EFFECTS OF DIRECT FED MICROBIALS ON CONCENTRATIONS OF GLUCOSE AND INSULIN IN PLASMA AND ANIMAL PERFORMANCE

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CHAPTER I

INTRODUCTION

Heifers that reach puberty prior to the breeding season have a greater ability to calve at two years of age and wean more pounds of calf in their reproductive lifetimes compared with heifers that fail to achieve puberty prior to the breeding season (Lessmeister et al., 1973). Conception rates are greater in heifers bred at their third rather than their pubertal estrus (Byerley et al., 1987). Development of beef heifers so that a large percentage is pubertal by 15 months of age is a major problem in the beef industry today. Most beef heifers are weaned in October, which nutrient intake is limited prior to breeding the next May. By reducing the age at the onset of puberty it is possible to increase pregnancy rate by increasing the number of estrous cycles prior to the breeding season. Heifers that becomes pregnant earlier in the breeding season produce calves that weigh more and have a greater value at weaning. Optimal heifer management can have a major effect on profitability since about 7 million heifers are added to the US cow herd each year.

Many experiments have evaluated mechanisms by which producers can reduce the age at puberty of beef heifers. Feeding high concentrate diets increase propionate production in the rumen and decrease age at puberty (Moseley et al., 1982). Increased energy intake and growth rate after weaning can reduce the age at puberty in beef heifers (Wiltbank et al., 1969; Arjie and Wiltbank, 1971; McShane et al., 1989).

Dietary treatments that increase concentrations of propionate in the rumen can increase concentrations of glucose and insulin in plasma. Increased concentrations of glucose and insulin in plasma of heifers have the potential to decrease the age at the onset of puberty and increase the number of pubertal heifers prior to the breeding season.

Feeding propionibacteria 169 (P169; Agtech Products Inc., Waukesha, WI) resulted in increased ruminal propionate concentrations and increased concentration of glucose and insulin in plasma of lactating dairy cows (Stein et al., 2006; Aleman, 2005). Lactating dairy cows fed yeast cultures for the first 6 wk of lactation increased concentrations of propionate in the rumen (Harrison et al., 1988). Feeding P169 and yeast culture to beef heifers prior to the breeding season may increase ruminal propionate concentrations and could increase plasma insulin and glucose concentrations, which would stimulate the onset of puberty and increase pregnancy rate. Propionibacteria and yeast culture increase pregnancy rate by 10 % and heifers become pregnant an average of 2 wk earlier in the breeding season. An increased profit of $ 23 per heifer exposed to a bull or artificially inseminated would be achieved.

An understanding of factors that influence the onset puberty in beef heifers may lead to management decisions to increase reproductive performance of heifers and enhance profitability of beef producers. Feeding direct fed microbials (DFM) may alter ruminal concentrations of propionate and plasma concentrations of glucose and insulin, and these changes could enhance animal and reproductive performance in beef and dairy cattle. Determination of weight at puberty relative to mature body weight will allow development of management strategies to increase the number of heifers that are pubertal before the breeding season.

CHAPTER II

REVIEW OF LITERATURE

Probiotics

Introduction

Probiotic is defined as a live naturally occurring microorganism that affects the host animal by improving its microbial balance (Fuller, 1989; Yoon and Stern, 1995). The addition of probiotics to animal diets has the potential to improve the production efficiency of animals, enhance the safety of the food produced by animals, and increase the health status of offspring. Probiotic is also known as direct fed microbials (DFM) which can improve gut microflora, reduce the risk of acidosis (Ghorbani et al., 2002) and increase weight gain (Yoon and Stern, 1995). Propionibacteria, yeast and fugal cultures are three types of probiotics that have been studied for inclusion into animal diets. The inclusion of yeast cultures in diets increased milk yield of lactating dairy cows (Piva et al., 1993) and body weight gain of early weaned calves (Hughes, 1998). Dry matter intake was increased by feeding the fungal culture, *Aspergillus Oryzae* (Gomez-Alarcon et al., 1990). Ruminal pH is increased by inclusion of yeast cultures to decrease production and increase utilization of lactic acid (Chaucheyras et al., 1996). Multiparous lactating dairy cows fed a combination of yeast and bacteria cultures from wk 3 prepartum to wk 10 postpartum, had increased DMI, milk yield and blood glucose concentrations (Nocek and Kautz, 2006). The combination of *Lactobacillus acidophilus*

NP 51 and *Propionibacterium freudenreichii* in the finishing diets of steers reduced fecal shedding of *Escherichia coli* O157 and have the potential to improve the ability of the beef industry to provide a safer food product (Elam et al., 2003). A probiotic strain, *Enterococcus faecium*, can reduce the rate of infection in piglets by obligate intracellular pathogens (Pollmann et al., 2005).

Propionibacteria

Propionibacteria are natural inhabitants of the rumen that make up about 1.4 % of the rumen microflora (Oshio et al., 1987), and their end product is propionic acid (Grinstead et al., 1992). Steers fed Propionibacteria 15 (P 15) had increased numbers of protozoa and concentrations of NH_3 in the rumen and decreased the numbers of amylolytic bacteria (Ghorbani et al., 2002).

Inclusion of Propionibacteria 63 (P63) in the diet of feedlot steers for 10 d did not influence gain or feed efficiency (Swinney-Floyd et al., 1999). Combination of P63 with a lactobacillus species increased gain and improved feed efficiency of steers during 10 d of treatment (Swinney-Floyd et al., 1999). Finishing diets that included bacteria producing a combination of propionic acid and lactic acid have improved growth rate by 2.6 % and increased carcass weight of steers by 6 kg (Krehbiel et al., 2003). Feeding (1 x 10^9 cfu/animal) P63 to heifers on a high concentrate diet for 126 d did not influence ADG, DMI or feed efficiency (Huck et al., 2000). If heifers were fed a high concentrate diet with P63 (1 x 10^9 cfu/animal) for 28 d, then lactobacillus acidophilus BG2FO4 (5 x 10^8 cfu/animal) for 120 d, daily gain was improved without an effect on feed efficiency (Huck et al., 2000). However, heifers fed a high concentrate diet with lactobacillus

acidophilus BG2FO4 (5 x 10^8 cfu/animal) for 28 d, then P63 (1 x 10^9 cfu/animal) for 120 d, had increased gains and improved feed efficiency by 5.0 % and 5.1 % respectively.

Inclusion of Propionibacteria 169 (P169; 6 x 10^{10} cfu/cow) in the diet of dairy cows for the first 12 wk postpartum did not influence concentration of glucose, insulin, cholesterol, insulin like growth factor binding protein III, IGF-I in plasma or reproductive function (Francisco et al., 2002). Adding 6 x 10^{10} or 6 x 10^{11} cfu/cow of P169 in the diet of multiparous and primiparous lactating dairy cows for 30 wk after calving increased milk production, but did not influence reproductive function (Stein et al., 2006). Feeding 6 x 10^{11} cfu/cow of P169 to multiparous and primiparous lactating dairy cows increased ruminal concentrations of propionate (Stein et al., 2006). This increase in ruminal propionate concentrations increased concentrations of insulin in plasma with no effect on glucose concentrations during the first 12 wk of treatment (Aleman, 2005; Stein et al., 2006). Feeding P169 (6 x 10^{10} cfu/cow) to primiparous lactating dairy cows for 30 wk increased concentrations of glucose by 6 to 9 % and decreased insulin during wk 13 to 25 of treatment (Aleman, 2005).

Propionibacteria naturally inhabits the rumen and can alter VFA production NH_3 production by influencing the bacteria content in the rumen. Increasing the number of propionibacteria in the rumen may increase ruminal concentrations of propionate and plasma concentration of glucose and insulin. Supplementation of propionibacteria species, after feeding a lactobacillus species, may improve feed efficiency and ADG.

Yeast Cultures

The feeding of yeast cultures has shown inconsistent results for DMI and no effect on ADG in dairy and beef cattle. Inclusion of yeast cultures in diets during early lactation increased DMI in dairy cows (Williams et al., 1991). Primiparous Holstein cows, fed 10 g of yeast culture (5×10^9 cfu of *Saccharomyces cerevisiae*/g), tended to have greater DMI during the first 4 wk of lactation which was associated with greater milk yield through the first 18 wk of lactation (Wohlt et al., 1991). Steers and heifers fed 28.4 g/steer of yeast culture had increase organic mater digestibility which possible led to increased DMI (Olson et al., 1994). In contrast, DMI of multiparous lactating dairy cows was not influenced by feeding 90 g/d of yeast culture (2×10^6 cfu of *Saccharomyces cerevisiae*/g) for 10 wk (Armabel et al., 1990). Body weight of crossbred dairy cows was not influenced by feeding yeast cultures during mid-lactation (Komari et al., 1999). Feeding 10 g/steer of yeast cultures (*Saccharomyces cerevisiae*, 5×10^9 live organisms/g of growth medium) daily to Hereford steers receiving high-grain or high-forage diet did not influence ADG or DMI (Mir and Mir, 1994). The inclusion of 10 or 20 g/d of yeast culture in the corn silage diet of mid lactating dairy cows did not influence DMI (Kung et al., 1997).

Yeast cultures have shown varied effects on concentrations of propionate in the rumen of dairy and beef cattle. Ruminal concentration of propionate was increased and acetate to propionate ratio was decreased during the 6 wk supplementation of yeast cultures to lactating dairy cows (Harrison et al., 1988). The acetate to propionate ratio was reduced in steers with inclusion of yeast cultures in the diet (Williams et al., 1991). In contrast, yeast cultures in the diet of lactating Holstein cows did not affect VFA

concentrations (Yoon and Stern, 1996). Feeding a concentrate diet containing 1.6 % yeast culture did not influence propionate concentrations in the rumen of Hereford steers (Malcolm and Kiesling, 1990). The inclusion of 3 g/d of yeast culture in a fescue based diet of Jersey steers for 10 d did not influence propionate concentrations in the rumen.

Limited research has been completed to determine the effects of yeast culture on concentrations of glucose and insulin in plasma. Piva et al. (1993) reported that inclusion of 10 g/d of yeast culture (*Saccharomyces cerevisiae*, 10 x 10^9 yeast cells/g) in the diet of lactating dairy cows for 6 wk did no influence plasma concentrations of insulin and glucose. Feeding a combination of 1 g of *Saccharomyces cerevisiae* (5 x 10^9 cfu/g) and 1 g of *Enterococcus faecium* strains (5 x 10^9 cfu/g) increased concentrations of glucose between wk 0 and 12 wk postpartum in lactating dairy cows (Nocek and Kautz, 2006).

Different cultures and concentrations of yeast may have different effects on cattle. Yeast cultures can increase DMI and milk production of lactating dairy cows. Yeast cultures can increase concentrations of propionate in the rumen but little research has been done to determine if this influences concentrations of glucose and insulin in plasma. The feeding of yeast cultures in combination with a bacteria species may increase concentrations of glucose in plasma.

Puberty in Heifers

Introduction

From a practical standpoint, the occurrence of puberty in the heifers is the culmination of a series of events that results in ovulation accompanied by estrus and normal luteal function (Moran et al., 1989). The onset of puberty is the result of increased secretions of Lutenizing Hormone (LH) due to a decline in estradiol negative feedback on the hypothalamus (Moran et al., 1989; Day and Anderson, 1998). The requisite stimulus for the occurrence of puberty is an increase in pulsatile LH secretion as a result of pulsatile release of gonadotropin releasing hormone (GnRH) from the hypothalamus (Day and Anderson, 1998). The increase in LH secretions occurs approximately 50 d preceding puberty in heifers (Day et al., 1984, 1987). The bovine ovary produces one or more dominate follicles during the period awaiting the prepubertal decline in estradiol negative feedback (Day and Anderson, 1998). The decline in estradiol negative feedback causes increased secretion of LH, ovulation of the dominant follicle, and formation of a corpus luteum which produces progesterone. Numerous factors affect the onset of puberty in heifers. Physiological, metabolic, and endocrine changes that occur prior to puberty may be hastened by increased nutrient intake, steroids, and feed additives.

Factors affecting the onset of puberty

Body weight is an important regulator of the onset puberty. Chelikani et al. (2003) concluded that dairy heifers attain puberty at a constant weight and body composition, independent of dietary manipulation. Heifers supplemented with cottonseed hulls were younger but had similar weight at conception compared with

heifers supplemented with soybean meal (Simpson et al., 1998). Body condition and body weight are important indicators of the onset of puberty and account for 55 % of the variation in age at first conception (Simpson et al., 1998).

Diet influences weight and age at the onset of puberty in beef heifers. Increasing gains from a moderate rate to high rate decreases age at puberty of beef heifers (Hall et al., 1995; Yelich et al., 1995). Heifers with greater energy in the diet were younger with a similar body weight, as moderate energy heifers (Hall et al., 1994). Energy intake is positively related to growth rate and inversely related to age at the onset of puberty (Arjie and Wiltbank 1971; McShane et al., 1989). Heifers on a high level of nutrition had increased energy reserves at puberty that may have caused an earlier age at puberty (Yelich et al., 1995).

Rate of gain influences age and weight at the onset of puberty (Yelich et al., 1995). Heifers fed to gain 0.5 kg/d tended to be older and weigh less at puberty than heifers fed to gain 1 kg/d (Hooper et al., 1993). Heifers on a high gain diet attained puberty at a heavier weight than heifers on a moderate gain diet (Hall et al., 1995; Yelich et al., 1995). An accelerated growth regimen for Holstein heifers decreased age at puberty by 32 d (Lammers et al., 1999). Increased weaning weight and post-weaning gain improve the probability that heifers would reach puberty before the breeding season (Buskirk et al., 1995).

Body weight, diet and rate of gain influence the onset of puberty. Increasing nutrient intake will increase rate of gain and hasten the onset of puberty. Carcass lipid deposition is greater for heifers on greater nutrient intake and this may decrease the age at puberty. Greater nutrient intake may increase body weight at the onset of puberty.

Reduced nutrient intake will decrease rate of gain and this could increase age and weight at the onset of puberty.

Prepubertal physiological, metabolic, and endocrine changes

Prepubertal development prepares the system for the establishment of pregnancy. Ovarian weight increased 2.7 times faster than body weight in Holstein heifers between 6 and 12 mo of age (Desjardins and Hafs, 1969). Vaginal weight increased slightly more rapid than body weight between 6 and 12 mo of age, and cervical weight gain was greater than body weight gain starting at 8 mo of age (Desjardins and Hafs, 1969). The rate of uterine growth was greater than body weight between 6 and 12 mo of age (Desjardins and Hafs, 1969), and uterine weight increased rapidly at approximately 50 d preceding puberty (Day et al., 1987). Ovulation of the dominant follicle is an important event in the onset of puberty and ovulation was delayed by decreased concentrations of insulin like growth factor I (IGF-I) in serum which impaired the ability of the ovary to synthesize adequate perovulatory concentrations of estradiol (Schoppee et al., 1996).

Immunization of heifers against GnRH delayed the initiation of normal ovarian cycles (Wettemann et al., 1994). Treatment with exogenous gonadotropins stimulated follicular growth, increased concentrations of estradiol in plasma, and induced ovulation in anovulatory heifers that were immunized against GnRH (Bishop et al., 1996). Weight gain was increased and age at puberty was reduced when heifers were treated twice daily with GnRH from 4 to 8 wk of age (Madgewick et al., 2005).

Concentrations of LH are a critical signal for the timing of puberty in heifers (Madgewick et al., 2005). Pulsatile release of LH gradually increases as beef heifers approach puberty (Day et al., 1984). The increase in LH concentrations in serum prior to

puberty commences at 16 wk of age in Holstein heifers and concentrations are maximal at puberty (Nakada et al., 2000). Average concentrations of LH and the frequency of release of LH, were greater in heifers 17 d before puberty compared with 40 d before puberty (Jones et al., 1991). Nutrient restriction delayed the onset of puberty in beef heifers by interrupting the pulsatile release of LH in peripubertal heifers through the estradiol dependent and ovary independent mechanisms (Kurz et al., 1990). The estradiol dependent mechanism involves prolong steroidal or ovarian inhibition of LH, while the ovary independent mechanism involves a direct inhibition of the hypothalamo-pituitary function suppressing LH secretion.

Concentrations of follicle stimulating hormone (FSH) in Holstein heifers increase 4 wk prior to puberty (Nakada et al., 2000). However concentrations of FSH are not directly associated with the onset of puberty (Dobson et al., 1988). Increased concentrations of FSH in plasma prior to puberty may stimulate production of estradiol and progesterone by bovine granulosa cells (Spicer et al., 1993).

Concentrations of estradiol 17β in plasma of Holstein heifers increased from 20 wk of age until puberty (Nakada et al., 2000). Inadequate serum concentrations of IGF-I are associated with decreased ovarian synthesis of estradiol, delayed stimulation of the LH surge, and delayed onset of puberty (Schoppee et al., 1996). Estradiol negative feedback on LH secretion decreases as puberty approaches in beef heifers (Day et al., 1984), which could be due to a decline in estradiol receptors in the anterior medial hypothalamus and the medial basal hypothalamus without changes in the medial preoptic area and the median eminence (Day et al., 1987). Bos tarus and Bos indicus heifers have

the same decline in estradiol negative feedback as they approach the onset of puberty (Rodrigues et al., 2002).

Progesterone is produced by luteal tissue in the ovary at approximately 9 d prior to the first estrus at puberty (Berardinelli et al., 1979). Progesterone increases the sensitivity of the ovaries to the endogenous gonadotropins, permits estradiol to cause estrus behavior and results in the formation of normal corpus luteum (Gonzalez-Padilla et al., 1975; Nakada et al., 2000).

Growth of the reproductive tract starts to increase at a faster rate than body weight at 6 mo of age. Increased pulsatile release of GnRH results in pulsatile release of LH, which causes ovulation and the attainment of puberty. Estradiol negative feedback is a possible mechanism that inhibits the preovulatory surge in LH. Ovarian hormone synthesis is associated with IGF-I production, and decreased serum concentrations of IGF-I could delay stimulation of the LH surge and the onset of puberty. Furthermore there is a decrease in estradiol receptors in the anterior and medial basal hypothalamus which may result in decreased estradiol negative feedback on LH secretions as puberty approaches in beef heifers. Stimulation of bovine granulosa cells with FSH results in production of estradiol and progesterone which may be associated with the onset of puberty.

Induction of puberty

Insulin-like growth factor I may be the signal that influences hypothalamic secretion of GnRH, because concentrations of IGF-I are decreased in plasma prior to the onset nutritionally induced anovulation in beef heifers (Bossis et al., 1999). Increased nutrient intake of heifers resulted in greater IGF-I concentrations and increased IGF-I was

associated with early attainment of puberty (Yelich et al., 1995; Yelich et al., 1996). Heifers fed low quality hay had decreased IGF-I concentrations and the onset of puberty was delayed compared with heifer fed hay plus a grain supplement (Granger et al., 1989). Immunization of pubertal heifers against growth hormone releasing factor caused decreased concentrations of IGF-I and estradiol in plasma, and delayed the onset of puberty (Armstrong et al., 1992; Schoppee et al., 1996). Insulin-like growth factor I stimulated proliferation of bovine granulosa cells, and production of estradiol and progesterone were increased (Spicer et al., 1993).

Administration of a progestin implant to heifers hastens the onset of puberty. Removal of progestin implants increases serum concentrations of lutenizing hormone by reducing the prepubertal estradiol negative feedback (Short et al., 1976; Anderson et al. 1996). The increase in serum lutenizing hormone due to progestin implants was correlated with the decline in estrogen receptors in the ventral medial nucleus of the anterior hypothalamus (Anderson et al., 1996). Age of the beef heifer is a critical factor influencing the efficacy of progestins to induce puberty (Hall et al., 1997). Insertion of an intravaginal releasing device containing progesterone in prepubertal heifers for 7 d increased the number of heifers in estrus and forming normal CL, and addition of estradiol benzoate will further enhanced these responses (Rasby et al. 1998).

Insulin binds specifically to receptors in the accurate nucleus and median eminence of hypothalamus (van Houten et al., 1983) and enhanced gonandotropin synthesis by rat pituitary cells in vitro (Adashi et al., 1981). Insulin stimulated proliferation of bovine granulosa cells and production of progesterone and estradiol were increased (Spicer et al., 1993). Greater nutrient intake increased serum concentrations of

insulin, increased the frequency of LH release, and resulted in an earlier age of puberty in Angus x Hereford heifer (Yelich et al., 1996). Immunization of Angus x Charolais heifers against growth hormone releasing factor delayed the onset of puberty which may have resulted from a decline in plasma concentrations of insulin (Simpson et al., 1991).

Concentrations of glucose in plasma are positively associated with nutrient intake in heifers (Yelich et al., 1996) and cows (Richards et al., 1989a). Heifer fed a higher level of nutrition (gain 1.36 kg/d) had greater concentration of glucose in the plasma compared with heifers that gained 0.23 kg/d (Yelich et al., 1996). When 9-month-old heifers were fed to gain 0.23 kg/d for 16 wk, then fed to gain 1.36 kg/d, concentrations of glucose and insulin in plasma increased linearly until the onset of puberty (Yelich et al., 1996). Minimal concentration of glucose in plasma did not provide adequate amounts of energy needed to cause secretion of LH and to stimulate ovarian function (Richards et al., 1989b). Increased nutrient intake of heifers resulted in greater concentrations of glucose in plasma and was associated with early attainment of puberty (Yelich et al., 1995).

Abomasal infusion of propionate enhanced the ability of prepuberal Brangus heifers to respond to a GnRH challenge with greater LH secretion (Rutter et al., 1983). Alteration of the ruminal fermentation pattern to increase ruminal propionate production decreased age at puberty (McCartor et al., 1979). In contrast, Lalman et al. (1993) found that addition of 400 g of a 50 % propionic acid 50 % water mixture sprayed on the roughage in the diet had no effect on age, or weight at puberty. Concentrations of propionate are positively associated with concentrations of insulin in plasma of heifers on concentrate diets (Burgwald-Balastad et al., 1995). Infusion of propionate in the rumen increased plasma insulin concentrations, concentrations of LH and amplitude of pulses of

LH in serum (DiCostanzo et al., 1999). The onset of puberty may be stimulated by increased ruminal propionate concentrations.

Addition of monensin to high roughage diets of beef heifers decreased age at puberty without an effect on weight or average daily gain (Moseley et al., 1982; Lalman et al., 1993). Feeding monensin to dairy heifers reduced age at puberty with no affect on body weight or composition (Meinert et al., 1992). Monensin fed at 200 mg per head per day in a diet composed of 80% alfalfa and 20% concentrate increased ruminal propionate concentration, reduced age at puberty and decreased body weight at puberty compared with control heifers (McCartor et al., 1979). However the effects of monensin on puberty are dependent on adequate ADG of the heifers during supplementation (Floyd et al., 2004).

Multiple mechanisms can be used to hasten the onset of puberty in heifers. Increased serum concentrations of IGF-I may increase serum concentrations of estradiol which may hasten the onset of puberty. The use of progestin implants induced the onset of puberty in heifers that have reached physiological maturity, by increasing lutenizing hormone after progestin withdrawal and reducing the prepubertal estradiol negative feedback. Greater nutrient intake increases concentrations of insulin in plasma. Insulin binds to receptors in the arcuate nucleus and median eminence and may stimulate GnRH production and thus hasten the onset of puberty. Plasma concentrations of insulin may also induce puberty by increasing the proliferation of ganulosa cells and production of estradiol and progesterone. Greater nutrient intake increases plasma concentrations of glucose which may hasten the onset of puberty. Monensin and high concentrate diets

increase concentrations of ruminal propionate, which increase insulin concentrations, and decrease age at puberty.

Summary and Conclusion

Direct fed microbial increase DMI and ruminal propionate concentrations, and this response is dose and species dependent. Propionibacteria strains and yeast cultures may increase ruminal propionate concentrations which will increase plasma concentrations of glucose and insulin. The attainment of puberty in heifers is associated with increased growth of the reproductive tract and increases in steroid hormones and blood metabolites. Factors such as body weight, rate of gain, and plane of nutrition influence the onset of puberty.

Propionibacteria strain P169 increased concentrations of ruminal propionate and plasma concentrations of glucose and insulin in lactating dairy cows. Yeast cultures increased organic mater digestibility in beef steers. Feeding yeast cultures increased DMI and propionate concentrations in lactating dairy cows. Therefore we hypothesize that feeding propionibacteria strains P169, P5 and XP-yeast will increase the concentrations of propionate in the rumen, increase concentrations of insulin and glucose in plasma, improve ADG and feed efficiency. The increase in glucose and insulin concentrations in plasma, and greater ADG will hasten the onset of puberty. Hastening the onset of puberty will increase the number of heifers that become pubertal prior to the breeding season and increase pregnancy rates.

CHAPTER III

Effects of direct fed microbials on concentrations of glucose and insulin in plasma and animal performance.

Abstract

Sixty-two Angus x Hereford heifers were used to determine the effects of feeding direct fed microbials on plasma concentrations of glucose and insulin, feed intake, average daily gain, age at puberty, age at conception, conception rate to AI,and pregnancy rate. Heifers were born from February to April and weaned in October. Heifers grazed native grass pastures, had access to hay and received 2.3 kg/d of 20 % CP supplement from weaning until March. Shrunk BWs (restriction of feed and water for 16 h) were taken at one week before trial (wk -1), and on wk 8 and wk 20 of trial. Heifers were stratified to treatment based on the wk -1 BW. Fifteen heifers were assigned to each of the two treatment drylot pens (20 x 20 m) and sixteen heifers were assigned to each of the two control drylot pens (20 x 20 m). The control heifers received a pelleted low starch diet (9.5 mm) and the treated heifers received the pelleted low starch diet plus 5 g of a mixture of Propionibacteria strains P169 and P5, and XP-yeast (Diamond V Mills Inc., Cedar Rapids, IA). Heifers were acclimated to the pelleted low starch dietand drylot for 1 wk. From wk 0 to 8 of the trial, treatments were fed daily for the first 2 wk, and then fed Monday, Tuesday, Thursday and Friday for the last 6 wk. Heifers received 1 kg/hd of prairie hay daily from wk 0 to 8 of trial. Blood sample were collected weekly

via tail venipuncture during the 8 wk of treatment and for 12 wk after treatment, and plasma concentrations of glucose, insulin and progesterone were quantified. Estrus was synchronized in half of the treated heifers and half of the control heifers at the end of treatment, and these heifers were artificially inseminated. At 10 d after treatment all heifers were exposed to two fertile bulls in the same grass pasture until 12 wk after treatment. From wk 14 to 20 of treatment, heifers were supplemented with 0.45 kg/hd/d of a 40% CP diet to meet nutritional requirements. Conception rate to AI was determined by ultrasound approximately 30 d after AI. Pregnancy was determined 3 mo after the breeding season. Body weight (control, 336 ± 6; treated, 350 ± 6 kg) and age (control, 437 ± 6; treated 442 ± 6 d) at the onset of ovarian luteal activity was not influenced by treatment. Feed intake (control, 9.32 ± 0.20; treated, 10.09 ± 0.33 kg/d) and ADG (control, 1.21 ± 0.1; treated, 1.27 ± 0.07 kg/d) were similar during treatment. The treated and control heifers had similar ADG (control, 0.46 ± 0.10; treated 0.45 ± 0.02 kg/d) after treatment while heifers grazed pasture. Concentrations of glucose were not influenced by treatment during the treatment period, but there was a treatment x wk effect during the pasture period. Concentrations of insulin were not influenced by treatment during the treatment period and after treatment period while heifers grazed pasture. Age at pregnancy, AI conception rate and total pregnancy rate were not influence by feeding of direct fed microbials. Further research is needed to determine the effective dose, administration procedure, and combination of propionibacteria and yeast culture that will increase concentrations of glucose and insulin in plasma and enhance animal and reproductive performance.

Introduction

The term probiotic has been defined as "a live microbial feed supplement, which beneficially affects the host animal by improving its intestinal microbial balance" (Fuller, 1989). Probiotics are viable microbial cultures or culture extracts, enzymes preparations, or various combinations (Yoon and Stern, 1995). Probiotic are also known as direct fed microbials (DFM) which can improve gut microflora, reduce the risk of acidosis (Ghorbani et al., 2002) and increase weight gain (Yoon and Stern, 1995). The term DFM includes specific and nonspecific yeast, fungi, bacteria, cell fragments and filtrates (Beharka et al., 1993; Sullivan and Martin, 1999; Knowleton et al., 2002). Other effects of DFM in ruminants are stimulation of celluolytic bacteria and lactate-utilizing bacteria, increased fiber digestion and increased flow of ruminal microbial protein (Newbold et al., 1996). A decline in ruminal pH may be prevented by the decreased lactic acid production and increase the utilization of lactic acid by microbes (Chaucheyras et al., 1996).

Feeding of yeast cultures to ruminants altered molar proportions of VFA (Harrison et al., 1988), increase numbers of ruminal bacteria (Wiedmeier et al., 1987), and increase nutrient digestibility (Williams et al., 1991). Inclusion of yeast culture in the diet of lactating dairy cows increased DMI (Wohlt et al., 1991). Addition of yeast culture to diets of weanling pigs increased intake and performance without altering the microflora or net concentrations of fermentation products (Mathews et al., 1998).

Propionibacteria are natural inhabitants of the rumen and produce propionate, a major precursor for glucose production by hepatic gluconeogenesis (Sauer et al., 1989). Theoretical efficiency of propionate as a source of energy for ATP is 108 % compared with glucose (McDonald et al., 2002), and thus directly feeding propionibacteria may be

a natural way to increase hepatic glucose production and positively influence metabolism (Francisco et al., 2002). Inclusion of propionibacteria 169 (P169) in the diet increased concentrations of glucose and insulin in plasma but did not influence reproduction of lactating dairy cows (Aleman, 2005; Stein et al., 2006).

Concentrations of glucose and insulin in plasma may be signals that stimulate the endocrine system and initiate the onset of puberty in heifers (Jones et al., 1991). A nutritional induced increase in insulin in heifers was associated with decreased age at puberty (Hall et al., 1995). Hypoglycemia has been associated with decreased fertility in beef cattle (Selk, 1986). Nutritionally induced decreases in insulin and glucose are associated with nutritional anestrous in cows (Richards et al., 1989a). Secretion of luteinizing hormone was increased in postpartum anovulatory cows byinfusion of glucose (Garmendia, 1986) or propionate (Rutter et al., 1983). Feeding monensin to prepubertal heifers increased concentrations of propionate in the rumen and enhanced ovarian response to gonadotropins (Bushmich et al., 1980).

The increase in plasma concentrations of insulin and glucose, as a result of increased ruminal propionate concentration, may mediate the hypothalamo-pituitary-ovarian axis and influence the onset of puberty. Our objective was to evaluate the effects of propionibacteria and yeast culture supplement to beef heifers fed a low starch diet, prior to the breeding season, on plasma concentrations of glucose and insulin, animal performance, and reproductive function.

Material and Methods

All experimental procedures were approved by the Oklahoma State University Animal Care and Use Committee.

Sixty-two Angus x Hereford heifers that were born from February to April, and weaned in October were used in the study. From weaning through March 9, heifers grazed native grass pastures with access to hay and received 2.3 kg daily of 20 % CP supplement. Shrunk BWs (restriction of feed and water for 16 h) were recorded one week before study (wk -1), and on wk 8 and wk 20 of study. Heifers were stratified to treatment based on the wk -1 BW. The control heifers received a pelleted low starch diet (9.5 mm; Table 3.1) and the treated heifers received the pelleted low starch diet plus 5 g of a mixture of Propionibacteria strains P169 and P5, and XP-yeast (Diamond V Mills Inc., Cedar Rapids, IA). The particular propionibacteria strain P169 was originally isolated from rumen fluid collected from fistulated dairy cows at the OSU Dairy Cattle Center (Davidson, 1998) and manufactured by Agtech Products Inc. (Waukesha, WI). Propionibacteria strain P5 is a strain of *Propionibacterium acidipropionici* and was selected for its ability to increase metabolism of nitrates and nitrites and to increase feed intake (Swartzlander, 1994). Feed ingredients and nutrient concentrations of the diet are in table 3.1. Sixteen heifers were assigned to each of the two control drylot pens (20 x 20 m) and 15 heifers were assigned to each of the two treated drylot pens. Heifers were acclimated to the drylot for 1 wk. During the acclimation period, heifers received 0.5 kg/d prairie hay and 3.6 kg/d of the pelleted low starch dietfor 3 d, then 4 kg/d for 4 d. Between wk 0 and wk 8 of trial, treatments were fed daily for the first 2 wk, and then on

Monday, Tuesday, Thursday and Friday for the last 6 wk. From wk 0 to wk 8 of trial heifers receive 1 kg/hd/d of prairie hay and had access to pelleted low starch diet for 14 h per day. Heifers were denied access topelleted low starch diet for 10 h prior to feeding the treatment to stimulate appetite. The freeze-dried cell preparation contained strains 75 g of a mixture of Propionibacterium strains P169, P5, and XP-yeast fermentation products. The package containing 75 gwas mixed in water (2L) and sprinkled on 15 kg of low starch pelleted diet (for a pen), and control heifers were fed 1 kg/hd of low starch pelleted diet without direct fed microbials. Direct fed microbials were sprinkled on the feed to increase adherence to the pelleted low starch diet. A minimum of sixteen inches of bunk space per heifer was provided. The metal corral fences between pens had an electric fence one meter from them to eliminate nose to nose contact and heifers and cross contamination of propionibacteria stains and yeast cultures between pens of heifers on different treatments. Each pen had a self feeder that held 500 kg of feed and one water source.

Blood samples were collected weekly during the 8 wk of the treatment period and for 12 wk after the end of the treatment period while heifers grazed pasture by tail venipuncture. Samples at wk 0 and wk 1 were collected after heifers had been restricted from feed and water for 10 h, and the remaining samples were collected when heifers had ad libitum access to feed and water. Blood was collected into tubes containing EDTA, placed on ice, and centrifuged at 1,800 x *g* within 4 h after collection. Plasma was aspirated and stored at -20º C until progesterone was quantified (Vizcarra et al., 1997) in all samples using a solid phase RIA (Coat-A-Count progesterone kit, Diagnostic Products Corp., Los Angeles, CA) to determine the onset of ovarian luteal activity. Intra- and

interassay coefficients of variation were 5.1 % and 6.9 % respectively. Concentrations of progesterone greater than 0.5 ng/ml for two consecutive samples was the criterion used to determine the onset of ovarian luteal activity. Date of the first of the consecutive samples was consider the date at the onset of ovarian luteal activity. Weight at the onset of ovarian luteal activity was expressed as shrunk body weight and determined by extrapolation of shrunk body weights taken at wk -1, 8 and 20. Concentrations of insulin in plasma were quantified (Bossis et al., 1999) by a solid-phase RIA for human insulin (Diagnostic Products Corp., Los Angeles, CA) with bovine pancreatic insulin as the standard (Sigma Chemical Co., St. Louis, MO). Intra- and interassay coefficients of variation are 4.1 % and 4.9 % respectively. Plasma concentrations of glucose were quantified using an enzymatic colorimetric procedure (Thermo DMA, Louisville, Colorado). Samples were analyzed for glucose in triplicate and intra- and inter assay coefficients of variation were 4.1 % and 6.2 % respectively.

At 3 d before the end of the 8 wk treatment period, half of the heifers (those that were pubertal) on each treatment were treated with prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$; 25 mg Lutalyse, Pfizer) to synchronize estrus for artificial insemination. Heifers were observed for estrus on d 1 to d 5 after $PGF_{2\alpha}$ treatment at 0700 and 1900 for 30 min. Heifers that exhibited estrus after treatment with $PGF_{2\alpha}$ were inseminated 12 h after first observed in estrus. Heifers that did not exhibit estrus after the first injection with $PGF_{2\alpha}$ were retreated 7 d later and inseminated. At 10 d after the end of treatment, inseminated and non-synchronized heifers were exposed to two fertile bulls in the same pasture for 75 d. From wk 14 to 20 of treatment, heifers were supplemented with 0.45 kg/hd/d of a 40% CP diet to meet nutritional requirements. Conception rate to AI was determined by

ultrasound approximately 30 d after AI. Pregnancy was determined by rectal palpation 3 mo after the end of the breeding season. Date of conception was estimated from the subsequent calving date.

Mixed-model ANOVA procedures of SAS (SAS Inst., Inc., Cary, NC) for repeated measures were performed to determine the effects of treatment on plasma concentrations of glucose and insulin. Concentrations of glucose and insulin were analyzed for the 8 wk treatment period and for the 12 wk pasture period. Fixed effects were treatment and date. Random effects were cow within treatment and block. Blocks were used to assign samples for lab analysis to remove assay variation. Each treatment was represented in a block, and allsamples for heifers were randomly assayed within the block. If a significant week effect occurred, orthogonal contrasts were used to compare week effects for the 8 wk treatment period (wk 0 vs. wk 1, wk 0 and wk 1 vs. wk 2 to 8, wk 2 vs. wk 3, wk 4 vs. wk 5, wk 2 and 3 vs. wk 4 and 5, wk 6 vs. wk 7 and 8, and wk 7 vs. wk 8) and the 12 wk pasture period (wk 9 to 14 vs. wk 15 to 20, wk 9 to 11 vs. wk 12 to 14, wk 15 to 17 vs. wk 18 to 20, wk 9 vs. wk 10 and 11, wk 10 vs. wk 11, wk 12 vs. wk 13 and 14, wk 13 vs. wk 14, wk 15 vs. wk 16 and 17, wk 16 vs. wk 17, wk 18 vs. wk 19 and 20, and wk 19 vs. wk 20). If a significant wk x treatment interaction was detected the effects of treatment x wk were compared using the SLICE option of LSMEANS statement of SAS. To evaluate the relationship between concentrations of glucose and insulin, correlation coefficients were calculated for the 8 wk treatment period and the 12 wk pasture period using MANOVA PRINTE option of GLM procedures of SAS. The model for simple correlation included treatment, cow within treatment, wk and wk x

treatment. The model for partial correlations (adjusted for week) included treatment and cow within treatment.

Treatment effects on BW, ADG, ADFI, feed efficiency and reproductive criteria were determined with one way ANOVA using GLM procedures of SAS. Heifer was the experimental unit for BW, ADG, and reproductive criteria. Pen was the experimental unit for ADFI and feed efficiency.

Results

Animal Performance

Body weight of control and direct fed microbial heifers were similar ($P > 0.10$) at the beginning of treatment, at the end of treatment, and at the end of the pasture period (Table 3.2). Treatment did not influence ($P > 0.10$) ADG during the treatment period or after treatment while heifers grazed pasture (Table 3.2).

Heifers receiving both treatments consumed similar amounts of the pelleted low starch diet between wk -1 and 8 of the trial (Table 3.3). Feed efficiency (kg gain/kg feed) was not influenced ($P > 0.10$) by feeding of direct fed microbials (Table 3.3).

Reproductive Performance

There was a tendency for a greater ($P < 0.09$) number of control heifers to have ovarian luteal activity compared with direct fed microbial heifers at the initiation of the study (table 3.4). However the number of heifers that initiated ovarian luteal activity during the 8 wk treatment period ($P < 0.21$) and during the 8 wk post treatment ($P <$

0.99) was not influenced by treatment. All heifers expressed luteal activity by wk 16 of the trial (Table 3.4). Diet did not influence the interval from the start of the treatment to the onset of ovarian luteal activity in the heifers that were anovulatory at wk 0 of trial (Control, 9.0 ± 0.3; Treated, 8.3 ± 0.3 wk; Table 3.5).

Onset of ovarian luteal activity occurred at a similar age and BW for control and treated heifers (Table 3.5). Body weight and age at the onset of ovarian luteal activity was similar for control and treated heifers ($P > 0.10$) when comparing only heifers that were anovulatory at wk 0. Conception rate to AI (Control, 69 ± 5 %; Treated, 73 ± 5 %) was not influenced ($P > 0.05$) by the feeding of direct fed microbials. W hen comparing only anovulatory heifers at wk 0 the feeding of direct fed microbials did not influence ($P > 0.05$) conception rate to AI (Control, 33 %; Treated, 72 %). Pregnancy rate of control (87 %) and direct fed microbial heifers (97 %) was not influenced ($P < 0.18$) by treatment. Analysis of pregnancy rate for only anovulatory heifers at the beginning of the trial resulted in a greater ($P < 0.05$) pregnancy rate for treated (100 %) than control (85 %) heifers. Age at conception (Control, 457 ± 4; Treated, 450 ± 4 d) for all heifers in the experiment was not influenced ($P < 0.44$) by treatment (Table 3.5) and treatment did not influence ($P > 0.10$) the age at conception in heifers that were anovulatory at wk 0 (Control, 470 ± 9; treated, 453 ± 9 d).

Concentrations of glucose and insulin

Concentrations of glucose and insulin were influenced by wk ($P < 0.05$; figure 3.1 but were not influenced by treatment or treatment x wk between wk 0 and wk 8 of trial. Concentrations of glucose during treatment averaged 80.5 ± 1.4 and 79.8 ± 1.4 mg/dL for

propionibacteria and control heifers, respectively. Concentrations of glucose in plasma were lesser ($P < 0.06$) on wk 0 and 1 (78.9 ± 0.6 mg/dL) when samples were obtained after water and feed had been restricted for 10 h, compared with concentrations in plasma on wk 2 to 8 (80.4 ± 0.3 mg/dL) when heifers had access to feed and water. Heifers had greater ($P < 0.05$) concentrations of glucose in plasma between wk 2 to 5 (81.4 ± 0.4 mg/dL) compared with wk 6 to 8 (79.3 ± 0.9 mg/dL). Plasma concentrations of glucose were greater ($P < 0.05$) at wk 3 (82.9 ± 0.8 mg/dL) vs. wk 2 (81.0 ± 0.8 mg/dL). At wk 6 plasma concentrations of glucose (82.1 ± 0.8 mg/dL) tended to be greater ($P < 0.10$) when compared with wk 7 and 8 (77.9 ± 0.6 mg/dL).

There was a treatment x wk interaction on concentrations of glucose in plasma from wk 9 to 20 of trial while heifers grazed pasture (Figure 3.2). Concentrations of glucose in plasma were greater ($P < 0.05$) in control (76.4 ± 1.4 mg/dL) than treated (71.3 ± 1.4 mg/dL) heifers at wk 11. In contrast, direct fed microbial heifers had greater concentrations of glucose (77.3 ± 1.5 mg/dL; $P < 0.05$) in plasma compared with control heifers (72.8 ± 1.4 mg/dL) at wk 18. Concentrations of glucose at wk 20 tended to be greater ($P < 0.10$) in direct fed microbial heifers vs. control heifers (77.2 ± 1.5 and 74.0 ± 1.4 mg/dL, respectively).

Concentrations of insulin in plasma between wk 0 and wk 8 of trial were influenced by wk (Figure 3.3; $P < 0.05$) but concentration were not influenced by treatment or treatment x wk. Concentrations of insulin in plasma during treatment averaged 1.53 ± 0.11 ng/mL and 1.55 ± 0.11 ng/mL in treated and control heifers, respectively. Concentrations of insulin in plasma were lesser ($P < 0.05$) at wk 0 to 1 (1.15 ± 0.04 ng/mL) when samples were obtained after feed and water had been restricted

for 10 h compared with concentrations in plasma on wk 2 to 8 (1.65 ± 0.02 ng/mL) when heifers had access to feed and water. Heifers tended to have greater ($P < 0.07$) concentrations of insulin in plasma during wk 2 to 5 compared with wk 6 to 8 (1.69 ± 0.03 ng/mL and 1.60 ± 0.04 ng/mL, respectively). Plasma concentrations of insulin were greater ($P < 0.05$) at wk 5 compared with wk 4 (1.92 ± 0.06 ng/mL and 1.63 ± 0.06 ng/mL, respectively). Insulin concentrations in plasma were greater ($P < 0.05$) on wk 7 compared with wk 8 (1.60 ± 0.06 ng/mL and 1.40 ± 0.06 ng/mL, respectively).

Concentrations of insulin in plasma (Figure 3.4) were influenced ($P < 0.05$) by wk but not by treatment or treatment x wk between wk 9 and 20 of the study. Concentrations of insulin in plasma during the pasture phase averaged 1.20 ± 0.06 and 1.23 ± 0.06 ng/mL in direct fed microbial and control heifers, respectively. Concentrations of insulin in plasma were greater ($P < 0.01$) at wk 15 to 20 (1.25 ± 0.01 ng/ml) compared with wk 9 to 14 (1.17 ± 0.01 ng/mL). Heifers had greater ($P < 0.01$) concentration of insulin in plasma on wk 18 to 20 compared with wk 15 to 17 (1.28 ± 0.02 ng/mL and 1.23 ± 0.02 ng/mL, respectively). On wk 16 and 17 (1.25 ± 0.02 ng/mL) concentrations of insulin were greater ($P < 0.05$) compared with wk 15 (1.19 ± 0.03 ng/mL).

Concentrations of glucose were positively correlated ($P < 0.01$) with insulin between wk 0 and wk 8 of the study (r = 0.21 and 0.27 for simple and partial correlations, respectively). Concentrations of glucose and insulin were not correlated ($P < 0.11$) between wk 9 and wk 20 of trial while heifers grazed pasture (r = 0.07 and r = 0.06 for simple and partial correlations, respectively).

Discussion

Feeding propionibacteria strains P169, P5 and yeast did not influence concentration of insulin or glucose during the 8 wk treatment period. It was hypothesized that propionibacteria and yeast culture would increase the concentration of propionate in the rumen and propionate would be converted to glucose and increase plasma concentrations of glucose and insulin. Abomasal infusion of propionate increased plasma insulin and decreased plasma glucose concentrations of prepubertal Brangus heifers (Rutter et al., 1983). Intraruminal infusion of 42 µmol of propionate per kg of BW per min to prepubertal Angus heifers for 6 h increased plasma concentrations of insulin, but did not influence plasma concentrations of glucose (DiCostanzo et al., 1999). Feeding of 57 g of *Sacharomyces cerevisiae* (2.40×10^6 cfu/g) for 6 wk increased concentrations of propionate in the rumen of Holstein cows (Harrison et al., 1988). In contrast, the inclusion of 57 g/d of *Sacharomyces cerevisiae* in diet of lactating dairy cows for 18 d did not influence concentrations of propionate in the rumen (Yoon and Stern, 1996). Ghorbani et al. (2002) reported no effect of feeding Propionibacteria P15 (10 g/steer/d; 1×10^9 cfu/g) for 3 wk on rumen concentrations of propionate and blood variable in feedlot steers. Feeding propionibacteria strain P169 (17 g) to lactating dairy cows from 2 wk before calving until 12 wk postpartum did not influenced concentrations of glucose and insulin in plasma (Francisco et al., 2002). Feeding P169 (6×10^{11} cfu/cow) increased molar percentages of ruminal propionate in lactating dairy cows compared with controls during 25 wk of treatment (Stein et al., 2006). The increase in ruminal propionate was associated with increased concentrations of insulin in plasma during the first 12 wk of treatment (Aleman, 2005).

Increased concentrations of propionate in plasma after the addition of propionate to the rumen may increase plasma concentrations of insulin and result in an increase, or no effect, on plasma concentrations of glucose. Investigation of P169 indicated that treatment of lactating dairy cows with P169 will increase molar percentages of ruminal propionate and increased plasma concentrations of insulin and glucose. The increase in insulin maintained normal concentrations of glucose in plasma. In the present study a lack of a treatment effect on insulin and glucose concentrations in plasma could be related to inadequate concentrations of direct fed microbials in the rumen to increase ruminal propionate production. The administrations procedure and dose of the propionibacteria that the heifers received may not have been adequate to increase concentrations of propionate in the rumen. Heifers were fed the direct fed microbials on Tuesday at 0800 and blood samples were taken on Wednesday at 0800 therefore time sampling relative to the time of feeding may not have been optimal to evaluate the effects of treatment on plasma concentrations of glucose and insulin. Previous studies have evaluated the effects of P169 in a high energy total mixed ration for multiparous and primiparous lactating dairy cows. The current study differed in that it evaluated the effects of propionibacteria strains P169, P5, and yeast culture fed to yearling heifers with ad libitum access to a pelleted low starch diet. Differences in age and type of animal, type of diet, and the propionibacteria strains might have influenced the lack of response in plasma concentrations of glucose and insulin.

Plasma concentrations of insulin and glucose were less in the first two weeks, when plasma samples were attained after water and feed were restricted for 10 h, compared with wk 2 to 8 when heifers had access to feed and water before sampling.

Restriction of water intake for 16 h from steers and bulls caused increased hematocrits probably associated with decreased plasma volume (Lents et al., 1996). Plasma concentrations of glucose in slaughter weight cattle decreased after 36 h without feed and water (Schaefer et al., 1990). Although the removal of water for 10 h may have decreased plasma volume, feed restriction may have decreased concentration of glucose and insulin in plasma.

Treatment did not influence concentrations of insulin while heifers grazed pasture phase. The treatment phase may not have influenced propionate production in the rumen since concentrations of insulin and glucose were similar between the direct fed microbial group and control group. Since the treatment did not positively influence propionate concentrations in the rumen it is likely the amount of propionate production in the rumen was not adequate during the pasture period to increase concentrations of glucose and insulin during the pasture period.

There was a week x treatment effect on plasma concentrations of glucose during the pasture period, however there was no pattern of a treatment effect on concentrations of glucose in plasma, and the effects can not be explained biologically. Plasma concentrations of insulin were influenced by wk during the pasture period. Variations in nutrient consumption while heifers grazed pasture could have influenced concentrations of glucose in plasma.

Animal Performance

Feeding direct fed microbials to beef heifers did not influence ADFI and feed efficiency. Supplementation of steers with 10 g/d of propionibacteria 15 (1 x 10^9 cfu/g)

did not influence DMI when steers were fed a steam-rolled barley, barley silage, and a protein and mineral supplement (Ghorbani et al., 2002). Feeding propionibacteria *freuenrichii* (P63) did not influence feed efficiency or DMI in finishing heifers during a 126 d period (Huck et al., 2000). Feeding of 10 g of *Sacharomyces cerevisiae* (5×10^9 cfu/g) to lactating dairy cows increased DMI in the first 6 wk of lactation (Wohlt et al., 1991). In contrast feeding lactating dairy cows 10 g of *Sacharomyces cerevisiae* (10×10^9 cfu/g) for the 4 wk did not influence DMI (Piva et al., 1993). Restriction of feed intake in beef heifers (Bossis et al., 1999) and non lactating beef cows (Richards et al., 1989) decreases concentrations of insulin and glucose in plasma. Realimentation of beef heifers (Bossis et al., 2000) and non-lactating beef cows (Richards et al., 1989) after nutritional restriction increased concentrations of glucose and insulin in plasma. Feed efficiency and DMI were not influenced by the feeding of direct fed microbials which possibly resulted in similar plasma concentration of insulin and glucose, for treated and control beef heifers.

Feeding direct fed microbials did not influence ADG during 8 wk treatment period or the 12 wk after treatment when heifers grazed pasture. It was anticipated that feeding direct fed microbials would increase ADFI and plasma concentrations of glucose and insulin, causing an improvement in ADG. Prepubertal heifers that were fed to achieve a gain of 1.36 kg/d had greater concentrations of insulin and glucose than heifers fed to gain 0.23 kg/d (Yelich et al., 1996). In contrast, heifers that were projected to gain 1.36, 0.68, or 0.23 had similar concentrations of insulin during the 16 wk of treatment (Yelich et al., 1995). Heifers projected to gain 0.68 kg/d had lower glucose concentrations in plasma compared with heifers fed to gain 0.23 kg/d (Yelich et al.,

1995). Supplementation with 2.27 kg/d of a mixture of 75 % cracked corn and 25 % soybean meal during the winter grazing period increased plasma concentrations of glucose and ADG when compared with heifer supplemented with 0.91 kg/d of soybean meal (Simpson et al., 1998). Since plasma concentrations of glucose and insulin and ADFI were not influenced by feeding heifers direct fed microbials, this may be the cause of a lack of treatment effects on ADG.

Reproductive performance

There was a tendency for more control heifers to have ovarian luteal activity at wk 0 of the trial. However the number of heifers with luteal activity during the 8 wk of treatment, and the 8 wk after treatment, was not influenced by treatment. Age at ovarian luteal activity was similar for direct fed microbials fed and control heifers. Feeding P169 to lactating dairy cows for the first 12 wk after calving did not affect plasma glucose and insulin concentrations or postpartum interval to ovulation (Francisco et al., 2002). In addition, inclusion P169 in the diet of lactating dairy cows during the first 30 wk after calving did not influence the interval to first ovulation (Stein et al., 2006). Dietary manipulation to increase plasma concentrations of insulin and glucose in prepubertal heifers resulted in a decreased age at puberty (Yelich et al., 1996). Feeding of direct fed microbials to heifers in the current experiment did not influence age at the onset of ovarian luteal activity, which possibly resulted from the lack of a treatment effect on plasma concentrations of glucose and insulin.

Feeding direct fed microbials did not influence weight at ovarian luteal activity. Heifers that received 400 g of a 50 % propionic acid and 50 % water mixture sprayed on

roughage for 120 d did not differ in body weight from controls at puberty (Lalman et al., 1993). Heifers that were projected to gain 1.36 kg/d had greater BW at puberty than heifers that were projected to gain 0.68 and 0.23 kg/d (Yelich et al., 1995). The heifers that gained 1.36 kg/d also had greater plasma concentrations of insulin and glucose in the 10 wk period prior to puberty. Nine-month-old heifers projected to gain 1.36 kg/d had greater plasma concentrations of insulin and glucose at d 68 and 84 of treatment but this did not influence weight at puberty when compared with heifers that gained 0.23 kg/d (Yelich et al., 1996). In contrast, heifers that were fed to gain 1 kg/d were heavier at puberty and had greater plasma insulin concentrations than heifers fed to gain 0.6 kg/d (Hall et al., 1995). Gain and plasma concentrations of glucose and insulin were not influenced by the feeding direct fed microbials therefore heifers weights at the onset of ovarian luteal activity did not differ.

Age at conception and pregnancy rate were not influenced by the feeding of direct fed microbials. This measure is biased because of the tendency for more control heifers to initiate ovarian luteal activity prior to treatment than direct fed microbials fed heifers. It is possible that heifer initiating ovarian luteal activity prior to the breeding season could have had multiple estrous cycles therefore increasing their probability of becoming pregnant (Byerley et al., 1987). Age at pregnancy is biased because heifers could have become pregnant earlier if they were exposed to a bull or AI prior to wk 8 of the study. Conception rate to AI was not influenced by the feeding of direct fed microbials. Age at pregnancy was similar and pregnancy rate was greater in treated than control heifers when comparing only heifers that had not initiated ovarian luteal activity before wk 0 of trial. Feeding 400 g of a 50 % propionic acid, 50 % water mixture sprayed to hay for 120

d did not influence pregnancy rate or glucose concentrations in beef heifers (Lalman et al., 1993). Heifers supplemented with corn and soybean meal during winter grazing had greater ADG, concentration of glucose in plasma, and conceived at a younger age compared with heifers supplemented with SBM (Simpson et al., 1998). The feeding of direct fed microbials did not influence plasma concentrations of glucose and insulin during treatment or the pasture period, therefore age at pregnancy and pregnancy rate were similar. Feeding heifers supplemental corn increased postweaning gain and the number of heifers pubertal before the breeding season, but did not influence pregnancy rate (Bushkirk et al., 1995). Feeding a high starch diet or low starch for 60 days prior to the breeding season increased ADG and this resulted in greater pregnancy rates in heifers with inadequate yearling weights (Ciccioli et al., 2005). The inability of direct fed microbials to influence gain of heifers resulted in similar pregnancy rates between treated and control heifers.

Energy and CP content of the diet in this experiment were similar to the experimental diets (high and low starch) used by Ciccioli et al. (2005). The study of Ciccioli et al. (2005) resulted in increased pregnancy rates when heifers were fed the high or low starch diets, for 60 d prior to the breeding season. In the current study, it is possible that the control diet that was fed 8 wk prior to the breeding season was adequate to increase energy and crude protein intake for heifers and to increase plasma concentrations of glucose and insulin. The adequate nutrient intake resulted in many pubertal heifers prior to the breeding season and resulted in acceptable pregnancy rates. Feeding the low starch diet may have increased intake, gain, plasma concentration of glucose and insulin, and reproductive performance, thus additional performance did not

occur by supplementation of direct fed microbials. Further research is needed to evaluate the effects of feeding propionibacteria and yeast culture with a lowstarch pelleted diet to determine if reproductive performance will be enhanced.

Conclusion

Supplementation of direct fed microbials with ad libitum access to a pelleted low starch diet for 8 wk prior to the breeding season did not improve ADFI or feed efficiency. The inability of direct fed microbial supplementation to influence intake resulted in similar plasma concentrations of glucose and insulin and ADG. Similar BW gains for control and direct fed microbial heifers during the treatment and pasture period contributed to similar ovarian function for control and treated heifers. Furthermore, age and weight at ovarian luteal activity were similar for control and treated heifers. Supplementation of direct fed microbials did not influence age at pregnancy or pregnancy rate of beef heifers.

Implications

Nutritional programs that result in adequate growth and development of replacement heifers will hasten the onset of puberty. Previous research determined animal growth and reproductive performance were increased when ruminal concentrations of propionate and concentrations of glucose and insulin in plasma were increased. Propionibacteria and yeast cultures may increase concentrations of ruminal propionate and concentrations of glucose and insulin in plasma. In the current study, supplementation of propionibacteria and yeast culture for 8 wk prior to the breeding season did not influence concentrations of insulin and glucose in plasma, animal growth

and reproductive performance. Further research is needed to evaluate the optimal amount and method of supplementation of propionibacteria and yeast culture to increase concentrations of propionate in the rumen, glucose and insulin concentrations in plasma, improve animal growth and reproductive performance.

Table 3.1. Ingredients and composition of the experimental diet

Diet ingredient	% (as-fed basis)
Corn Dent No. 2	10.0
Soybean Meal 47.7%	6.0
Soybean hulls	66.0
Dicalcium Phosphate	0.3
Zinc Sulfate	0.001
Manganous Oxide	0.003
Wheat midds	7.0
Limestone 38%	0.4
Salt	0.3
Vitamin A-30,000[a]	0.02
Selenium 600	0.005
Cottonseed hulls	10.0
Calculated composition [b]	
CP %	13.59
$NE_{m,}$ Mcal/kg	2.00
NE_g, Mcal/kg	1.24
Ca, %	0.46
P, %	0.33

[a]30,000 IU/g, 90 % DM.
[b]Based on NRC (2000).

Table 3.2. Effect of feeding direct fed microbials on body weight and ADG of beef heifers

Item	Treatment		SE	P-value
	Control[a]	Treated[b]		
Heifers, no.	32	30		
BW, kg				
wk -1	283.8	281.1	3.5	0.57
wk 8	360.5	361.6	3.3	0.81
wk 20	399.6	400.0	3.3	0.99
ADG, kg/d				
wk -1 to 8	1.21	1.27	0.07	0.28
wk 9 to 20	0.46	0.45	0.02	0.81

[a]Heifers were fed a low starch diet from wk 0 to 8.
[b]Heifers were fed a low starch diet plus 5 g of direct fed microbial mixture of propionibacteria strains P169 and P5 and of XP yeast from wk 0 to 8.

Table 3.3. Effects of feeding direct fed microbials on feed intake and growth efficiency of beef heifers

Item	Treatment		SE	P-value
	Control[a]	Treated[b]		
Pens, no.	2	2		
Heifers per pen, no.	16	15		
ADFI, kg				
wk -1 to 8	9.32	10.09	0.33	0.24
G:F, kg/kg				
wk -1 to 8	0.13	0.12	0.01	0.34

[a]Heifers were fed a low starch diet from wk 0 to 8.
[b]Heifers were fed a low starch diet plus 5 g of direct fed microbial mixture of propionibacteria strains P169 and P5 and of XP yeast from wk 0 to 8.

Table 3.4. Effect of feeding direct fed microbials on the onset of luteal activity of beef heifers

Item	Treatment		P-value
	Control[a]	Treated[b]	
Heifers, no.	32	30	
Heifers with luteal activity[c]			
At wk 0	10	4	0.09
At wk 4	12	6	0.13
At wk 8	17	17	0.78
At wk 12	29	29	0.34
At wk 16	32	30	0.99

[a]Heifers were fed a low starch diet from wk 0 to 8.
[b]Heifers were fed a low starch diet plus 5 g of direct fed microbial mixture of propionibacteria strains P169 and P5 and of XP yeast from wk 0 to 8.
[c]Number of heifers that attained luteal activity by each wk.

Table 3.5. Effects of feeding direct fed microbials on reproductive characteristics beef heifers.

Items	Treatment		SE	P-value
	Control[a]	Treated[b]		
All heifers				
Heifers, no.	32	30		
Age at ovarian luteal activity, d	437	442	5	0.62
Weight at ovarian luteal activity, kg	336	350	6	0.12
Age at pregnancy, d[c]	457	450	4	0.44
Start of treatment to pregnancy, d[c]	67	66	2	0.93
AI Conception Rate[g]	68	73	5	0.78
Pregnancy Rate, %	87	97	5	0.18
Anovulatory heifers[d]				
Heifers, no.	22	26		
Age at ovarian luteal activity, d	458	450	5	0.26
Weight at ovarian luteal activity, kg	354	361	5	0.38
Age at pregnancy, d[e]	470	453	8	0.13
Start of treatment to luteal activity, wk	9.0	8.3	0.3	0.36
Start of treatment to pregnancy, d[e]	69	68	6	0.91
AI Conception Rate[h]	33	72	5	0.13
Pregnancy Rate, %	85	100	5	0.05

[a]Heifers were fed a low starch diet from wk 0 to wk 8.
[b]Heifers were fed a low starch diet plus 5 g of direct fed microbial mixture of propionibacteria strains P169 and P5 and of XP yeast from wk 0 to 8.
[c]Analysis included 16 control and 18 treated heifers.
[d]Includes all heifers that were anovulatory at wk 0 of treatment.
[e]Analysis included 7 control and 14 treated heifers because calving date is not available for all heifers.
[g]Analysis included 16 control and 15 treated heifers.
[h]Analysis included 6 control and 11 treated heifers.

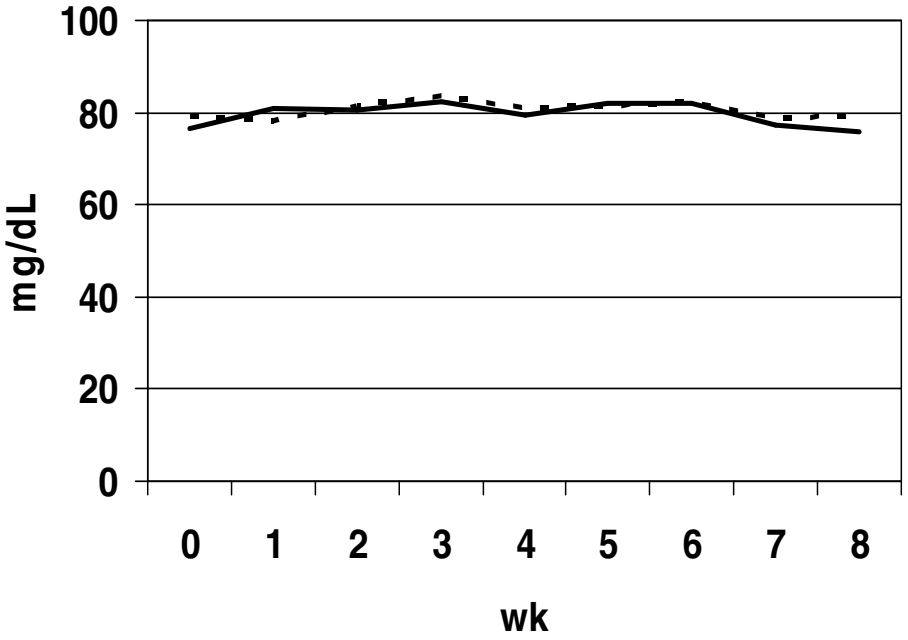

Figure 3.1. Concentrations of glucose in plasma of control (----------) and treated (——————) heifers during treatment. (trt $P > 0.05$, day $P < 0.05$)

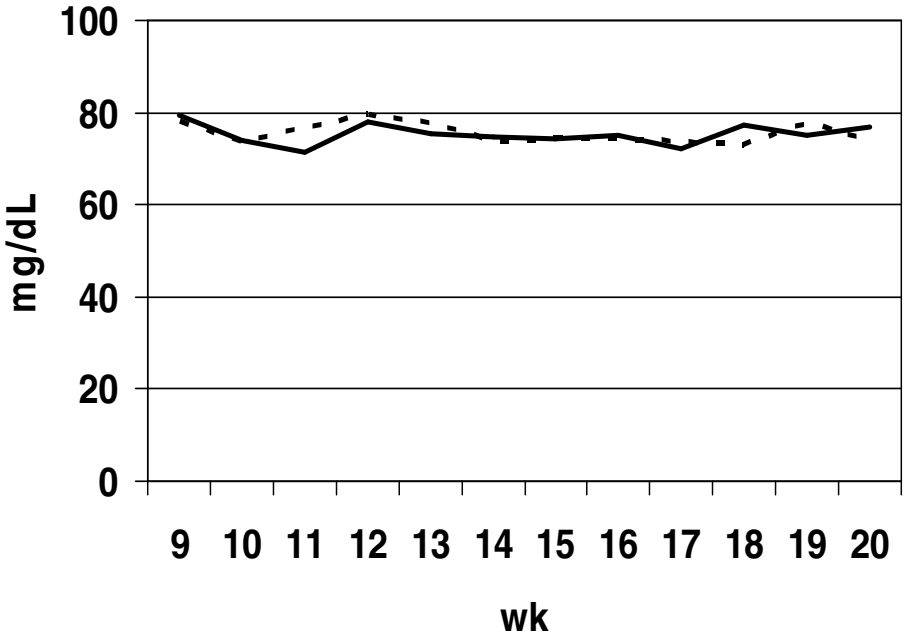

Figure 3.2. Concentrations of glucose in plasma of treated (----------) and controlled (————) heifers during pasture period. (trt $P > 0.05$, day $P < 0.05$, trt x day $P < 0.05$)

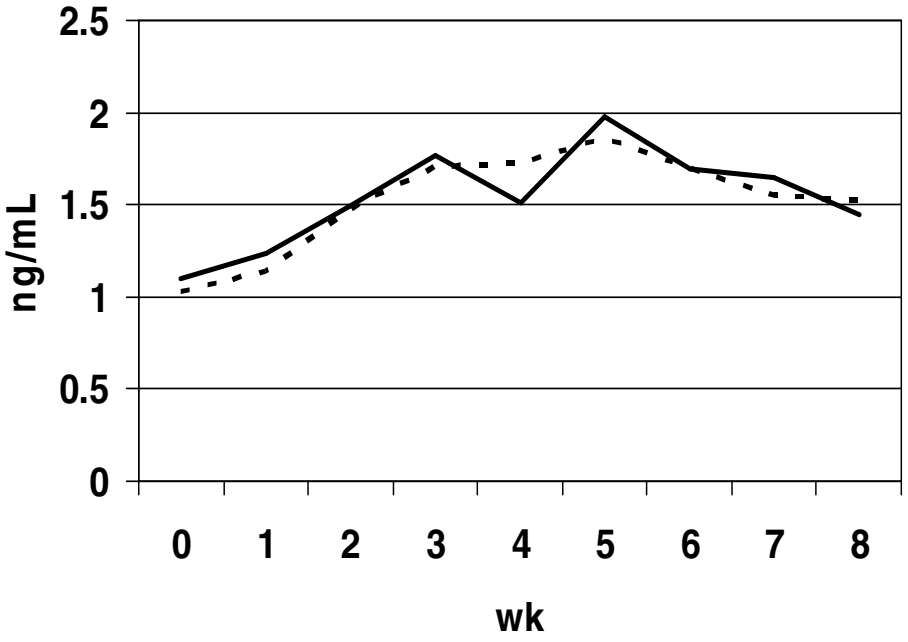

Figure 3.3. Concentrations of insulin in plasma of control (----------) and treated (‾‾‾‾‾‾‾‾) heifers during treatment. (trt $P > 0.05$, day $P < 0.05$)

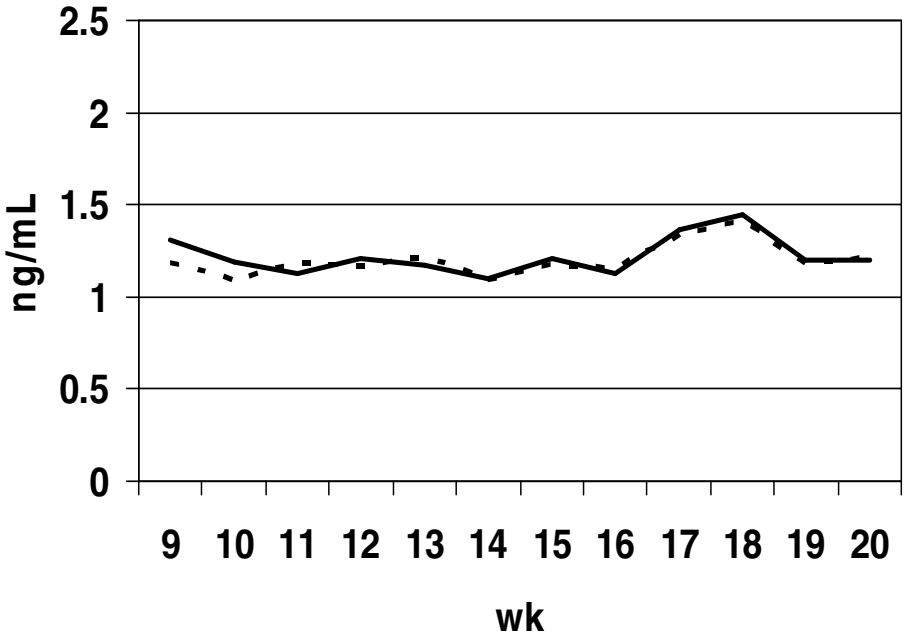

Figure 3.4. Concentrations of insulin in plasma of control (----------) and treated (——————) heifers during the pasture period. (trt $P > 0.05$, day $P < 0.05$)

CHAPTER IV

Relationship between weight at puberty and mature weight in beef cattle

Abstract

The relationship between weight at puberty and mature weight was evaluated in Angus x Hereford heifers born in the spring of 1997 (n = 9), 1998 (n = 5), and 2003 (n = 13). Weight at puberty was calculated from shrunk body weights taken before and after the onset of luteal activity as determined from concentrations of progesterone in plasma. Mature body weight was a shrunk body weight, adjusted to BCS 5. Mature weights were taken at 5 to 7 yr of age for 1997 and 1998 born heifers and 2.5 yr of age for 2003 born heifers. Weights at puberty were similar ($P > 0.05$) for heifers born in the spring of 1997, 1998 and 2003 (320 ± 11, 312 ± 15, and 336 ± 9 kg respectively). The 1997 and 1998 heifers had greater ($P > 0.05$) BW at 5 to 7 yr of age compared with BW of 2003 heifers at 2.5 yr of age (591 ± 16, 550 ± 22, 454 ± 14 kg respectively). Heifers born in 1997 and 1998 had similar percentage of mature weight at the onset of puberty (54 ± 2 and 58 ± 2 % respectively). Heifers born in 2003 weighed 74 ± 1 % of their 2.5 yr old weight at puberty. Additional observations are necessary to determine if heifers initiate ovarian luteal activity at approximately 56 % of their mature weight.

Introduction

Age at which a heifer calves for the first time is an important factor that determines the profitability of a beef herd. Heifers that reach puberty prior to the breeding season have a greater ability to calve at two years of age and wean more pounds of beef in their reproductive lifetime (Lessmeister et al., 1973). When puberty is not attained before the start of the breeding season, fertility (Byerley et al., 1987) and potential income of cow-calf producer is reduced (Werth et al., 1991). Manipulation of the diet, rate of gain, and plasma metabolites and hormones can hasten the onset of puberty (Yelich et al., 1996). Management decisions should be made to hasten the onset of puberty in heifers, increase the number of pubertal heifers prior to the breeding season, and increase the number of heifers that calve at two years of age to improve profitability.

The relationship between body weight at puberty and mature weight in beef cattle is not established. Maas (1987) presented a comparison and suggested that the percentage of mature weight at which a heifer reaches puberty is 66%, but data were not provided to support this conclusion. An accurate determination of the percentage of mature weight that a beef heifer needs to achieve for puberty to occur will allow producers to manage heifers to increase the number pubertal prior to breeding season.

There is a genetic and environmental component that influences mature weight in beef cattle. Mature weight is heritable and can be altered by selection (Kaps et al., 1999). Selection for weaning weight and yearling weights will indirectly increase mature weight. Rate of maturing in Polled Hereford cows is moderately heritable and other factors such as environment may influence mature weight (Meyer, 1995). An environment that is conductive to early structural growth would lend itself to increased mature weight when

the animals are fully developed (Bullock et al., 1993). The environment, including type of nutrient consumption, may influence mature weight of beef cattle. Brahman heifers that grazed Bermuda grass had greater mature weights than Brahman cattle that grazed fescue (Sandelin el al., 2002).

Development of replacement heifers is critical for profitable beef production. An understanding of the relationship of the weight at puberty with mature weight will allow development of management strategies to enhance performance. Beef producers must consider resources and environmental conditions when developing feeding programs for replacement heifers to increase the probability that heifers will calve at two years of age. The objective of this study was to determine the relationship between weight at puberty and mature weight in Angus x Hereford cattle.

Materials and Methods

Weights at puberty and mature weights were obtained for Angus x Hereford heifers born in the spring of 1997 (n = 9), 1998 (n = 5) and 2003 (n = 13). The heifers were maintained in the research herd and used for a variety of projects to study reproductive physiology at the Oklahoma Agricultural Experiment Station. Weights obtained at 5 to 7 yr of age were taken after cows were withdrawn from feed and water for 16 h and BW adjusted to a BCS of 5 (Tennant et al., 2002). Mature weights for 2003 spring born heifers were obtained at 2.5 years of age after withdrawal from feed and water for 16 h , and adjusted to a BCS of 5 (Tennant et al., 2002).

Weight at puberty was expressed as shrunk body weights (restriction of feed and water for 16 h) and determined by extrapolation of shrunk body weights taken monthly

for 1997 and 1998 heifers and at 8 to 12 wk intervals before and after the onset of puberty for the 2003 heifers.

Blood was collected weekly from heifers into tubes containing EDTA, placed on ice, and centrifuged at 1,800 x *g* within 4 h after collection. Plasma was aspirated and stored at – 20º C until progesterone was quantified (Vizcara et al., 1997) with a solid phase RIA (Coat-A-Count progesterone kit, Diagnostic Products Corp., Los Angeles, CA) to determine the onset of ovarian luteal activity. Concentration of progesterone greater than 0.5 ng/mL for two consecutive samples was the criterion to determine the onset of puberty. Date of the first of the two consecutive samples with progesterone greater than 0.5 ng/ml was considered age at the onset of puberty.

Percentage of mature weight at puberty was determined for each animal. Differences in weight at puberty, mature weight and percentage of mature weight at puberty for the years of birth were analyzed as one way ANOVA using GLM procedures of SAS. When year was significant, orthogonal contrasts (1997 and 1998 vs. 2003, 1997 vs. 1998) were used to compare years. Regression analysis was used to evaluate the relationship between weights at puberty and mature weights.

Results

Weights at puberty and maturity are in Table 4.1. Spring born heifers in yr 1997, 1998 and 2003 attained puberty at similar (P>0.05) weights (320 ± 11, 312 ± 15, and 336 ± 9 kg respectively). Heifers born in 1997 and 1998 had similar ($P > 0.05$) mature weights (591 ± 16 and 550 ± 22, kg respectively). The 2.5 yr old weight of 2003 born heifers (454 ± 14 kg) was less compared with mature BW of heifers born in 1997 and 1998. The percentage of mature weights at puberty is presented in Table 4.1. Spring

born heifers in 1997 attained puberty at 54 ± 2 % of their mature weight. Puberty was attained at 58 ± 2 % of mature weights for heifers born in 1998. Heifers born in 2003 reached puberty at average 74 ± 1 % of their 2.5 yr old weight.

The average percentage of mature weights that heifers reached puberty did not differ between heifers born in 1997 and 1998. Regression analysis of mature weight and weight at puberty for spring born heifers in 1997 and 1998 revealed that mature weight accounted for 57 % of the variation in weight at puberty ($P < 0.01$). However heifers born in yr 2003 attained puberty at a greater ($P < 0.05$) percentage of the 2.5 yr old than that of mature weights for heifers born in 1997 and 1998.

Discussion

Prepubertal growth and development of the heifers in this experiment was similar to normal management used in the beef industry of the Great Plains. Heifers born in 1997 and 1998 attained puberty at a similar percentage of mature weight (56 %) which was less than the estimate given by Mass (66%; 1987). Heifers born in 2003 were pubertal at a greater percentage of their 2.5 yr old weight than that of mature weight for heifers born in 1997 and 1998. At 2.5 yr of age heifers born in 2003 had not attained their mature weight. With continue growth, heifers born in 2003 may have a similar percentage of mature weight at the attainment of puberty as the heifers born in 1997 and 1998. Comparisons should be made between similar aged cows because age at which you obtain the mature weights can influence the percentage of mature weight at which the heifer reaches puberty.

Conclusion

In this study Angus x Hereford heifers reached puberty at approximately 56% of their mature weight (5 to 7 yr of age) and at a BCS of 5. Breed of heifers may influence the percentage of mature BW at puberty. Additional observations are necessary to determine if different breeds and all crossbred heifers initiate ovarian luteal activity at approximately 56 % of their mature weight.

Implications

The determination of a specific percentage of mature weight at which a heifer attains puberty can be a management tool to increase the probability that heifers will become pregnant and calve by 2 yr of age. In the current study, the percentage of mature weight the heifer attained puberty was consistent, when the value was calculated using the age of 5 to 7 yr for maturity and BCS 5. This is an easily measurable indicator that producers can use to increase reproductive performance, productive life,and profitability of their herd.

Table 4.1. Weight at puberty, mature weight, and percentage of mature weight at the attainment of puberty in beef cattle

Items	Birth Year			SE	P-value
	1997	1998	2003		
Cows, no.	9	5	14		
Weight at Puberty, kg	320	313	336	12	0.34
Mature Weight, kg[a]	591 [b]	550 [b]	454 [c]	17	0.01
Percentage of Mature weight at puberty, kg/kg	54 [b]	58 [b]	74 [c]	5	0.01

[a]All weights were adjusted to BCS 5, and weights for 1997 and 1998 born heifers were calculated from 5 to 7 yr old weights and 2003 born heifers were calculated using 2.5 yr old weights.

[b,c] Means within rows differ ($P < 0.05$)

CHAPTER V

SUMMARY AND CONCLUSION

Increasing the number of heifers that calve at two years of age will increase the lifetime productivity of beef cows. Increased pregnancy rates of heifers and heifers calving earlier in the breeding season will increase the profit margin of cow-calf producers. Ovulation of the dominate follicle and the formation of a CL, combined with expression of estrous behavior, is defined as puberty. Pulsatile release of GnRH causes pulsatile release of LH and maturation of the dominant follicle, ovulation and formation of CL. Decreased sensitivity to estradiol negative feedback is associated with pulsatile release of LH. Increased concentrations of insulin, glucose and IGF-I are associated with hastening the onset of puberty. These hormones may act on the hypothalamus, pituitary, and ovary to stimulate the onset of puberty

Direct fed microbial can influence the mechanism that causes the onset of puberty. Feeding propionibacteria, yeast, and fungal cultures increased concentrations of propionate in the rumen of lactating dairy cows. Feeding yeast cultures and propionibacteria increased DMI and nutrient digestibility, which can increase concentrations of glucose and insulin in plasma. Propionibacteria 169 increased ruminal propionate concentrations, and concentrations of insulin and glucose in plasma. An increase in ruminal propionate concentrations, either by propionate infusion or feeding monensin, decreased the age at the onset of puberty in beef heifers. Therefore, feeding

P169 has the potential to hasten onset of puberty by increasing concentrations of ruminal propionate and concentrations of insulin and glucose in plasma.

A major challenge in the beef industry is the management of replacement heifers so that they are pubertal prior to the breeding season. It has been suggested that heifer reach puberty at 66 % of mature weight (Maas, 1987). However the relationship between pubertal weight and mature weight has not been established. Since mature weight is dependent on genetics and environment, a producer can predict the mature weight of their herd. Determination of the percentage of mature weight at which heifers attain puberty, will allow producers to design strategies that will increase the number of heifers that have initiated normal estrous cycles prior to breeding season and increase the probability that heifers will calve at two years of age. Two experiments were conducted to evaluate effects of propionibacteria and yeast supplementation on pubertal development and to determine the percentage of mature weight at puberty. In experiment one, the effects of feeding propionibacteria strains P169, P5 and XP yeast, for 8 wks prior to breeding, season on concentrations of insulin and glucose in plasma, growth rate and reproductive performance was evaluated in beef heifers. The comparison between weight at the onset of puberty in beef cattle and mature weight was evaluated in the second experiment.

In experiment one, insulin and glucose concentrations were monitored for the 8 wk treatment period and for 12 wk after treatment while heifers grazed pasture. Feed intake, feed efficiency and ADG were monitored during the treatment period and ADG was monitored while heifers grazed pasture. Concentrations of progesterone were used to determine age and weight at the onset of ovarian luteal activity. Conception rate to AI

was determined by ultrasound approximately 30 d after insemination. Pregnancy rate was determined by rectal palpation. Age and date at pregnancy was determined by subsequent calving date.

Feeding direct fed microbials did not influence concentrations of insulin and glucose in plasma during the treatment or pasture periods. Feed intake and feed efficiency during treatment were not influenced by feeding direct fed microbials. Direct fed microbial fed heifers had similar ADG when compared with control heifers during treatment and while heifers grazed pastures. Feeding direct fed microbials did not influence age or weight at the onset of ovarian luteal activity. Feed direct fed microbials did not influence conception rate to AI of beef heifers. Furthermore, direct fed microbials fed heifers had similar pregnancy rates and age at pregnancy when compared with control heifers.

Feeding direct fed microbials did not influence DMI. A lack of effect of treatment on concentrations of glucose and insulin in plasma may have resulted from inadequate concentrations of direct fed microbials to increase concentrations of propionate in the rumen. Feeding direct fed microbials did not influence ADG during treatment and while heifers grazed pasture which could be related to similar concentrations of insulin and glucose in plasma of treated and control heifers. Reproductive performance was not influenced by feeding direct fed microbials, which could be the result of similar concentrations of glucose and insulin in plasma for control and treated heifers.

Management strategies should result in heifers calving at two years of age. The determination of a specific percentage of mature weight at which heifers attain puberty will allow producers to develop management programs to increase reproductive performance of the beef heifers. In experiment 2, mature weight and weight at the onset of puberty were evaluated. Data were collected from spring born heifers in 1997, 1998, and 2003. For heifers born in 1997 and 1998 puberty occurred at approximately 56 % of the mature weight when mature weight of mid gestating cows was adjusted to a BCS 5. For heifers born in 2003, puberty occurred at 74 % of the body weight at 2.5 yr of age (mid gestation, and adjusted for a BCS 5).

In conclusion, feeding propionibacteria and yeast culture to beef heifers for 8 wk prior to the breeding season did not influence concentrations of glucose and insulin in plasma, animal growth and reproductive performance. Further investigation of the amount of propionibacteria and yeast used to treat heifers is needed to ensure that an adequate amount of propionibacteria and yeast is added to the rumen to increase ruminal concentrations of propionate, concentrations of glucose and insulin in plasma, and to improve animal growth and reproductive performance.

Heifer reached puberty at 56 % of their mature weight adjusted to BCS 5. Further investigation is needed to determine the effects of frame size and on the relationship between weight at puberty and mature weight.

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VITA

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Candidate for the Degree of

Master of Science

Thesis: EFFECTS OF DIRECT FED MICROBIALS ON CONCENTRATIONS OF GLUCOSE AND INSULIN IN PLASMA AND ANIMAL PERFORMANCE

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Title of Study: EFFECTS OF DIRECT FED MICROBIALS ON CONCENTRATIONS OF GLUCOSE AND INSULIN IN PLASMA AND ANIMAL PERFORMANCE

Pages in Study: 67 Candidate for the Degree of Master of Science

Major Field: Animal Science

Scope and Method of Study:

Sixty-two Angus x Hereford heifers were used to determine the effects of feeding direct fed microbials for 8 wk prior to the breeding season on concentrations of insulin and glucose in plasma, animal performance and reproductive performance. Animal were grouped fed in four drylot pens with two pens per treatment. Treatments were a pelleted low starch diet (control) and the control diet plus 5 g of a mixture of propionibacteria strains P169, P5 and XP-yeast (Diamond V). Treatments were fed daily for 2 wk then fed four days a week for the next 6 wk. Concentrations of glucose, insulin and progesterone in plasma were quantified in blood sample taken weekly by tail venipuncture. Shrunk body weights were taken at 1 wk prior to treatment and at wk 8 and 20 of trial. Feed intake and feed efficiency were measured on a pen basis and animal and reproductive performance were measured on an animal basis.

Heifers born in spring of year 1997, 1998 and 2003 were used to evaluate the percentage of mature body weight at puberty. Weights at puberty were an extrapolation of shrunk body weights taken 4 to 6 wk before or after puberty. Mature weights were shrunk body weights taken at 5 to 7 yr of age for the heifers in 1997 and 1998 and 2.5 year of age for heifers in 2003. Mature weights were adjusted to BCS 5.

Findings and Conclusions:

Feeding propionibacteria P169, P5, and XP-yeast for 8 wk prior to the breeding season did not influence concentrations of insulin and glucose in plasma and animal and reproductive performance in beef heifers. Further research is needed to determine the proper combination and administration procedure for propionbacteria and yeast that will increase plasma concentration of insulin and glucose and positively influence animal performance.

Heifers will attain puberty at approximately 56 % of mature body weight (5 to 7 yr of age). Puberty occurred at 74 % of their 2.5 yr weight. Additional observations are necessary to determine if heifers initiate ovarian luteal activity at 56 % of mature BW.

ADVISER'S APPROVAL: Dr. Robert Wettemann