 7, 1995, Gering, Nebraska.

Teeri J.A., and L.G. Stowe. 1976. Climatic patterns and the distribution of C4 grasses in North America. Oecol.23:1–12.

Thompson, W. R., J. C. Meiske, R. D. Goodrich, J. R. Rust, and F. M. Byers. 1983. Influence of body composition on energy requirements of beef cows during winte J.Anim.Sci. 56:1241-1252.

Thompson, W. R., D. H. Theuninck, J. C. Meiske, R. D. Goodrich, J. R. Rust, and F. M. Byers. 1983. Linear measurements and visual appraisal as estimators of percentage empty body fat of beef cows. J. Anim. Sci. 56:755-760.

Tsai, H. F., J. S. Liu, C. Staben, M. J. Christensen, G. C. Latch, M. R. Siegel et al. 1994. Evolutionary diversification of fungal endophytes of tall fescue grass by hybridization with epichloë species. PNAS. 91: 2542-2546.

Vallee, B.L., and W.E.C. Wacker. 1956. Zinc, a component of rabbit muscle lactic dehydrogenase. J. Am. Chem. Soc. 78: 1771-1772

Van Soest, P. J. 1982. Nutritional Ecology of the Ruminant. O&B Books, Corvallis, OR.

Van Soest, P. J. 1993. Cell wall matrix interactions and degradation- session synopsis. In: H. G. Jung, D. R. Buxton, R. D. Hatfield, and J. Ralph (Ed.) Forage Cell Wall Structure and Digestibility. p 377. ASA-CSSA-SSSA, Madison, WI.

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

Washizu T., M. Nakamura, N. Izawa, E. Suzuki, S. Tsuruno, M. Washizu, S. Nakajo, and T. Arai. The activity ratio of the cytosolic MDH/LDH and the isoenzyme pattern of LDH in the peripheral leukocytes of dogs, cats and rabbits. 2002. Vet. Res. Commun.26 :341-6.

Wasserman, K., B. J. Whipp, S. N. Koyl, and W. L. Beaver. 1973. Anaerobic threshold and respiratory gas exchange during exercise. Journal of Applied Physiology. 35:236-243.

Wilson, J. R., R. H. Brown, and W. R. Windham. 1983. Influence of leaf anatomy on the dry matter digestibility of C3, C4, and C3/C4 intermediate types of Panicum species. Crop Sci. 23:141-146.

Wiltbank, J. N., W. W. Rowden, J. E. Ingalls, K. E. Geegoey, and R. M. Koch. 1962. Effect of energy level on reproductive phenomena of mature hereford cows. J. Anim. Sci. 21:219-225.

Wiltbank, J. N., W. W. Rowden, J. E. Ingalls, and D. R. Zimmerman. 1964. Influence of post-partum energy level on reproductive performance of hereford cows restricted in energy intake prior to calving. J. Anim. Sci. 23:1049-1053.

Wrobleweski, F. 1958. The Mechanisms of alternation of lactic dehydrogenase activity in body fluids. Ann. N.Y. Acad. Sci.75:322-338.

Yates, S. G., R. D. Plattner, and G. B. Garner.1985. Detection of ergopeptine alkaloids in endophyte-infected, toxic Ky-31 tall fescue by mass spectrometry/mass spectrometry. J. Agri. Food Chem. 33:719-722.

The Effects of Lactate Dehydrogenase B Haplotypes and Body Condition on Beef Cow Production

O. ALAAMRI

Cell and Molecular Biology Program, University of Arkansas, Fayetteville 72701

2.0 ARTICLE I

2.1 Abstract

Lactate dehydrogenase (LDH) catalyzes the conversion of the pyruvate to lactate (forward) or lactate to pyruvate (reverse) in the last step of glycolysis. The objectives were to document effects of LDH-B haplotypes and their SNPs, found in the promoter and coding sequence site, on beef cow production. Four single nucleotide polymorphisms (SNP) were detected, and eight haplotypes were assigned with the order of SNPs: G-348A, A-261G, N-222D, and C541A. Specific primers were designed for polymerase chain reaction and amplification of 452-base pair fragment and 457-base pair fragments of the bovine LDH-B coding sequence and promoter, respectively. Brahman-influenced cows (n = 109) managed to achieve either low (BCS = 4.3± 0.1) or moderate (BCS = 6.4± 0.1) body condition. Cows grazed stockpiled and spring growth, endophyte-infected tall fescue pastures during 60-d breeding period to obtain desired BC; serum samples were collected on d -35. Cows that were heterozygous (GA) had a lower calving rate than homozygous with the major allele (53.3 vs. 79.1 %, respectively) at base position G-348A. In addition, IGF-I was affected ($P < 0.05$) by body condition and G-348A. A-261G had an effect ($P < 0.05$) on LDHf and follicle size. Deletion of six nucleotides (GGCCGC) was detected at base N-222D. LDHf of fifty-five cows that were either hetero or homozygous for the deletion were affected ($P < 0.02$) by the N-222D. Interaction between BC x C541A had an effect ($P < 0.05$) on LDHr. NEFA (non essential fatty acid) was affected ($P < 0.02$) by base position C541A. Haplotype 1 had the same sequence as that published at GenBank "No SNP". Haplotypes had an effect on LDHf ($P < 0.05$), and IGF-I, PRL, T3, and T4 were significantly affected by BCS. Our results demonstrated that the

polymorphism of the coding sequence and promoter region can be used as a genetic marker for the selection of cows with high fertility.

Key Words: LDHf, LDHr, SNP, Haplotypes, BCS, IGF-I, PRL, T3, T4

2.2 Introduction

Lactate dehydrogenase (LDH) can be found in wide variety of organisms such as mammals. Lactate dehydrogenase consists of at least two structural-function domains. In mammals and birds, lactate dehydrogenase is present in five somatic isozymes in which activity and regulation can be organ specific. These five somatic isozymes are tetrameric combinations of two 35-kDa subunits, including M (muscles) and H (heart) (Li et al., 1985; Cardinet, 1997). Lactate dehydrogenase, as a cytoplasmic enzyme, catalyzes the conversion of the pyruvate to lactate (forward; LDHf) in the absence of oxygen or lactate to pyruvate (reverse; LDHr) in the presence of oxygen. Stressors such as toxic tall fescue and cardiac stress have been reported to have effects on LDH activity (Galyean et al., 1981; Crookshank et al., 1979). Looper et al. (2002) suggested that the increase in reproduction in cattle is related to decrease LDHr activity in heifers. In addition, it has been determined the influence of BC at calving and postpartum on endocrine function and reproduction of beef cows. The study demonstrated that high energy intake and increase in BCS after calving increased the concentration of IGF-1, leptin, insulin, glucose, and thyroxin in plasma and shorten PPI (Ciccioli et al., 2003). Single nucleotide polymorphism of bovine LDH- B has been identified in previous study (Williamson et al., 2010). The study reported the effects of an interaction between the genotype with the SNP within LDH-B gene, and forage on calving rate. In humans, Both LDH-B and LDH-A genes have been analyzed and shown that their protein coding sequences are interrupted with six introns at homologous positions (Maekawa et al., 1993). In addition, LDH-B deficiency has been reported

43

in individuals homozygous for LDH-B (Maekawa et al., 1993). Additionally, the molecular

nature of genetic mutation in two individuals with unstable LDH-B has been characterized as

missense mutation from serine to arginine (Maekawa et al., 1993). Moreover, amino acid

replacement has been found in LDH-B gene mutant alleles, as a result of three single base

substitutions (transversion), may cause conformational changes in neighboring residue.

Therefore, these changes may affect the arrangement of the active site resulting in loss of the

enzyme activity (Maekawa et al., 1993). Our objective was to determine the effect of LDH-B

haplotypes on reproductive performance of Brahman-influenced cows.

2.3 Materials and Methods

2.3.1 Description of Animal Management

Spring-calving crossbred multiparous Brahman-influenced cows (n = 109) were

managed to achieve low or moderate body condition (BC) at parturition. Cows grazed on

stockpiled and spring growth, endophyte-infected tall fescue (*Lolium arundinaceum* (Schreb.)

S.J. Darbyshire) pastures before the breeding season to obtain desired BC at a stocking rate of

either 1 cow/3237.49 m² (low BC) or 1 cow/8093.712 m² (moderate BC) for approximately 162

d prior to the breeding season. Mean BCS of low (n = 55; mean BW = 422.3 kg± 15.79 kg) and

moderate (n = 54; mean BW = 530.204 kg± 16.0118 kg) BC cows ware 4.3 ± 0.1 and 6.1 ± 0.1

based on the 1 to 9 scale (1 = emaciated to 9 = obese), respectively (Wagner et al., 1988).

2.3.2 Blood Collection

Blood samples were collected from cows at 35 d before the breeding season. Plasma and

buffy coats were harvested within 8 h of blood collection. Blood samples were maintained at 4

ºC until centrifuged (1,500 × g for 25 min). Plasma samples were stored at -20 ºC and buffy

coats were stored at -80 ºC. Serum concentrations of hormones were determined in duplicate

aliquots using radioimmunoassay procedures. Serum samples collected on d -35, and Genomic

DNA was harvested using a commercially available QIAmp kit provided by QIAGEN.

2.3.3 Polymerase Chain Reaction (PCR)

A Peltier thermal cycler 100 (MJ Research, Waltham, Mass.) was used for amplification.

The thermal cycler began with a denaturation temperature of 94 ºC for 2 min and then cycled at

94 ºC for 30 s, 55 ºC for 1 min and 68 ºC for 1 min. After cycling 35 times, a final extension

occurred at 68 ºC for 10 min. Each PCR were 5 µl of 20 ng/µl genomic DNA, 1 µl of 10 μM

forward primer, 1 µl of 10μM reverse primer and 43 µl of Platinum PCR Supermix (Invitrogen,

Carlsbad, CA, USA) for a total volume of 50 µl. Amplicons were visualized by electrophoresis

in 1.2% agarose gels stained with ethidium bromide in 1.0· Tris/Boric Acid/EDTA.

Amplification products were purified using QIAquick PCR purification kit (Qiagen). Samples

were held at 8 ºC until sequenced. Purified PCR products were sequenced at the University of

Arkansas DNA Core Lab.

2.3.4 Primers

Four primers were designed for PCR amplification and sequencing. Two primers were

designed based on the conserved region, which is downstream from the start codon,   to amplify

a 452-base pair (bp) fragment (bases 489 to 940 of accession number AJ_401268) of the bovine

LDH-B coding sequence (cds) (5′-GTACAGTCCTGCCTGCATCA -3′ and 5′-

CCATTGTTGACACTGGGTGA -3′). The other two primers Pro FOR 5'-

ACACACCAGCAGCATCTCAG-3'and REV (5'–GATAAGGGCTGCACGAAGAC-3') were

designed to amplify a 457-base pair fragment from position -269 to 30 of accession number

NW_001495083 of the bovine LDH-B promoter. Products were sequenced, SNP sites

determined, and haplotypes assigned.

2.3.5 DNA Sequencing

Sequencing was performed by the DNA Core Lab using the ABI Prism 3100 Genetic

Analyzer (Applied Biosystems, Foster City, Calif.). The primers used for sequencing were (5′-

GTACAGTCCTGCCTGCATCA -3′ and 5′ CCATTGTTGACACTGGGTGA -3′) and Pro FOR

(5'-ACACACCAGCAGCATCTCAG-3') and Pro REV (5'–GATAAGGGCTGCACGAAGAC-

3'). Sequences were analyzed and compared for sequence identity using the web-based software

package ClustalW (European Bioinformatics Institute, Cambridge, UK). Three genotypes were

observed for each SNP: G-348A homozygous guanine (GG), homozygous adenine (AA) and

heterozygous (GA), A-261G homozygous adenine (AA), homozygous (GG), and heterozygous

(AG), N-222D, homozygous normal (NN), homozygous deletion (DD), heterozygous (ND), and

C541A, homozygous cytosine (CC), homozygous adenine (AA), and heterozygous (CC).

Homozygous and heterozygous alleles were identified by assessing sequence chromatograms

using the ABI PRISM Sequence Scanner V1.0 (Applied Biosystems) and BIOEDIT (Hall, 1999).

2.3.6 Lactate Dehydrogenase Activities

LDH activities were determined using modified colormetric kinetic assay on Spectra Max

250 (CV within 10% Molecular Devices, Sunnyvale, CA). Forward reaction of LDH was

determined using reagents β-nicotinamide adenine dinucleotide (0.0194mmol/L; Sigma, Sr.

Louis, MO) and pyruvic acid (16.2mmol/L; Sigma). Samples were analyzed at 340nm four

times, one minute apart. Reverse reaction of LDH was determined using reagents NAD$^+$, Free

Acid (7mmol/L; Calbiochem, San Diego, CA) and L-lactic acid lithium salt (50mmol/L; Tokyo

Co., Tokyo, Japan). Samples were analyzed at 340nm three times, 30 seconds apart.

2.3.7 Statistical Analysis

Calving percentage was analyzed by Chi-square. The effects of haplotypes and

genotypes on calving date were determined using analysis of variance.

2.4 Results and Discussion

2.4.1 Identification of Polymorphisms

Four single nucleotide polymorphism (SNP) sites were detected. Three SNP on the

promoter region and one SNP on the coding sequence were identified by amplification and

sequencing of the 452-bp and 457-bp fragments of the bovine LDH-B coding sequence and

promoter regions (gene bank accession numbers aj401268 and NW_001495083, respectively).

These SNPs were: G-348A, A-261G, N-222D, and C541A. Two transitions, deletion of six

nucleotides, and one transversion were detected. A transition from guanine to adenine at

position -348, and from adenine to guanine at position -261 on the promoter site (non coding

sequence) may affect the transcription. The transcription of LDH-B was not measured in our

study. A transversion from cytosine to adenine at position 541 on the coding sequence resulted

in amino acid change from threonine (Thr) to proline (Pro) at position 181(Thr^{181}Pro) based on

the full nucleotides sequence which was used as a reference sequence (gi 8979738) (Fig. 1).

Cows were either heterozygous or homozygous with the minor allele (Table 1). The single cow

that was homozygous for the minor allele was used with the heterozygous cows for statistical

analysis.

2.4.2 Base Position -348

At base -348, a transition was determined from a guanine to adenine (G to A). Since the transition occurred on the promoter site (non coding sequence), the mutation can affect the transcription. The transcription was not measured in our study. The effect on transcription provides future research opportunities. Twenty cows were either homozygous or heterozygous with the minor allele (Table 1). Calving rate was affected ($P < 0.05$) by the genotype at G-348A of this group of cows. Cows that were heterozygous (GA) had a lower calving rate than homozygous with the major allele (53.3 vs. 79.1 %, respectively; Fig. 2), but this genotype did not affect ($P > 0.10$) the calving date. Reproductive hormones (PRL and T3) were influenced ($P < 0.02$) by body condition but not by the genotype. IGF-I concentrations were affected ($P < 0.05$) by body condition and genotype, and a tendency ($P= 0.08$) for a BC x genotype interaction. Neither LDHr nor LDHf were influenced ($P > 0.10$) by body condition or genotype.

2.4.3 Base Position-261.

At base -261, transition was detected from an adenine to guanine (A to G) in the promoter site which may affect the transcription. Genotype did not influence ($P > 0.10$) the calving rate or calving date. Twenty- five cows were either homozygous or heterozygous with the minor allele (Table 1). This SNP had an effect ($P < 0.05$) on LDHf, but it did not affect ($P> 0.10$) LDHr. Follicle size was influenced ($P < 0.02$) by the genotype and by BCS. Our results were in agreement with one of the previous research that determined large follicles in cows with high energy levels (Perry et al., 1991). PRL was not affected ($P > 0.05$) by the SNP, but it was affected ($P < 0.02$) by BC. IGF-I and T3 were affected ($P < 0.05$) by body condition, but the SNP had a tendency ($P= 0.07$) affect on IGF-I and T3. There was no significant effect ($P > 0.05$) on T4 by the genotype.

2.4.4 Base Position -222

Deletion of six nucleotides (GGCCGC) was detected at base -222 in the promoter sequence. This deletion can cause reading frame shift mutation due to the triplet nature of gene expression by codons. Since the deletion occurred on the non coding region (promoter), this mutation will not alter the protein produced. In this case, the deletion may affect the transcriptional control. Fifty-five cows were either hetero or homozygous for the deletion (Table 1). Calving rate and calving date were not influenced ($P > 0.10$) by the SNP (N-222D). Our results did not agree with other results that indicated the influence of BCS on the calving date (Bellows et al., 1982; Randel, 1990; Lalman et al., 1997; Spitzer et al., 1995), but it did agree with another documentation that determined that BCS did not affect the calving date (Selk, et al., 1988). LDHf was affected ($P < 0.02$) by the genotype. No significant effects ($P > 0.10$) of the SNP on T3, T4, IGF-I were determined. The SNP had a tendency ($P < 0.10$) effect on PRL. In contrast, body condition had significant effects ($P < 0.05$) on T4, PRL, IGF-I, and T3 which was agreed with other results that indicated an increase in gonadotropic hormones in cows with moderate BCS (Short and Adams, 1988; Rutter and Randel, 1984; Richards et at., 1991; Ciccioli et al., 2003).

2.4.5 Base Position 541

A Transversion was detected from a cytosine to adenine (C to A) in the coding region (exone). The transversion resulted in amino acid change from threonine (Thr) to proline (Pro) at position 181(Thr[181]Pro) based on the full nucleotides sequence which was used as a reference sequence (Fig. 1). This kind of point mutation is called missense mutation in which a single nucleotide is changed resulting in a different amino acid. The codon that formed as a result of transversion from C to A coded for different amino acid (Pro instead of Thr). Proline has

different properties than threonine in which Pro is a non polar amino acid (hydrophobic), while Thr is a polar amino acid (hydrophilic). Thr can be phosphorylated, as well. This missense mutation is non conservative because the new amino acids were translated (Thr) and have different properties than the wild type amino acid (Pro). The SNP, C541A, tended (P= 0.09) to affect PRL. The genotype had no effect (P > 0.10) on IGF-I, T3, LDHr, and LDHf; whereas, the interaction between BC x C541A had an effect (P < 0.05) on LDHr. BCS affected (P < 0.02) IGF-I, T3, and PRL. In addition, the calving rate and calving date were not influenced (P > 0.10) by the SNP. The genotype affected (P < 0.02) NEFA.

2.4.6 LDH-B Haplotypes

Eight unique haplotypes were derived from the four SNP sites (Table 2). The range of those haplotypes is from two to thirty-three. Haplotype 1 had the same sequence as that published at GenBank (GenBank accession numbers AJ401268 and NW001495085). Thus, the cows (n = 33) with haplotype 1 were classified as "No SNP". Fifty- one cows represented six haplotypes which were classified as "Deletion". The remaining 25 cows with SNP other than deletion were classified as "Yes SNP" (Table 2).

Haplotypes did not affect calving rate or calving date. Those haplotypes had an effect on LDHf (P < 0.04), but did not influence LDHr (P > 0.10). IGF-I, T3, T4, and follicle size were not influenced (P > 0.10) by haplotypes. However, they had a tendency (P < 0.10) to affect PRL. Additionally, haplotypes had a tendency (P = 0.06) to affect NEFA. Reproductive hormones (PRL, T3, and IGF-I) were affected (P < 0.02) by body condition; whereas, LDHf and LDHr were not affected (P > 0.10) by body condition.

Our results documented that body condition had significant effects on hormones at different base positions which is in agreement with several published results. As reported by

50

Looper et al., (2010), the IGF-I and PRL concentrations were low in cows with thin body condition which is similar to our result. IGF-I, PRL, T3, and T4 concentrations were greater in moderate-BC cows compared with low-BC cows (Flores et al., 2008). In contrast, the genotypes did not show significant effects on hormones except for the SNP (G-348A) that had an effect (P < 0.02) on IGF-I. Furthermore, those genotypes did not affect the calving rate and the calving date with an exception of the SNP (G-348A) that had an effect on the calving rate. Activity of LDHr was not affected by the haplotypes and the SNPs; whereas, LDHf was affected by the haplotypes and SNP (A-261G and N-222D) and SNP (541) had an interaction (BC x SNP) effect on LDHr. The genotypes did not have any effect on the follicle size, while body condition had an effect (P < 0.05) on the follicle size.

2.5 Implications

The SNP identified in the promoter and coding sequence regions of the bovine LDH-B gene may be used as genetic markers for selecting cows which are prone to have greater calving rates. Our results suggested that more investigation is needed to document any interactions between genotypes and environment (body condition, forage, heat stress, etc) on cattle to improve reproductive performance which is associated with cattle profitability.

2.6 Tables

Table 1. Description of SNP in (452-bp and 457-bp) amplicon of the bovine LDH-B coding sequence and promoter, respectively

| Polymorphism[1] | Genotype Distribution[2] | | | MAF[3] |
	Homo	Hetero	homo	
G-348A	102	17	3	9.4
A-261G	97	21	4	11.9
N-222D	67	49	6	25
C541A	68	63	2	25.2

[1] Single nucleotide polymorphism (SNP) occurred at the number indicated. First letter the primary allele and the letter following the digits is the minor allele (D represent deletion of six nucleotides).

[2] Number of cows that were homozygous for the major allele (Homo), heterozygous (Hetero), and homozygous fort the minor allele.

[3] Minor allele frequency expressed as a percent.

Table 2. Haplotype frequency in a (452-bp and 457-bp) amplicon of the bovine LDH-B coding sequence and promoter, respectively

| | Haplotype[1] | |
Number	Sequence	No. observations
1	GANC	33
2	AADA	10
3	AADC	10
4	GADA	8
5	GADC	2
6	GANA	25
7	GGDA	12
8	GGDC	9

[1] All of these haplotypes have the same order of single nucleotide polymorphisms (SNP): G-348A, A-261G, N-222D, and C541A. Haplotype one represents the publish sequence (GenBank accession numbers AJ_401268 and NW_001495083). Deletion of a six nucleotides is represented as D.

2.7 Figures

AJ401268
MATLKEKLIAPVAEEETTIPNNKITVVGVGQVGMACAISILGKSLTDELALVDVLEDKLK 60
LDHB_a541c   --

AJ401268
GEMMDLQHGSLFLQTPKIVADKDYSVTANSKIVVVTAGVRQQEGESRLNLVQRNVNVFKF 120
LDHB_a541c   --

AJ401268
IIPQIVKYSPACIIIVVSNPVDILTYVTWKLSGLPKHRVIGSGCNLDSARFRYLMAEKLG 180
LDHB_a541c   -------
YSPACIIIVVSNPVDILPYVTWKLSGLPKHRVIGSGCNLDSARFRYLMAEKLG 53
               ******************.*********************************

AJ401268
IHPSSCHGWILGEHGDSSVAVWSGVNVAGVSLQELNPEMGTDNDSENWKEVHKMVVESAY 240
LDHB_a541c
IHPSSCHGWILGEHGDSSVAVWSGVNVAGVSLQELNPEMGTDNDSENWKEVHKMVVESAY 113
       ****

AJ401268
EVIKLKGYTNWAIGLSVADLIESMLKNLSRIHPVSTMVKGMYGIENEVFLSLPCILNARG 300
LDHB_a541c   EVIKLKGYTNWAIGLSVADLIESMLKNLSRIHPVSTM---------------------- 150
       ****

AJ401268   LTSVINQKLKDEEVAQLKKSADTLWGIQKDLKDL 334
LDHB_a541c   -----------------------------------

Figure1. Amino acid sequence alignments showing the conserved region (*) within the full-length sequence of bovine LDH-B. The single-nucleotide polymorphism A541C identified in the nucleotide sequence resulted in an amino acid change from Thr to Pro at position 181(:) relative to the reference sequence (NCBI)
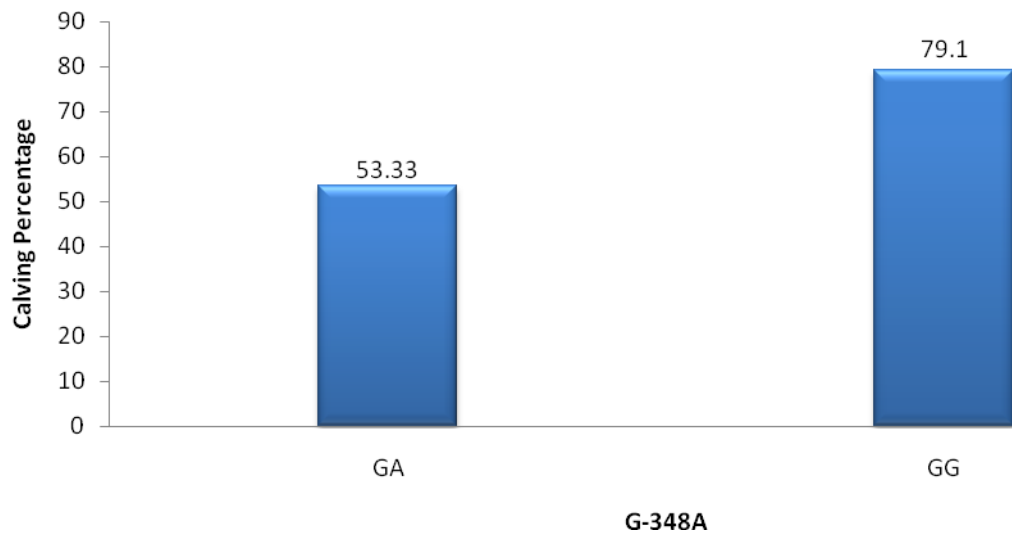
53

Figure2. Percentage of cows calving presented by LDH-B promoter single nucleotide polymorphism (SNP) G-348A. The genotype distribution of this SNP was 102 and 17 for GG and GA, respectively.

2.8 Literature Cited

Bellows, R. A., R. E. Short, and G. V. Richardson. 1982. Effects of sire, age of dam and gestation feed level on dystocia and postpartum reproduction. J. Anim. Sci. 55:18-27.

Cardinet, G.H. 1997. Skeletal muscle function. In: Kaneko, J.J, J.W. Harvey, M.C. Bruss, eds. Clinical biochemistry of domestic animal. 5th ed. Academic Press; p407-440. San Diego, CA

Ciccioli, N. H., R. P. Wettemann, L. J. Spicer, C. A. Lents, F. J. White, and D. H. Keisler. 2003.Influence of body condition at calving and postpartum nutrition on endocrine function and reproductive performance of primiparous beef cows. J. Anim. Sci. 81:3107-3120.

Crookshank, H. R., M. H. Elissalde, R. G. White, D. C. Clanton, and H. E. Smalley. 1979. Effect of transportation and handling of calves upon blood serum composition. J. Anim. Sci. 48:430-435.

Flores, R., M. L. Looper, R. W. Rorie, D. M. Hallford, and, C. F. Rosenkrans. 2008. Endocrine factors and ovarian follicles are influenced by body condition and somatotropin in postpartum beef cows. J. Anim. Sci. 86:1335-1344.

Galyean, M. L., R. W. Lee, and M. E. Hubbert. 1981. Influence of fasting and transit on ruminal and blood metabolites in beef steers. J. Anim. Sci. 53:7-18.

Hall, T. A., 1999: BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symposium Series 41, 95–98.

Lalman, D. L., D. H. Keisler, J. E. Williams, E. J. Scholljegerdes, and D. M. Mallett. 1997. Influence of postpartum weight and body condition change on duration of anestrus by undernourished suckled beef heifers. J. Anim. Sci. 75:2003-2008

LI, S. S.-L., H. F. Tiano, K. M. Fukasawa, K. Yagi, M. Shimizu, F. S. Sharief et al. 1985. Protein structure and gene organization of mouse lactate dehydrogenase-A isozyme. European Journal of Biochemistry. 149:215-225.

Looper, M. L., S. T. Reiter, B. C. Williamson, M. A. Sales, D. M., and C. F. Rosenkrans, 2010. Effects of body condition on measures of intramuscular and rump fat, endocrine factors and calving rate of beef cows grazing common Bermudagrass or endophyte-infected tall fescue. J. Anim. Sci. 88:4133-4141.

Looper, M.L., T.P. Neidecker, C.W. Wall, S.T. Reiter, R. Flores, A.H. Brown, r, J,
Z.B. Johnson, and, C.F. Rosenkrans. 2002. Relationship of Lactate Dehydrogenase Activity with Body Measurements of Angus x Charolais Cows and Calves. Prof. Anim. Sci. 18:120.

Maekawa, M., K. Sudo, S. S.-L. Li, and T. Kanno. 1993. Analysis of genetic mutations in human lactate dehydrogenase-A(M) deficiency using DNA conformation polymorphism in combination with polyacrylamide gradient gel and silver staining. JF Human Genetic. 19:163-168.

Perry, R. C., L. R. Corah, R. C. Cochran, W. E. Beal, J. S. Stevenson**M**, J. E. Minton et al. 1991. Influence of dietary energy on follicular development, serum gonadotropins, and first postpartum ovulation in suckled beef cows. J. Anim. Sci. 69:3762-3773.

Randel, R. D. 1990. Nutrition and postpartum rebreeding in cattle. J. Anim. Sci. 68:853-862.

Richards, M. W., R. P. Wettemann, and H. M. Schoenemann. 1989. Nutritional anestrus in beef cows: Body weight change, body condition, luteinizing hormone in serum and ovarian activity. J. Anim. Sci. 67:1520-1526.

Richards, M. W., R. P. Wettemann, L. J. Spicer, and G. L. Morgan. 1991. Nutritional anestrus in beef cows: Effects of body condition and ovariectomy on serum luteinizing hormone and insulin-like growth factor-I. Biology of Reproduction. 44:961-966.

Rutter, L. M., and R. D. Randel. 1984. Postpartum nutrient intake and body condition: Effect on pituitary function and onset of estrus in beef cattle. J. Anim. Sci. 58: 265-274.

Selk, G. E., R. P. Wettemann, K. S. Lusby, J. W. Oltjen, S. L. Mobley, R. J. Rasby et al. 1988. Relationships among weight change, body condition and reproductive performance of range beef cows. J. Anim. Sci. 66:3153-3159.

Spitzer, J. C., D. Short, R. E., and D. C. Adams. 1988. Nutritinal and hormonal interrelationships in beef cattle reproduction. J. Anim. Sci. 68:29-39.

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

Williamson, B. C., C. F. Rosenkrans, Jr., S. T. Reiter, J. D. Thompson, M. A. Sales, and M. L. Looper. 2010. Identification of Single Nucleotide Polymorphisms of the Lactate Dehydrogenase-B Gene and Association with Cow and Calf Performance. Prof. Anim. Sci.

Effects of Lactate Dehydrogenase A Haplotypes and Body Condition on Beef Cow Production

O. ALAAMRI

Cell and Molecular Biology Program, University of Arkansas, Fayetteville 72701

3.0 ARTICLE II

3.1 Abstract

Lactate dehydrogenase (LDH) catalyzes the conversion of pyruvate to lactate (forward) or lactate to pyruvate (reverse) in the last step of glycolysis enzyme. Objectives were to document effects of LDH-A haplotypes and there SNPs, found in the promoter and coding sequence on beef cow productivity. Five single nucleotide polymorphisms (SNP) were detected, and four haplotypes were assigned with the order of SNPs: T-327G, D-263C, G390A, A406G, and T530C. Specific primers were designed for polymerase chain reaction and amplification of 507-base pair (bp) fragment and 555-base pair (bp) fragment of the bovine LDH-A coding sequence and promoter, respectively. Brahman-influenced cows (n=109) were managed to achieve either low (BCS=4.3± 0.1) or high (BCS=6.4± 0.1) body condition (BC). Cows grazed stockpiled and spring growth endophyte-infected tall fescue pastures during 60-d breeding period to obtain desired BC; serum samples collected on d -35. NEFA were affected ($P < 0.02$) by T-327G; whereas, BCS does not have such effect on NEFA. Deletion of six nucleotides (GGCCGC) was detected at base D-263C with an insertion of cytosine. LDHf and LDHr were not affected ($P > 0.10$) by genotype or BCS. However, the interaction between BC and D-263C affected ($P < 0.05$) LDHf. No significant effects ($P > 0.10$) of the SNPS on T3, IGF-I, and PRL were determined. In contrast, BC affected ($P < 0.05$) PRL, IGF-I, and T3, but it did not affect LDHf and LDHr. Haplotype 1 had the same sequence as that published at GenBank "No SNP". Haplotypes had no effect on LDHf or LDHr ($P > 0.10$), while the interaction between BC X haplotypes affected LDHf. Calving date was not affected by genotype or haplotype. Our results demonstrated that polymorphisms of the LDH-A coding sequence and promoter region can be used as genetic markers for the selection of cows with high fertility.

3.2 Introduction

Lactate dehydrogenase can be found in wide variety of organisms such as mammals. It is found in liver, skeletal and heart muscles, lung, spleen, pancreas and kidney, and each of these organs have different enzymatic activity (Wroblewski, 1958). Lactate dehydrogenase (LDH) consists of at least two structural-function domains. In mammals and birds, lactate dehydrogenase was presented in five somatic isozymes in which activity and regulation can be organ specific. These five somatic isozymes are tetrameric combinations of two 35-kDa subunits, including M (muscles) and H (heart; Li et al 1985; Cardinet, 1997). As a cytoplasmic enzyme, LDH catalyzes the conversion of the pyruvate to lactate (forward; LDHf) in the absence of oxygen or lactate to pyruvate (reverse; LDHr) in the presence of oxygen. Weaning stress in calves, fasting, and grazing toxic tall fescue have been reported to have effects on LDH activity (Galyean et al., 1981; Crookshank et al., 1979). Looper et al., (2002) suggested that improved cattle reproduction was related to the decreased LDHr activity in heifers. In addition, it has been demonstrated that high energy intake and increased BCS after calving increased the concentration of IGF-1, leptin, insulin, glucose, and thyroxin in plasma and shortened post-partum interval (PPI) (Ciccioli et al., 2003). Single nucleotide polymorphisms of bovine LDH- B were identified and they represented an interaction between genotype and forage on calving rate (Williamson et al., 2010). In human, Both LDH-B and LDH-A genes have been analyzed and shown that their protein coding sequences are interrupted with six introns at homologous positions (Maekawa et al., 1993). Additionally, it has been estimated that the carrier frequency of LDH-A and LDH-B subunit deficiencies, in Japan, to be 0.185% and 0.159%, respectively (Maekawa et al., 1993). Moreover, the individuals were identified as being heterozygous for the

LDH-B by examination the ratio of LDH-B to LDH-A subunits in erythrocytes. Our objective was to determine the effect of LDH-A haplotypes on reproductive performance of Brahman-influenced cows.

3.3 Materials and Methods

3.3.1 Description of Animal Management

Spring-calving crossbred multiparous Brahman-influenced cows (n =99) were managed to achieve low or moderate body condition (BC) at parturition. Cows grazed on stockpiled and spring growth, endophyte-infected tall fescue (*Lolium arundinaceum* (Schreb.) S.J. Darbyshire) pastures to the breeding season to obtain desired BC. Stocking rates were either 1 cow/3238 m² (low BC) or 1 cow/8094 m² (moderate BC) for approximately 162 d prior to the breeding season. Mean BCS of low (n = 50; mean BW = 422 kg ± 15 kg) and moderate (n = 49; mean BW = 530 kg ± 16 kg) BC cows was 4.3 ± 0.1 and 6.1 ± 0.1 based on the 1 to 9 scale (1 = emaciated to 9 = obese), respectively (Wagner et al., 1988).

3.3.2 Blood Collection

Blood samples were collected from cows at 35 d before the breeding season. Plasma and buffy coats were harvested within 8 h of blood collection. Blood samples were maintained at 4 ℃ until centrifuged (1,500 × g for 25 min). Plasma samples were stored at -20 ℃ and buffy coats were stored at -80 ℃. Serum concentrations of hormones were determined in duplicate aliquots using radioimmunoassay procedures. Serum samples collected on d -35, and Genomic DNA was harvested using a commercially available QIAmp kit provided by QIAGEN.

3.3.3 Polymerase Chain Reaction (PCR)

A Peltier thermal cycler 100 (MJ Research, Waltham, Mass.) was used for amplification. The thermal cycler began with a denaturation temperature of 94 ℃ for 2 min and then cycled at

94ºC for 30 s, 55 ºC for 1 min and 68 ºC for 1 min. After cycling 35 times, a final extension

occurred at 68 ºC for 10 min. Each PCR were 5 µl of 20 ng/µl genomic DNA, 1 µl of 10 µM

forward primer, 1 µl of 10µM reverse primer and 43 µl of Platinum PCR Supermix (Invitrogen,

Carlsbad, CA, USA) for a total volume of 50 µl. Amplicons were visualized by electrophoresis

in 1.2% agarose gels stained with ethidium bromide in 1.0· Tris/Boric Acid/EDTA.

Amplification products were purified using QIAquick PCR purification kit (Qiagen). Samples

were held at 8 ºC until sequenced. Purified PCR products were sequenced at the University of

Arkansas DNA Core Lab.

3.3.4 Primers

Four primers were designed for PCR amplification and sequencing. Two primers were

designed based on the conserved region, which is downstream from the start codon, to amplify a

507-base pair (bp) fragment (bases 151 to 659) of accession number NM_174099) of the bovine

LDH-A coding sequence (cds) (F 5' AGTCCAAGATGGCAACTCTCAA-3' and R 5'-

TGAATCCAGATTGCAACCAC - 3'). The other two primers were designed to amplify a 555-

base pair (bp) fragment (bases -508 to 64) of accession number NW_001494522) of the bovine

LDH-A promoter (Pro FOR 5' ATTCTGGAGGCCATTGACTG-3' and Rev 5'-

TGCTGACACTCCGACTTCTC-3'). Products were sequenced, SNP sites determined, and

haplotypes assigned.

3.3.5 DNA Sequencing

Sequencing was performed by the DNA Core Lab using the ABI Prism 3100 Genetic

Analyzer (Applied Biosystems, Foster City, Calif.). The primers used for sequencing were (5'-

AGTCCAAGATGGCAACTCTCAA-3' and R 5'- TGAATCCAGATTGCAACCAC - 3') and

bovine LDH-A promoter (Pro FOR 5' ATTCTGGAGGCCATTGACTG-3' and Rev 5'-

TGCTGACACTCCGACTTCTC-3'). Sequences were analyzed and compared for sequence identity using the web-based software package ClustalW (European Bioinformatics Institute, Cambridge, UK). Two genotypes were observed for each SNP: T-327G homozygous thymine (TT), and heterozygous (TG), D-263C homozygous deletion (DD) and heterozygous (DC), G390A, homozygous guanine (GG) and heterozygous (GA), A406G, homozygous adenine (AA) and heterozygous (AG), and T530C homozygous thymine (TT) and heterozygous (TC). Homozygous and heterozygous alleles were identified by assessing sequence chromatograms using the ABI PRISM Sequence Scanner V1.0 (Applied Biosystems) and BIOEDIT (Hall, 1999).

3.3.6 Lactate Dehydrogenase Activities

LDH activities ware determined using a colormetric kinetic assay on Spectra Max 250 (CV within 10% Molecular Devices, Sunnyvale, CA). Forward reaction of LDH was determined using reagents β-nicotinamide adenine dinucleotide (0.0194 mmol/L; Sigma, Sr. Louis, MO) and pyruvic acid (16.2mmol/L; Sigma). Samples were analyzed at 340nm four times, one minute apart. Reverse reaction of LDH was determined using reagents NAD^+ (7mmol/L; Calbiochem, San Diego, CA) and L-lactic acid lithium salt (50mmol/L; Tokyo Co., Tokyo, Japan). Samples were analyzed at 340nm three times, 30 seconds apart.

3.3.7 Statistical Analysis

Calving percentage was analyzed by Chi-square. The effects of haplotypes and genotypes on calving date were determined by analysis of variance.

3.4 Results and Discussion

3.4.1 Identification of Polymorphisms

Five single nucleotide polymorphisms (SNP) sites were detected. Two SNP in the promoter region and three SNP on the coding sequence site were identified by amplification and sequencing of the 507-bp and 555-bp fragments of the bovine LDH-A coding sequence and promoter regions (gene bank accession numbers NM_174099 and NW_001494522), respectively. Those SNPs were: T-327G, D-263C, G390A, A406G, and T530C (Table 1). The point mutation in which a single nucleotide was changed in the promoter site could change the transcriptional control or change gene expression, but would not change the protein polypeptide (silent mutation). A transition consisting of adenine to guanine at position 390 in the coding sequence resulted in an amino acid change from valine to isoleucine at position 129 (Val^{129}Ile) based on the full length sequence that was used as a reference (gi 31342847) (Fig. 1). The missense mutation that leads to amino acid change is a result of a single nucleotide change resulting in codons coded for a different amino acid. This kind of missense mutation is conservative because the properties of the amino acids remain the same. A transition from thymine to cytosine at position 530 based on the full nucleotide sequence that was used as reference (gi 31342847) resulting in silent mutation (no amino acid was changed; Fig. 1). For statistical analyses, the single cow that was homozygous for the minor allele was used with the heterozygous cows.

3.4.2 *Base Position -327*

At base -327, a transversion was determined from a thymine to guanine (G to A) in the promoter region. Six cows were heterozygous with the minor allele (Table 1). Calving date was affected neither ($P > 0.10$) by the genotype nor by BCS. Our result did not agree with other results that indicated the influence of BCS on the calving date (Randel, 1990; Lalman et al., 1997; Spitzer et al., 1995), but it did agree with another documentation that determined that BCS did not affect the caving date (Selk, et al., 1988). Reproductive hormones (PRL and T3) were influenced ($P < 0.02$) by body condition but not by the genotype. IGF-I was affected ($P < 0.05$) by body condition; however, LDHr and LDHf were not influenced ($P > 0.10$) by body condition or genotype. Genotype affected ($P < 0.02$) NEFA.

3.4.3 *Base Position -263*

Deletion of six nucleotides (GGCCGC) was detected at base -263 with an insertion of cytosine. The deletion /insertion could influence gene expression or the transcriptional control since they happened in the promoter regionmost of the cows (n=83) were either heterozygous or homozygous for the deletion (Table 1). Calving date was not influenced ($P > 0.10$) by the SNP (D-263C) or and by BCS. This result was not in agreement with previous research which indicated that BCS influenced the calving date (Bellows et al., 1982; Rutter and Randel, 1984; Richards et al., 1989). LDHf and LDHr were not affected ($P > 0.10$) by the genotype or BCS. However, the interaction between BCS and genotype affected ($P < 0.05$) LDHf. No significant effects ($P > 0.10$) of the SNP on T3, IGF-I, and PRL were determined. In contrast, body condition had significant effects ($P < 0.05$) on PRL, IGF-I, and T3 which was in agreement with other results that indicated an increase in gonadotropic hormones in cows with moderate BCS (Short and Adams, 1988; Rutter and Randel, 1984; Richards et at., 1991; Ciccioli et al., 2003).

Spicer et al., (1989) indicated that IGF-I was not influenced by BCS but decreased IGF-I was due to decreasing in LH concentrations in low body condition cows.

3.4.4 LDH-A Haplotypes

Four unique haplotypes were derived from the 4 SNP sites (Table 2). Frequency of those haplotypes was from two to 58. Haplotype 1 had the same sequence as that published at GenBank (GenBank accession numbers NM_174099 and NW_001494522). Thus, the cows (n =58) with haplotype 1 were classified as "No SNP". 66 cows represented three haplotypes which were classified as "Deletion".

Haplotypes did not affect (P > 0.10) calving date, LDHf, and LDHr (P > 0.10). The interaction between BC X haplotypes had an effect on LDHf. IGF-I, T3, PRL, or NEFA were not influenced (P > 0.10) by the haplotypes. Hormones (PRL, T3, and IGF-I) were affected (P < 0.02) by body condition.

Our results documented that body condition had significant effects on hormones which is in agreement with previous reports. As reported by Looper et al., (2010), the IGF-I and PRL concentrations were low in cows with thin body condition which is similar to our result. IGF-I, PRL, T3, and T4 concentrations were greater in moderate-BC cows compared with low-BC cows (Flores et al., 2008). In contrast, the genotypes did not show significant effects on hormones. Furthermore, those genotypes did not affect the calving date. LDHr and LDHf were not affected by the haplotypes and the SNPs; whereas, LDHf was affected by an interaction between haplotype and BC.

3.5 Implications

The SNP identified in the promoter and coding sequence regions of the bovine LDH-A genes may be used as genetic markers for cows which are prone to have a greater calving rate.

Our results suggested that more investigation is needed to document interactions between

genotypes and environment (body condition, forage, heat stress, etc) on cattle reproductive

performance which is associated with cow/calf enterprise profitability.

3.6 Tables

Table 1. Description of SNP in (507-bp and 555-bp) amplicon of the bovine LDH-A coding sequence and promoter, respectively

| Polymorphism[1] | Genotype Distribution[2] | | | MAF[3] |
	Homo	Hetero	homo	
T-327G	77	6	0	7.3
D-263C	69	14	0	16.9

[1] Single nucleotide polymorphism (SNP) occurred at the number indicated. First letter the primary allele and the letter following the digits is the minor allele (D represent deletion of six nucleotides).

[2] Number of cows that were homozygous for the major allele (Homo), heterozygous (Hetero), and homozygous fort the minor allele.

[3] Minor allele frequency expressed as a percent.

0

Table 2. Haplotype frequency in a (507-bp and 555-bp) amplicon of the bovine LDH-A coding sequence and promoter, respectively

| | Haplotype[1] | No. observations |
Number	Sequence	
1	TDGAT	58
2	TDGAC	2
3	TCGAT	14
4	GDGAT	6

[1] All of these haplotypes have the same order of single nucleotide polymorphisms (SNP): T-327G, D-263C, G390A, A406G, and C530A. Haplotype one represents the published sequence (GenBank accession numbers NM_174099 and NW_001494522). Deletion of six nucleotides is represented as D.

3.7 Figures

```
NM_174099.2
MATLKDQLIQNLLKEEHVPQNKITIVGVGAVGMACAISILMKDLADEVALVDVMEDKLKG 60
LDHA_t530c
MATLKDQLIQNLLKEEHVPQNKITIVGVGAVGMACAISILMKDLADEVALVDVMEDKLKG 60
LDHA_g390a
MATLKDQLIQNLLKEEHVPQNKITIVGVGAVGMACAISILMKDLADEVALVDVMEDKLKG 60


************************************************************


NM_174099.2
EMMDLQHGSLFLRTPKIVSGKDYNVTANSRLVIITAGARQQEGESRLNLVQRNVNIFKFI 120
LDHA_t530c
EMMDLQHGSLFLRTPKIVSGKDYNVTANSRLVIITAGARQQEGESRLNLVQRNVNIFKFI 120
LDHA_g390a
EMMDLQHGSLFLRTPKIISGKDYNVTANSRLVIITAGARQQEGESRLNLVQRNVNIFKFI 120


*****************:******************************************


NM_174099.2      IPNIVKYSPNCKLLVVSNPVDILTYVAWKISGFPKNRVI 159
LDHA_t530c       IPNIVKYSPNCKLLVVSNPVDILTYVAWKISGFPKNRVI 159
LDHA_g390a       IPNIVKYSPNCKLLVVSNPVDILTYVAWKISGFPKNRVI 159
        ***************************************
```

Figure 1. Amino acid sequence alignments showing the conserved region (*) within the full-length sequence of bovine LDH-A. The single-nucleotide polymorphism T530C identified in the nucleotide sequence did not result in an amino acid change at position 530 (:) relative to the reference sequence (NCBI). The single-nucleotide polymorphism G390A identified in the nucleotide sequence resulted in an amino acid change from valine to isoleucine at position 129 (:) relative to the reference sequence (NCBI).

3.8 Literature Cited

Bellows, R. A., R. E. Short, and G. V. Richardson. 1982. Effects of sire, age of dam and gestation feed level on dystocia and postpartum reproduction. J. Anim. Sci. 55(1): 18-27.

Cardinet, G.H. 1997. Skeletal muscle function. In: Kaneko, J.J, J.W. Harvey, M.C. Bruss, eds. Clinical biochemistry of domestic animal. 5th ed. Academic Press; p407-440. San Diego, CA.

Ciccioli, N. H., R. P. Wettemann, L. J. Spicer, C. A. Lents, F. J. White, and D. H. Keisler. 2003. Influence of body condition at calving and postpartum nutrition on endocrine function and reproductive performance of primiparous beef cows. J. Anim. Sci. 81:3107-3120.

Crookshank, H. R., M. H. Elissalde, R. G. White, D. C. Clanton, and H. E. Smalley. 1979. Effect of transportation and handling of calves upon blood serum composition. J. Anim. Sci. 48:430-435.

Flores, R., M. L. Looper, R. W. Rorie, D. M. Hallford, and, C. F. Rosenkrans. 2008. Endocrinefactors and ovarian follicles are influenced by body condition and somatotropin in postpartum beef cows. J. Anim. Sci. 86:1335-1344.

Galyean, M. L., R. W. Lee, and M. E. Hubbert. 1981. Influence of fasting and transit on ruminal and blood metabolites in beef steers. J. Anim. Sci. 53:7-18.

Hall, T. A., 1999: BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symposium Series 41, 95–98.

Lalman, D. L., D. H. Keisler, J. E. Williams, E. J. Scholljegerdes, and D. M. Mallett. 1997. Influence of postpartum weight and body condition change on duration of anestrus by undernourished suckled beef heifers. J. Anim. Sci. 75:2003-2008

LI, S. S. -., H. F. Tiano, K. M. Fukasawa, K. Yagi, M. Shimizu, F. S. Sharief et al. 1985. Protein structure and gene organization of mouse lactate dehydrogenase-A isozyme. European Journal of Biochemistry. 149:215-225.

Looper, M. L., S. T. Reiter, B. C. Williamson, M. A. Sales, D. M., and C. F. Rosenkrans, 2010. Effects of body condition on measures of intramuscular and rump fat, endocrine factors and calving rate of beef cows grazing common Bermudagrass or endophyte-infected tall fescue. J. Anim. Sci. 88:4133-4141.

Looper, M.L., T.P. Neidecker, C.W. Wall, S.T. Reiter, R. Flores, A.H. Brown, r. J, Z.B. Johnson, and, C.F. Rosenkrans. 2002. Relationship of Lactate Dehydrogenase Activity with Body Measurements of Angus x Charolais Cows and Calves. Prof. Anim. Sci. 18:120.

Maekawa, M., K. Sudo, S. S.-L. Li, and T. Kanno. 1993. Analysis of genetic mutations in human lactate dehydrogenase-A(M) deficiency using DNA conformation polymorphism in combination with polyacrylamide gradient gel and silver staining. JF Human Genetic. 19:163-168.

Randel, R. D. 1990. Nutrition and postpartum rebreeding in cattle. J. Anim. Sci. 68:853-862.

Richards, M. W., R. P. Wettemann, and H. M. Schoenemann. 1989. Nutritional anestrus in beef cows: Body weight change, body condition, luteinizing hormone in serum and ovarian activity. J. Anim. Sci. 67: 1520-1526.

Richards, M. W., R. P. Wettemann, L. J. Spicer, and G. L. Morgan. 1991. Nutritional anestrus in beef cows: Effects of body condition and ovariectomy on serum luteinizing hormone and insulin-like growth factor-I. Biology of Reproduction. 44:961-966.

Rutter, L. M., and R. D. Randel. 1984. Postpartum nutrient intake and body condition: Effect on pituitary function and onset of estrus in beef cattle. J. Anim. Sci. 58:265-274.

Selk, G. E., R. P. Wettemann, K. S. Lusby, J. W. Oltjen, S. L. Mobley, R. J. Rasby et al. 1988. Relationships among weight change, body condition and reproductive performance of range beef cows. J. Anim. Sci. 66:3153-3159.

Spicer L.J., W.B. Tucker, and G.D. Adams . 1990. Insulin-Like Growth Factor-I in Dairy Cows: Relationships Among Energy Balance, Body Condition, Ovarian Activity, and Estrous Behavior1. J. Dai. Sci. 73:929-937.

Spitzer, J. C., D. Short, R. E., and D. C. Adams. 1988. Nutritinal and hormonal interrelationships in beef cattle reproduction. J. Anim. Sci. 68:29-39.

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

Williamson, B. C., C. F. Rosenkrans, Jr., S. T. Reiter, J. D. Thompson, M. A. Sales, and M. L. Looper. 2010. Identification of Single Nucleotide Polymorphisms of the Lactate Dehydrogenase-B Gene and Association with Cow and Calf Performance. Prof. Anim. Sci.

Wrobleweski, F. 1958. The Mechanisms of alternation of lactic dehydrogenase activity in body fluids. Ann. N.Y. Acad. Sci.,75: 322-338.

4.0 CONCLUSION

The SNP identified in the promoter and coding sequence regions of the bovine LDH-A and LDH-B gene may be used as genetic markers for selecting cows which are prone to have a greater calving rates. Our results suggested that more investigation is needed to document interactions between the genotypes and environment (body condition, forage, heat stress, etc) on cattle reproductive performance which is associated with cow/calf enterprise profitability.