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Serum progesterone concentration and conception rate of beef cows supplemented with ground corn after a fixed-time artificial insemination protocol

[*Concentração sérica de progesterona e taxa de concepção em vacas de corte suplementadas com milho moído após inseminação artificial em tempo fixo*]

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ABSTRACT

The effects of different levels of finely ground corn (FC) supplementation to grazing beef cows after fixed-time AI (TAI) on serum progesterone (P4) concentrations on day 7 and conception rates on day 28 after TAI were investigated. Three hundred and sixty-four lactating multiparous Brangus cows had follicular and luteal activity synchronized by treatment with estradiol benzoate (Estrogin; 2.0mg IM) and insertion of intra-vaginal P4 releasing device (CIDR) on day -11, followed by treatment with PGF2α (Lutalyse; 25mg IM) on day -4, CIDR and calf removal on day -2, and treatment with GnRH (Fertagyl; 100μg IM) TAI and calf return on day 0. On day 0, cows were randomly allotted in one of the following FC supplement treatments: G1 - 2kg/day from day 0 to 21; G2 - 2kg/day from day 0 to 7, and 6kg/day from day 8 to 21; G3 - 6kg/day from day 0 to 7, and 2kg/day from day 8 to 21; and G4 - 6kg/day from day 0 to 21. Blood samples were collected on day 7, and pregnancy was determined by ultrasonography indicating the presence of a fetus on day 28. Cows supplemented with 2kg/d of FC had higher serum concentration of P4 on day 7 than cows supplemented with 6kg/d (1.58 vs. 1.28ng/mL; $P<0.01$; SEM = 0.08). Cows from G4 had higher conception rates compared to G1 cows (58.4 vs. 41.9%, respectively; $P<0.05$). The level of supplemental energy intake after TAI is negatively associated with following serum P4 concentrations, but positively associated with conception rates of grazing beef cows.

Keywords: beef cow, conception rate, energy intake, progesterone

RESUMO

Avaliaram-se os efeitos de diferentes níveis de ingestão de suplemento com milho moído finamente (MF) em vacas de corte, mantidas em pasto, após inseminação artificial em tempo fixo (IATF), sobre a concentração sérica de progesterona (P4) no dia 7, e sobre a concepção no dia 28 pós IATF. Trezentas e sessenta e quatro vacas Brangus, multíparas lactantes, tiveram as atividades folicular e luteal sincronizadas por tratamento com benzoato de estradiol (Estrogin; 2,0mg IM) e inserção de dispositivo intravaginal de P4 (CIDR) no dia -11, seguido por tratamento com PGF2α (Lutalyse; 25mg IM) no dia -4, retirada do CIDR e remoção temporária de bezerros no dia -2, e tratamento com GnRH (Fertagyl; 100μg IM), IATF e retorno dos bezerros no dia 0. No dia 0, as vacas foram aleatoriamente distribuídas para receber um dos quatro tratamentos: G1 - 2kg/dia de MF do dia 0 ao dia 21; G2 - 2kg/dia de MF do dia 0 ao dia 7, e 6kg/dia de MF do dia 8 ao dia 21; G3 - 6kg/dia de MF do dia 0 ao dia 7, e 2kg/dia de MF do dia 8 ao dia 21; G4 - 6kg/dia de MF do dia 0 ao dia 21. Amostras de sangue foram colhidas no dia 7, e o diagnóstico de gestação foi realizado por ultrassonografia no dia 28. As vacas suplementadas com 2kg/dia de MF apresentaram maior concentração sérica de P4 no dia 7 em relação às vacas

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suplementadas com 6kg/dia (1,58 vs. 1,28ng/mL; P<0,01, EPM=0,08). As vacas do G4 apresentaram maior taxa de concepção em relação às vacas do G1 (58,4 vs. 41,9%, respectivamente; P<0,05). O nível de consumo do suplemento energético após a IATF é negativamente associado às concentrações séricas de P4, porém positivamente associado à taxa de concepção em vacas de corte em pasto.

Palavras-chave: vaca de corte, taxa de concepção, ingestão de energia, progesterona

INTRODUCTION

The primary nutrient consideration for optimum reproductive performance of cattle is energy (Mass, 1987). Beef cows consuming inadequate amounts of energy in postpartum typically experience extended anestrous and decreased conception and pregnancy rates (Santos and Amstalden, 1998). Tropical forages usually do not meet the energy demand of lactating beef cows (Moore et al., 1991); therefore, the development of nutrition programs that emphasize energy supplementation to maintain or enhance reproductive efficiency is often required, if not essential, for grazing cow-calf operations based on tropical forages. Increased feed intake reduces circulating progesterone (P4) concentrations (Sangsritavong et al., 2002; Vasconcelos et al., 2003; Cooke et al., 2007) in dairy and beef females.

Circulating P4 is critical for the onset and maintenance of pregnancy, especially during the early phases (Spencer and Bazer, 2002) because P4 prepares the uterine environment for gestation, inhibits the activity of hormones that may disrupt pregnancy (Vallet and Lamming, 1991; Lau et al., 1992), and is important for conceptus growth and development (Garrett et al., 1988). Pregnancy rates are positively influenced by blood concentration of P4 after breeding (Robinson et al., 1989; Lopez-Gatius et al., 2004), and recent findings further suggest that P4 concentrations during the initial seven days following AI greatly influence conception rates in cattle (Stronge et al., 2005; Demetrio et al., 2007). Therefore, it can be speculated that high amounts of supplemental energy after breeding decrease blood P4 concentrations and are consequently detrimental to the establishment of pregnancy in beef cows.

The objective of the present experiment was to investigate if different levels of finely ground corn supplementation after fixed-time AI (TAI) would affect serum P4 concentrations and conception rates of lactating mature beef cows maintained on tropical pastures.

MATERIAL AND METHODS

The experiment was carried out at the NP Brangus Farm, located in Bauru, SP.

Three hundred and sixty four lactating multiparous Brangus cows, averaging (mean±SD) 134±30d postpartum and 5.6±0.9 of body condition score (BCS; 1= emaciated to 9= obese; Wagner et al., 1988), assessed on d -11 of the study (d 0 = TAI), had follicular and luteal functions synchronized by treatment with estradiol benzoate (2.0mg IM; Estrogin - Farmavet) and insertion of intra-vaginal releasing device with 1.9g of P4 (CIDR - Pfizer Saúde Animal), followed by treatment with PGF2α (25mg IM; Lutalyse - Pfizer Saúde Animal) on d -4, CIDR and calf removal on d -2, and treatment with GnRH (100µg IM; Fertagyl - Intervet) and calf return on d 0. All cows were submitted to TAI following the GnRH treatment. From d -2 and GnRH + TAI on d 0, estrus was detected by visual observation (from 7:00 to 8:30 a.m. and from 5:00 to 6:30 p.m.) and determined if cows accepted mount from other females. All cows were inseminated by the same technician, with semen from four different bulls, equally distributed among treatments to minimize semen source effect. Following TAI, cows were randomly assigned to one of the four FC supplement treatments: G1 - 2kg/d from d 0 to 21; G2 - 2kg/d from d 0 to 7, and 6kg/d from d 8 to 21; G3 - 6kg/d from d 0 to 7, and 2kg/d from d 8 to 21; and G4 - 6kg/d from d 0 to 21. The BCS (mean±SD) and percent of cows detected in estrus were 3.2±0.4 and 50%, 3.2±0.4 and 46.8%, 3.4±0.4 and 48.2%, and 3.3±0.4 and 41.6%; for G1, G2, G3, and G4, respectively.

Cows from all treatments were maintained in a single group within a rotational grazing system throughout the study. Pasture (*Brachiaria decumbens*) availability (1,300kg of DM/ha) was adequate to assure ad libitum intake. Cows also

had free access to mineral. Treatments consisted of finely ground corn (DM: 88%; CP: 9.1%; NDF: 14%) daily offered at 06 a.m., at different amounts according to the treatment group. Cows were daily separated according to treatment immediately before feeding, and were grouped in the same pasture after feeding. Cows within the same treatment were fed in a single group; however, to prevent competition for feed and ensure that all cows had similar opportunity to consume the treatment, bunk space was approximately 1.0m/cow. Prior to the assignment to treatments, all cows received 2kg/d of FC for an 11d adaptation period (d -11 to d 0).

On day 7, from 3 to 5h after supplement treatments had been offered, blood samples were collected from coccygeal vein into commercial blood collection tubes (Vacutainer, 10mL – Becton Dickinson), immediately placed on ice, maintained at 4°C for 24h, and centrifuged at 1500 × g for 15 min for serum separation and collection. Serum was frozen at -20°C. Concentrations of serum P4 were determined using Coat-A-Count Kit (DPC - Diagnostic Products Inc.) solid phase ^{125}I radioimmunoassay. The intra-assay coefficient of variation was 4.8%.

Ovulation was determined by transrectal ultrasonography (Aloka SSD 500V, 7.5 MHz linear-array intrarectal transducer). Ovulation was confirmed if follicle ≥9mm presented on day 0 was absent on day 2. Conception rate was determined by the presence of a fetus also using transrectal ultrasonography on day 28.

Progesterone data were analyzed using the PROC MIXED of SAS. The model statement contained the effects of treatment, estrus (detected or not), BCS (≥5 or <5), and the two-way interactions. Main effects and interactions with P-value greater than 0.20 were excluded from the model. Data were analyzed using cow (treatment) as the random variable and Satterthwaite approximation to determine the denominator df for the tests of fixed effects. Results are reported as least square means. Means were separated using LSM.

Conception rates were analyzed as binomial data with the LOGIT function of the PROC GENMOD of SAS. The model statement contained the effects of treatment, P4 concentration on day 7 after TAI, estrus (detected or not), BCS (≥5 or <5), and the two-way interactions. Interactions and main effects with P-value greater than 0.20 were excluded from the model. Results are reported as means, which were separated using DIFF.

For all analyses, cow was considered the experimental unit. Significance was set at $P \leq 0.05$, and tendencies were determined if $P > 0.05$ and ≤ 0.10. Interactions are reported only if significant.

RESULTS

Only data from animals that responded to the synchronization protocol were analyzed (ovulation rate = 89.8%; 327/364). Additionally, 46.8% of the synchronized cows were detected in estrus from day -2 to day 0 of the study (153/327). For P4 analysis, treatments G1 and G2 were combined, likewise for G3 and G4, because these groups were receiving the same amount of supplement on day 7 after TAI (2 and 6kg/d of FC, respectively). For conception rates, treatments were individually analyzed and compared to each other.

Cows fed 6kg/d of FC (G3 and G4; n= 162) had reduced P4 concentration ($P<0.01$) compared to cows fed 2kg/d (G1 and G2; n= 165) (1.28 vs. 1.58ng/mL, respectively; SEM = 0.08). Regardless of treatment, cows detected in estrus (n = 153) had higher ($P<0.05$) serum P4 concentrations compared to cows (n = 174) not detected in estrus (1.54 vs. 1.31ng/mL, respectively; SEM = 0.08), and cows (n = 201) with BCS≥5.0 had higher (P = 0.05) serum P4 concentration compared to cows (n = 126) with BCS<5.0 (1.55 vs. 1.30ng/mL, respectively; SEM = 0.09).

Conception rate in G1 (41.9%; 36/86) was lower ($P<0.05$) than G4 (58.4%; 45/77). No further differences were observed among treatments (55.7%; 44/79 and 52.9%; 45/85 conception rates for G2 and G3, respectively). Cows detected in estrus had higher ($P<0.05$) conception rates (56.2%; 86/153) compared to cows not detected in estrus (48.3%; 84/174).

DISCUSSION

The hypothesis of the present study was that increased energy supplement intake after TAI (d

0) would be negatively associated with serum P4 concentrations on day 7 and consequently with conception rates detected on day 28 in lactating grazing beef cows. According to the NRC model (Nutrient…, 1996), the cows utilized in this experiment, grazing typical amounts of forage dry matter and supplemented with 2kg/d of corn, consumed approximately 90% of their daily requirements in net energy (11.6 Mcal/day consumed and 12.9 Mcal/d), whereas cows supplemented with 6kg/d of corn consumed approximately 135% of their daily net energy requirements (17.4 Mcal/d consumed). As result, cows fed 2kg/d of FC had higher P4 concentrations than cows fed 6kg/d of FC on day 7 after TAI. Previous data support the findings of the present study. Non-lactating Holstein cows fed 150% of their maintenance nutrient requirements had decreased P4 concentrations 4h after feeding compared to cows fed 50% of requirements or cows remained unfed, and this difference was likely caused by an increased hepatic clearance of P4 in response to greater supplement intake of cows fed 6kg/d of corn (Sangsritavong et al., 2002). Grazing pubertal heifers offered energy-based supplements three times weekly had decreased P4 concentrations on days that supplements were offered, and forage-fed cows similarly supplemented had decreased P4 concentrations 4h after supplements had been offered (Cooke et al., 2007). Dairy cows fed 100% of the daily diet at once, or half of the diet every 12h had decreased P4 concentrations by 1h after feeding, and concentrations remained depressed until 8 to 9h after feeding compared to cows fed a quarter of their diet every 6h or unfed animals (Vasconcelos et al., 2003).

Circulating concentrations of P4 after breeding have been positively associated with conception rates of cattle because P4 is critical for the maintenance of pregnancy (Spencer and Bazer, 2002). Cows with greater blood P4 concentrations or supplemented with exogenous sources of P4 after AI had increased conception rates compared to non-supplemented or cows with reduced blood P4 concentrations (Robinson et al., 1989; Lopez-Gatius et al., 2004). Stronge et al. (2005) indicated that reduced P4 concentrations from day 5 to day 7 after AI were associated with low fertility in dairy cows, whereas Demetrio et al. (2007) reported a positive relationship between P4 concentrations on day 7 and conception rates on day 28 after AI in dairy cattle. In the present study, however, conception rates were not affected by P4 concentrations on day 7 after TAI, and G4 cows had decreased P4 concentrations, but greater conception rates compared to G1 cows (1.28 vs. 1.60ng/mL of P4, respectively; $P<0.05$; SEM = 0.10). Energy intake has been positively associated with blood concentrations of insulin-like growth factor I (IGF-I) in beef cattle (Lapierre et al., 2000; Hersom et al., 2004). Previous studies reported that IGF-I seems to enhance the rate of embryo development to the blastocyst stage and increase embryonic cell numbers (Moreira et al., 2002a, b) in cattle, which allows greater secretion of interferon tau, positively contributing to pregnancy establishment and maintenance after AI (Bilby et al., 2006). Therefore, it can be speculated that cows from G4 had greater IGF-I concentrations compared to G1-cows during the experimental period, leading to the treatment effects detected for conception rates.

Regardless of treatment, cows detected in estrus had higher P4 concentrations on day 7 and higher conception rates on day 28 after TAI compared to cows not detected in estrus. Cows inseminated after estrus detection had higher conception rates compared to cows submitted to TAI after estrus synchronization by hormone-based protocols (Stevenson et al., 1996; Pursley et al., 1997; Stevenson et al., 1999). Bovine follicles are naturally able to ovulate when they reach approximately 10mm diameter (Sartori et al., 2001), but GnRH treatment as part of synchronization protocols may induce ovulation of smaller follicles (Perry et al., 2005). These authors indicated that ovulation of follicles lesser than 11mm diameter by exogenous GnRH stimulation is detrimental to conception rates in cattle because of decreased estradiol concentrations on the day of AI, and impaired P4 concentrations after AI. Bello et al. (2006) also indicated that circulating concentrations of estradiol at the final GnRH of Ovsynch were positively associated with pregnancy rates. Therefore, cattle detected in estrus in this experiment likely had increased estradiol concentrations at TAI and had larger ovulatory follicle compared to cattle not detected in estrus, resulting in larger corpus lutea and consequent P4 concentrations on day 7, and improved conception rates on day 28 after TAI.

It was also observed that BCS was positively associated with P4 concentrations on day 7 after TAI. Stevenson et al. (1999) reported that for each unit of BCS increase in dairy cows, P4 concentrations increased by approximately 0.5ng/mL. Beal et al. (1978) indicated that P4 concentrations in energy-restricted cattle are decreased compared to P4 concentrations in cattle consuming adequate amounts of energy. Progesterone synthesis by ovarian tissues is enhanced by blood hormones associated with energy intake, such as insulin and IGF-I (Spicer et al., 1993); therefore, it can be speculated that the improved energy status of cows with BCS≥5.0 may have enhanced their P4 synthesis compared to cows with BCS<5.0.

In conclusion, it was observed that level of energy supplementation after TAI was negatively associated with following P4 concentrations, but positively associated with conception rates of beef cows. Cows that received 6kg/d of FC from day 0 to day 21 after TAI had reduced P4 concentrations on day 7, but showed higher conception rates compared to cows offered 2kg/d of FC during the same period.

REFERENCES

BEAL, W.E.; SHORT, R.E.; STAIGMILLER, R.B. et al. Influence of dietary energy intake on bovine pituitary and luteal function. *J. Anim. Sci.*, v.46, p.181-188, 1978.

BELLO, N.M.; STEIBEL, J.P.; PURSLEY, J.R. Optimizing ovulation to first GnRH improved outcomes to each hormonal injection of Ovsynch in lactating dairy cows. *J. Dairy Sci.*, v.89, p.3416-3424, 2006.

BILBY, T.R.; SOZZI, A., LOPEZ, M.M. Pregnancy, bovine somatotropin, and dietary n-3 fatty acids in lactating dairy cows: I. ovarian, conceptus, and growth hormone–insulin-like growth factor system responses. *J. Dairy Sci.*, v.89, p.3360-3374, 2006.

COOKE, R.F.; ARTHINGTON, J.D.; STAPLES, C.R. et al. Effects of supplement type on performance, reproductive, and physiological responses of Brahman-crossbred females. *J. Anim Sci.*, v.85, p.2564-2574, 2007.

DEMETRIO, D.G.B.; SANTOS, R.; VASCONCELOS, J.L.M. Factors affecting conception rates following artificial insemination or embryo transfer in lactating Holstein cows. *J. Dairy Sci.*, v.90, p.5073-5082, 2007.

GARRETT, J.E.; GEISERT, R.D.; ZAVY, M.T. et al. Evidence for maternal regulation of early conceptus growth and development in beef cattle. *J. Reprod. Fertil.*, v.84, p.437-447, 1988.

HERSOM, M.J.; WETTEMANN, R.P.; KREHBIEL, C.R. et al. Effect of live weight gain of steers during winter grazing: III. Blood metabolites and hormones during feedlot finishing. *J. Anim Sci.*, v.82, p.2059-2068, 2004.

LAPIERRE, H.; BERNIER, J.F.; DUBREUIL, P. et al. The effect of feed intake level on splanchnic metabolism in growing beef steers. *J. Anim. Sci.*, v.78, p.1084-1099, 2000.

LAU, T.M.; GOW, C.B.; FAIRCLOUGH, R.J. Differential effects of progesterone treatment on the oxytocin-induced prostaglandin F2a response and the levels of endometrial oxytocin receptors in ovariectomized ewes. *Biol. Reprod.*, v.46, p.17-22, 1992.

LOPEZ-GATIUS, F.; SANTOLARIA, P.; YANIZ, J. et al. Progesterone supplementation during the early fetal period reduces pregnancy loss in high-yielding dairy cattle. *Theriogenology*, v.62, p.1529-1535, 2004.

MASS, J. Relationship between nutrition and reproduction in beef cattle. *Vet. Clin. N. Am. Food Anim. Pract*., v.3, p.633-646, 1987.

MOORE, J.E.; KUNKLE, W.E.; BROWN, W.F. Forage quality and the need for protein and energy supplements. In: ANNUAL FLORIDA BEEF CATTLE SHORT COURSE. 40., 1991, Gainesville, FL. *Proceedings...* Gainesville, FL: University of Florida, 1991. p.113-123.

MOREIRA, F.; BADINGA, L.; BURNLEY, C. et al. Bovine somatotropin increases embryonic development in superovulated cows and improves posttransfer pregnancy rates when given to lactating recipient cows. *Theriogenology*, v.57, p.1371-1387, 2002a.

MOREIRA, F.; PAULA-LOPES, F.F.; HANSEN, P.J. et al. Effects of growth hormone and insulin-like growth factor-I on development of in vitro derived bovine embryos. *Theriogenology*, v.57, p.895-907, 2002b.

NUTRIENT requirements of beef cattle. Washington, DC: National Academy Press, 1996.

PERRY, G.A.; SMITH, M.F.; LUCY, M.C. et al. Relationship between follicle size at insemination and pregnancy success. *Proc. Natl. Acad. Sci. USA*, v.102, p.5268-5273, 2005.

PURSLEY, J.R.; WILTBANK, M.C.; STEVENSON, J.S. et al. Pregnancy rates per artificial insemination for cows and heifers inseminated at a synchronized ovulation or synchronized estrus. *J. Dairy Sci.*, v.80, p.295-300, 1997.

ROBINSON, N.A.; LESLIE, K.E.; WALTON, J.S. Effect of treatment with progesterone on pregnancy rate and plasma concentrations of progesterone in Holstein cows. *J. Dairy Sci.*, v.72, p.202-207, 1989.

SANGSRITAVONG, S.; COMBS, D.K.; SARTORI, R. et al. High feed intake increases blood flow and metabolism of progesterone and estradiol-17β in dairy cattle. *J. Dairy Sci.*, v.85, p.2831-2842, 2002.

SANTOS, J.E.; AMSTALDEN, M. Effects of nutrition on bovine reproduction. *Arq. Fac. Vet. UFRGS*, v.26, p.19-89, 1998.

SARTORI, R.; FRICKE, P.M.; FERREIRA, J.C. et al. Follicular deviation and acquisition of ovulatory capacity in bovine follicles. *Biol. Reprod.*, v.65, p.1403-1409, 2001.

SPENCER, T.E.; BAZER, F.W. Biology of progesterone action during pregnancy recognition and maintenance of pregnancy. *Front. Biosci.*, v.7, p.1879-1898, 2002.

SPICER, L.J.; ALPIZAR, E.; ECHTERNKAMP, S.E. Effects of insulin, insulin-like growth factor I, and gonadotropins on bovine granulosa cell proliferation, progesterone production, estradiol production, and(or) insulin-like growth factor I production in vitro. *J. Anim. Sci.*, v.71, p.1232-1241, 1993.

STEVENSON, J.S.; KOBAYASHI, Y.; SHIPKA, M.P. et al. Altering conception of dairy cattle by gonadotropin-releasing hormone preceding luteolysis induced by prostaglandin $F_{2\alpha}$. *J. Dairy Sci.*, v.79, p.402-410, 1996.

STEVENSON, J.S.; KOBAYASHI, Y.; THOMPSON, K.E. Reproductive performance of dairy cows in various programmed breeding systems including Ovsynch and combinations of gonadotropin-releasing hormone and prostaglandin $F_{2\alpha}$. *J. Dairy Sci.*, v.82, p.506-515, 1999.

STRONGE, A.J.H.; SREENAN, J.M.; DISKIN, M.G. et al. Post-insemination milk progesterone concentration and embryo survival in dairy cows. *Theriogenology*, v.64, p.1212-1224, 2005.

VALLET, J.L.; LAMMING, G.E. Ovine conceptus secretory proteins and bovine recombinant interferon α1-1 decrease endometrial oxytocin receptor concentrations in cyclic and progesterone-treated ovariectomized ewes. *J. Endocrinol.*, v.131, p.475-482, 1991.

VASCONCELOS, J.L.M.; SANGSRITAVONG, S., TSAI, S.J. et al. Acute reduction in serum progesterone concentrations after feed intake in dairy cows. *Theriogenology*, v.60, p.795-807, 2003.

WAGNER, J.J.; LUSBY, K.S.; OLTJEN, J.W. et al. Carcass composition in mature Hereford cows: Estimation and effect on daily metabolizable energy requirement during winter. *J. Anim. Sci.*, v.66, p.603-612, 1988.