Effects of Feeding a Vitamin and Mineral Supplement and/or an Energy Supplement to Beef Heifers during the First 84 Days of Pregnancy on Heifer Performance, Concentrations of Progesterone, and Corpus Luteum Size and Fetal Body Measurements

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Our objectives were to determine the influence of feeding vitamin and mineral (VTM) and energy (NRG) supplements to beef heifers during the first 84 days of pregnancy on growth performance, circulating concentrations of progesterone (P4), corpus luteum (CL) size and fetal body measurements. By design, heifers receiving NRG had greater average daily gain (ADG), compared with NoNRG heifers (0.85 vs. 0.34 ± 0.04 kilograms per day [kg/d], respectively; P < 0.0001). Providing NRG supplements during early gestation resulted in heavier CLs, greater circulating concentrations of P4 and greater fetal femur growth, whereas providing VTM supplements enhanced fetal liver growth.

Summary

The objectives were to determine the influence of feeding vitamin and mineral (VTM) and energy (NRG) supplements to beef heifers during the first 84 days of pregnancy on growth performance, circulating concentrations of progesterone (P4), and corpus luteum (CL) size and fetal body measurements. Crossbred beef heifers (n = 35; initial body weight [BW] = 359.5 ± 7.1 kg) were blocked by weight and assigned to treatments in a 2 × 2 factorial arrangement with main factors of VTM (NoVTM or VTM) and NRG (NoNRG or NRG) supplement (eight or nine heifers per treatment group).

Heifers were fed a basal total mixed ration (TMR) once daily with VTM and NRG top-dressed. The VTM factor was initiated 71 to 148 days before artificial insemination (AI). Heifers were AI bred to a single sire, and the NRG was initiated at AI with target gains of 0.28 kg/d for NoNRG and 0.79 kg/d for NRG.

Body weights and serum samples were collected on days 14, 28, 42, 56, 70 and 84 after AI, and the serum samples were analyzed for concentrations of P4. On day 83 ± 0.27 after AI, gravid reproductive tracts were collected and fetuses were dissected. Performance and body measurement data were analyzed using the MIXED procedure of SAS and P4 data were evaluated as repeated measures in time.

By design, heifers receiving NRG had greater ADG, compared with NoNRG heifers (0.85 vs. 0.34 ± 0.04 kg/d, respectively; P < 0.0001). An NRG × day interaction ($P = 0.006$) was observed for P4, with concentrations being similar on days 14 to 56, a tendency ($P = 0.09$) for divergence between NRG and NoNRG at day 70, and with concentrations being greater ($P = 0.002$) on day 84 for NRG, compared with NoNRG heifers (6.74 vs. 4.85 ± 0.43 nanograms per milliliter [ng/mL], respectively).

No interactions ($P \geq 0.22$) were present for CL or gravid uterine weights, or fetal body, liver, heart, pancreas, hind limb, femur or brain weights. No

impact of main effects ($P \geq 0.24$) were observed for gravid uterine weight or fetal body, heart, pancreas, hind limb or brain weights.

However, NRG heifers had greater CL weights, compared with NoNRG heifers (4.86 vs. 3.94 ± 0.32 gram [g], respectively; $P = 0.003$). Furthermore, fetuses from NRG dams had greater ($P = 0.009$) femur weights than fetuses from NoNRG dams (0.39 vs. 0.34 g, respectively).

Interestingly, fetal liver weight was greater ($P = 0.05$) from dams fed VTM than NoVTM (4.80 vs. 4.42 ± 0.12 g, respectively). Overall, providing an NRG supplement during early gestation resulted in heavier CLs, greater circulating concentrations of P4 and greater fetal femur growth, whereas providing a VTM supplement enhanced fetal liver growth.

Introduction

Energy supplementation often is provided to maintain targeted production goals for growth and reproductive performance (Schillo et al., 1992; Ciccioli et al., 2005; Cappellozza et al., 2014). In addition, during the first breeding season, pregnant heifers still need to grow while maintaining a pregnancy. Energy intake modulates BW gain and circulating concentrations of progesterone, which is a steroid required for maintenance of pregnancy and is important for conceptus growth and development (Garrett et al., 1988).

When providing supplemental energy and/or mineral to their herds, wide variation exists among beef producers; some producers do not provide any supplements to their herds at any time of the year, whereas others will ensure energy and/or minerals is provided 365 days a year. Inadequate trace mineral consumption can compromise reproduction, animal health and animal growth (NRC, 2005; NASEM, 2016).

Furthermore, providing a dietary supply of trace minerals to meet animal requirements is essential for the immediate and long-term well-being of the embryo, fetus and neonate (Ashworth and Antipatis, 2001). Trace minerals also play key roles in vitamin synthesis, hormone production, enzyme activity, tissue synthesis, oxygen transport and energy production (Underwood, 1999).

Therefore, supplementation at different stages of production may have greater impacts on reproduction and animal performance, which may affect fetal development. Grace et al. (1986) indicated that an exponential increase of mineral accumulation of ovine fetuses occurs in the mid to late stages of gestation, where they eventually peak at late gestation. However, we do not know if we have a similar accumulation of minerals in the first stage of gestation in a bovine model.

Our lab has demonstrated that production efficiencies of beef cattle are compromised with moderate nutrient restriction. Moreover, genes in functional categories in tissues such as the fetal liver are where metabolic pathways and protein kinases also can be affected by moderate nutrient restriction (Crouse et al., 2017).

In the previous model of early pregnancy, all cattle received supplemental trace minerals. Not providing supplemental trace minerals in diets likely will lead to deficiencies at some stages of production. With such a large variation in mineral and energy supplementation strategies in place on beef operations, understanding the impacts that pre-breeding trace mineral and energy supplementation have on reproductive processes and fetal growth and development would be a great benefit to our industry.

Therefore, our objectives were to determine the influence of feeding vitamin and mineral (VTM) and energy (NRG) supplements to beef heifers during the first 84 days of pregnancy on heifer performance, concentrations of progesterone (P4), and corpus luteum (CL) size and fetal body measurements.

Materials and Methods

The North Dakota State University Institutional Animal Care and Use Committee approved all animal procedures (A19012).

Animals, Housing and Diet

Crossbred beef heifers (n = 72; initial BW = 359.5 ± 7.1 kg) at the Central Grasslands Research Extension Center near Streeter, N.D., were used in a randomized complete block design. Prior to treatment allocation, heifers underwent consecutive day weights and transrectal ultrasonography.

One technician scanned each ovary using an Aloka-500 linear array transrectal probe (7.0-MHZ transducer, 500 V Aloka, Wallingford, Conn.) to count small (3 to 5 millimeter [mm]), medium (6 to 10 mm) and large (greater tjhan 10 mm) follicles. Follicles counted on each ovary were summed to determine the antral follicle count (**AFC**).

To initiate the 2 × 2 factorial arrangement, heifers were stratified by weight and AFC into two respective treatments: 1) heifers received a mineral supplement (**VTM**; n = 36) or 2) heifers received no mineral supplement (**NoVTM**; n = 36). The diet was delivered once daily via total mixed ration (**TMR**) and consisted of triticale hay, corn silage, modified distillers grains plus solubles, ground corn, and if indicated by treatment, mineral premix (delivered at a 0.45 kg feeding rate to target 113 grams per day [g/d] of mineral and vitamins). The mineral premix consisted of ground corn and a loose mineral supplement (Purina® Wind & Rain® Storm® All-Season 7.5 Complete, Land O'Lakes Inc., Arden Hills, Minn.).

Later, heifers were transported and housed at the Animal Nutrition and Physiology Center (**ANPC**) in Fargo, N.D., where feed intake was measured individually using the Calan Gate system (American Calan, Northwood, N.H.). Upon arrival at the ANPC, heifers were weighed individually, stratified by weight and allotted to one of six breeding groups.

The VTM factor was initiated 71 to 148 days before artificial insemination (**AI**). At breeding, to complete the factorial arrangement, heifers were assigned to one of four treatment groups: 1) heifers received a vitamin and mineral supplement and an energy supplement (**VTM + NRG**, n = 18), 2) heifers received no vitamin and mineral supplement but received energy supplement (**NoVTM + NRG**, n = 18), 3) heifers received vitamin and mineral supplement but no energy supplement (**VTM + NoNRG**, n = 18) and 4) heifers received no vitamin and mineral supplement and no energy supplement (**NoVTM + NoNRG**, n = 18).

At the ANPC, the diet was delivered once daily via TMR (53.01% dry matter [DM]; Table 1) and consisted of prairie grass hay, corn silage and dried distillers grains plus solubles, and supplemented with a **VTM** or **NoVTM** digestible fiber-based carrier supplement. The VTM supplement was a pelleted product fed at a 0.45 kg feeding rate to a target 113 g/d of mineral and vitamins (Purina® Wind & Rain® Storm® All-Season 7.5 Complete, Land O'Lakes Inc., Arden Hills, Minn.).

The **NRG** supplement was based on a commercially available product (Purina® Accuration® Range Supplement 33) fed at a rate reflective of pasture-based consumption (fed at 0.58% as-fed basis of BW per day). The NRG supplement factor was initiated at AI with target gains of 0.28 kg/d for NoNRG and 0.79 kg/d for NRG (0.433 and 0.304 megacalorie per kilogram [Mcal/kg] net energy for maintenance [NEm] and net energy for gain [NEg], respectively).

Estrous Synchronization and Breeding

All heifers were estrus synchronized using the seven-day CO-Synch plus controlled internal drug release (**CIDR**) and timed-AI (**TAI**) protocol. Additionally, all heifers received an estrus detection patch (Estrotect; Rockway Inc., Spring Valley, Wis.) to determine their heat state. Heifers were bred using female-sexed semen from a single sire by artificial insemination (**AI**).

Pregnancy diagnosis was performed 42 days after AI using transrectal ultrasonography to determine AI pregnancy rates. Fetal measurements via transrectal ultrasonography were performed at 56, 70, and 82 days following AI.

After pregnancy diagnosis, the final treatment numbers were: 1) **VTM + NRG**, n = 8; 2) **NoVTM + NRG**, n = 9; 3) **VTM + NoNRG**, n = 9; and 4) **NoVTM + NoNRG**, n = 9.

Blood Sampling and Analyses
Serum samples were collected every 14 days via jugular venipuncture into serum tubes (10 milliliters [mL]; Becton Dickinson Co., Franklin Lakes, N.J.), allowed to clot for 30 minutes and centrifuged at 1,500 × g at 4 C for 20 minutes. Serum samples were separated and stored in plastic vials at minus 20 C until further analysis.

Serum samples were analyzed for progesterone (P_4) concentrations by competitive chemiluminescent immunoassay using the Immulite 1000 (Siemens, Los Angeles, Calif.).) Briefly, a 50-microliter (µL) sample of maternal serum was analyzed in duplicate. Lesser, medium and greater P_4 pools were assayed in duplicate (0.94 ± 0.12, 8.44 ± 0.94 and 19.6 ± 0.54 ng/mL, respectively). The intra- and inter-assay coefficients of variation (CV) were 4.15% and 8.96%, respectively.

Tissue Collection and Analysis
Ovariohysterectomy procedures were conducted at day 83 ± 0.27 of gestation for collection of utero-placental and fetal tissues as previously described by McLean et al. (2016). Briely, the ovariohysterectomy was conducted as a standing procedure with a left flank incision.

Ovarian and uterine arteries were sutured and ligated. Additionally, sutures were placed around the cervix. The uterus was clamped caudal to the bifurcation and incised along the clamp, thereby collecting the entire uterine body and horns, along with the attached ovaries.

Immediately following the ovariohysterectomy, gravid uterine, ovary and CL weights were recorded. The fetus was removed from the gravid uterus and weighed, and the collection of the fetal liver, heart, intestine, pancreas, hind limb and brain occurred. Tissues were weighed and stored individually for further analysis.

Statistical Analysis
Data were analyzed as a completely randomized block design with heifer as the experimental unit for all analyses. Body weight and progesterone data were analyzed using the MIXED procedure of SAS (9.4, SAS Inst. Inc., Cary, N.C.), and the Kenward-Roger approximation was used to determine the denominator degrees of freedom for the tests of fixed effects.

The model statement contained the effects of treatment, day and all interactions, with the breeding group as the random effect. The specified term for the repeated statement was day, and heifer was included as the subject.

The covariance structure was compound symmetry by providing the smallest Akaike information criterion for all variables analyzed. Maternal performance, uterine measurements and fetal measurements also were analyzed using PROC MIXED, and the Kenward-Roger approximation was used to determine the denominator degrees of freedom for the tests of fixed effects.

The model statement contained the effects of VTM, NRG and all interactions. Results are reported as least square means using the LSMEANS statement and separated using PDIFF. For all analyses, significance was set at $P \leq 0.05$, and tendencies were determined if $P > 0.05$ and $P \leq 0.10$.

Results and Discussion

An NRG × day interaction ($P < 0.0001$) was observed for body weight being greatest on day 84 for NRG, compared with NoNRG heifers (426.8 vs. 387.6 ± 5.9 kg, respectively; $P < 0.0001$). We observed no interactions ($P = 0.35$; Table 2) for ADG.

By design, heifers receiving NRG had greater ADG, compared with NoNRG heifers (0.85 vs. 0.34 ± 0.04 kg/d, respectively; $P < 0.0001$). We found no effect of VTM treatment on day 84 ADG ($P = 0.72$). Average consumption of the NRG supplement was 1.95 kg/d.

We found no interactions or effect of VTM treatment ($P \geq 0.11$) on total dry-matter intake (DMI). However, NRG heifers consumed more feed than NoNRG heifers (7.72 vs. 4.98 ± 0.004 kg, respectively; $P < 0.0001$), which was by design so that targeted gains would be met. Moreover, we observed no interactions or effect of VTM treatment

($P \geq 0.10$) on the gain-to-feed (G:F) ratio, but NRG heifers had a higher G:F ratio, compared with NoNRG heifers ($P < 0.0001$).

Similar gains have been reported by Ciccioli et al. (2005) when beef heifers were fed a high- or low-starch supplement for 60 days prior to the breeding season. Furthermore, Cappellozza et al. (2014) noted ADG was greater for energy and protein supplemented heifers, compared with control heifers, when supplemented at intakes of 0.54% and 0.50% of heifer BW, respectively. Collectively, these results provide evidence that to support BW gains, beef heifers consuming low-quality forages equally can utilize nutrients provided by supplements based on protein or energy ingredients.

An NRG × day interaction ($P = 0.006$; Figure 1) was observed for P concentrations being similar on days 14 to 56, a tendency ($P = 0.09$) for divergence at day 70, and concentrations being greater ($P = 0.002$) on day 84 for NRG, compared with NoNRG heifers (6.74 vs. 4.85 ± 0.43 ng/mL, respectively). Progesterone is necessary for maintenance of pregnancy and regulating changes in the uterine environment, and is important for conceptus growth and development (Garrett et al., 1988).

We found no interactions ($P \geq 0.49$; Table 3) present for dam CL or gravid uterine weights. Furthermore, no impact of main effects ($P \geq 0.43$) was observed for gravid uterine weight. However, NRG heifers had greater CL weights, compared with NoNRG heifers (4.86 vs. 3.94 ± 0.32 g, respectively; $P = 0.003$)

A VTM × NRG interaction ($P = 0.03$; Table 3) was present for fetal intestine weight where the intestinal weight was greatest in fetuses from dams receiving VTM + NRG and lowest in those from VTM + NoNRG dams. No interactions ($P \geq 0.22$) were present for fetal body, liver, heart, pancreas, hind limb, femur or brain weights.

Furthermore, no impact of main effects ($P \geq 0.24$) was observed for fetal body, heart, pancreas, hind limb or brain weights. However, fetuses from NRG dams had greater ($P = 0.009$) femur weights than fetuses from NoNRG dams (0.39 vs. 0.34 g, respectively).

Interestingly, fetal liver weights were greater ($P = 0.05$) from dams fed VTM than NoVTM (4.80 vs. 4.42 ± 0.12 g, respectively). Researchers have noted that the fetus is completely dependent on the dam for its supply of nutrients, including trace elements (Hidiroglou and Knipfel, 1981).

Overall, body weights diverged during the first 84 days of pregnancy, which was by design and resulted in NRG heifers gaining more than NoNRG heifers. Additionally, providing NRG supplements during early gestation resulted in heavier CLs that produced more P4 and greater fetal femur growth, whereas providing VTM supplements enhanced fetal liver growth.

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Table 1. Dietary ingredient and nutrient compositions.

	% DM basis			
Item	TMR[1]	NoVTM[2]	VTM[3]	NRG[4]
Ingredient				
Corn silage	38	-	-	-
Prairie hay	55	-	-	-
Dried distillers grains with solubles	7	-	-	-
Wheat middlings	-			-
Ground corn	-	-	-	
Ground meal byproduct	-	-	-	
Nutrient Analysis				
DM	53.01	86.6	89.6	87.7
Ash	11.54	5.3	25.1	2.4
CP	9.98	15.6	14.8	17.5
N	1.60	2.5	2.4	2.8
NDF	65.85	41.9	27.6	19.4
ADF	39.28	16.4	13.0	4.3
Ether Extract	1.52	-	-	9.1
Ca	0.61	-	-	0.02
P	0.31	-	-	0.59
Mineral Analysis, mg/kg				
Fe		222	1,646	57
Cu		172.6	3,903	42.7
Zn		139.1	1,092.5	35.4
Mo		9.2	28.2	< 5.0
Mn		112.4	1,188.2	27.7

[1] TMR: Composite samples for the feeding period.

[2] NoVTM: No vitamin and mineral supplement was a pelleted product fed at a 0.45 kg feeding rate with no added mineral and vitamin supplement.

[3] VTM: Vitamin and mineral supplement was a pelleted product fed at a 0.45 kg feeding rate to target 113 g/d of mineral and vitamins (Purina® Wind & Rain® Storm® All-Season 7.5 Complete, Land O'Lakes Inc., Arden Hills, Minn.).

[4] NRG: Energy supplement was based on Purina® Accuration® Range Supplement 33 fed at a rate reflective of pasture-based consumption (fed at 0.58% of body weight per day).

Table 2. Effect of feeding a vitamin and mineral supplements and/or an energy supplement to beef heifers during the first 84 days of pregnancy on performance and intake.

	NoVTM[1]		VTM[2]			*P*-values		
Item	NoNRG	NRG[3]	NoNRG	NRG	SEM	VTM	NRG	VTM × NRG
No.	9	9	9	8	--	--	--	--
ADG, kg/d	0.35	0.82	0.32	0.88	0.04	0.72	< 0.0001	0.35
Forage DMI, kg/d	4.24	5.53	4.91	5.22	0.25	0.49	0.003	0.08
NRG DMI, kg/d	-0.01	1.95	0.02	1.95	0.06	0.70	< 0.0001	0.91
Total DMI, kg/d	4.63	7.87	5.34	7.57	0.29	0.50	< 0.0001	0.11
G:F, kg/kg	0.035	0.047	0.026	0.055	0.004	0.72	< 0.0001	0.10

[1] NoVTM: No vitamin and mineral supplement was a pelleted product fed at a 0.45 kg feeding rate with no added mineral and vitamin supplement.

[2] VTM: Vitamin and mineral supplement was a pelleted product fed at a 0.45 kg feeding rate to target 113 g/d of mineral and vitamins (Purina® Wind & Rain® Storm® All-Season 7.5 Complete, Land O'Lakes Inc., Arden Hills, Minn.).

[3] NRG: Energy supplement was based on Purina® Accuration® Range Supplement 33 fed at a rate reflective of pasture-based consumption (fed at 0.58% of body weight per day).

Table 3. Effect of feeding vitamin and mineral supplements and/or energy supplements to beef heifers during the first 84 days of pregnancy on the gravid reproductive tract and fetal body measurements.

	NoVTM[1]		VTM[2]			*P*-values		
Item	NoNRG	NRG[3]	NoNRG	NRG	SEM	VTM	NRG	VTM × NRG
No.	9	9	9	8	--	--	--	--
Maternal, grams								
Uterine weight	1,916.9	1,765.4	1,763.5	1,757.0	100.0	0.44	0.43	0.49
Dam CL	3.98	4.78	3.89	4.93	0.32	0.79	0.003	0.82
Fetal, grams								
Fetal body weight	117.5	117.24	116.16	125.78	4.54	0.41	0.27	0.28
Liver	4.50	4.34	4.70	4.90	0.19	0.05	0.90	0.33
Heart	1.02	1.00	1.10	1.07	0.06	0.27	0.66	0.89
Intestine	2.56^{a}	2.47^{a}	2.42^{a}	2.87^{b}	0.11	0.24	0.09	0.03
Pancreas	0.292	0.269	0.265	0.292	0.044	0.96	0.97	0.57
Hind limb	7.71	7.70	7.51	7.52	0.49	0.69	0.99	0.98
Femur	0.337	0.363	0.347	0.422	0.019	0.08	0.009	0.22
Brain	3.67	3.51	3.60	3.67	0.19	0.84	0.79	0.56

[ab] Means within row lacking common superscript differ ($P < 0.05$).

[1] NoVTM: No vitamin and mineral supplement was a pelleted product fed at a 0.45 kg feeding rate with no added mineral and vitamin supplement.

[2] VTM: Vitamin and mineral supplement was a pelleted product fed at a 0.45 kg feeding rate to target 113 g/d of mineral and vitamins (Purina® Wind & Rain® Storm® All-Season 7.5 Complete, Land O'Lakes Inc., Arden Hills, Minn.).

[3] NRG: Energy supplement was based on Purina® Accuration® Range Supplement 33 fed at a rate reflective of pasture-based consumption (fed at 0.58% of body weight per day.

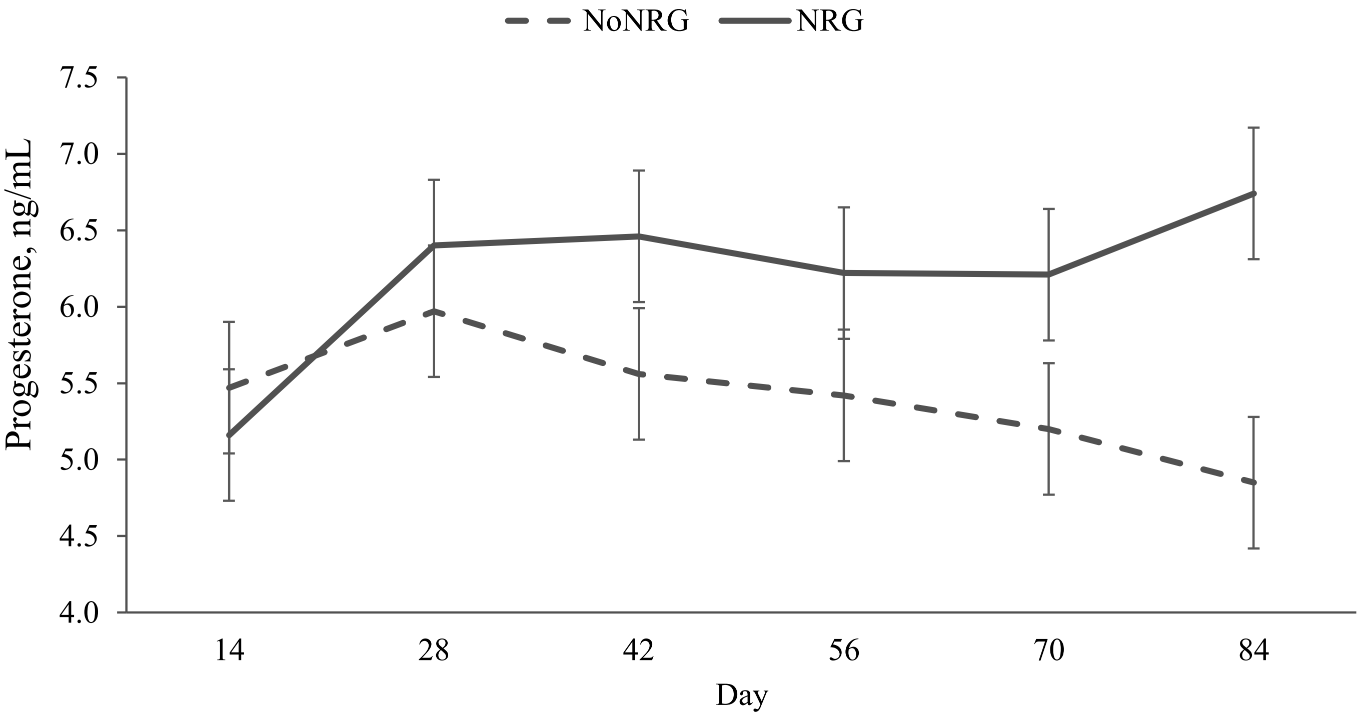

Figure 1. Effect of feeding an energy supplement to beef heifers during the first 84 days of pregnancy on circulating concentrations of progesterone (P4). An NRG × day interaction (*P* = 0.006) was observed for P4 concentrations, which were similar between NoNRG and NRG on days 14 to 56, tended to differ (*P* = 0.09), as indicated by *, on day 70, and were greater (*P* = 0.02) for NRG, compared with NoNRG, on day 84, as indicated by **.