Late gestation supplementation of corn dried distiller's grains plus solubles to beef cows fed a low-quality forage: III. effects on mammary gland blood flow, colostrum and milk production, and calf body weights

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ABSTRACT: Objectives were to investigate the effects of supplementation with corn dried distiller's grains plus solubles (DDGS) to late gestating beef cows on arterial blood flow to the mammary glands during late gestation and early lactation; colostrum and milk production; dystocia and immunity; and calf BW. Cows were fed a control (CON; $n = 15$; 5.1% CP; 36.2% ADF) diet consisting of 90% corn stover and 10% corn silage on a dry basis offered ad libitum or CON diet with supplementation of DDGS (0.30% of BW; SUP $n = 12$). Mammary gland blood flow was assessed on day 245 of gestation. At parturition, maternal and calving parameters were assessed; colostrum and jugular blood was sampled; and dams were weighed. Mammary gland blood flow and milk production was measured on day 44 of lactation. Calves were weighed fortnightly for 8 wk and at weaning. Colostrum production tended to be greater in SUP dams than in CON dams (837 vs. 614 ± 95 g, $P = 0.10$). Calves of SUP dams were heavier at birth and 24 h (0 h, 43.2

vs. 39.8 ± 1.0 kg, $P = 0.02$; 24 h, 44.0 vs. 40.4 ± 1.1 kg, $P = 0.02$). At birth and 24 h, blood $p\text{CO}_2$ was greater in calves born to SUP dams (6.82 vs. 6.00 ± 0.41 kPa, $P = 0.04$). Serum IgG did not differ ($P = 0.21$) at 24 h. Ipsilateral mammary gland blood flow of SUP cows was greater than CON cows (2.76 vs. 1.76 ± 0.30 L/min; $P = 0.03$); however, when summed with contralateral, total blood flow was similar ($P = 0.33$). Hemodynamic measures on day 44 of lactation were similar ($P \geq 0.32$). Milk production tended to be increased (13.5 vs. 10.2 ± 1.2 kg/d, $P = 0.07$) in SUP vs. CON cows. Despite similar BW through 56 d, calves from SUP cows were heavier ($P = 0.04$) at weaning (309.7 vs. 292.0 ± 6.0 kg). In conclusion, we accept our hypothesis that DDGS supplementation during gestation influenced mammary blood flow, milk production and calf weights. These findings implicate maternal nutrition's leverage on both nutrient and passive immunity delivery to the calf early in life as well as potential advantages on long-term performance.

Key Words: beef cow, colostrum, lactation, mammary blood flow

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J. Anim. Sci. 2019.97:3337–3347

doi: 10.1093/jas/skz201

This work was supported by the North Dakota Corn Council, the USDA National Institute of Food and Agriculture, and Hatch Multistate projects number ND01744, ND01755, ND01756, and ND01785. The authors would like to thank Trent Gilbery, Sarah Underdahl, and Jim Kirsch for their assistance with the animal portion of the project.

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Received March 28, 2019.

Accepted June 8, 2019.

INTRODUCTION

Maternal nutrition is a key component to fetal and placental development, consequently influencing lifetime performance of the offspring (Funston et al., 2010; Lan et al., 2013). Oftentimes, forage quality and quantity are limited during the peripartum period, especially in spring calving herds. As gestation advances, there is an increased need for maternal dietary protein to supply the increased amino acid demands for the fetus in utero (Bell, 1995). Supplementation of corn dried distiller's grains plus solubles (DDGS), which provides protein and energy, during late gestation could positively influence blood flow to the mammary glands, affecting colostrum, milk production, and eventually calf growth trajectories (McSweeney et al., 1993; Svennersten-Sjaunja and Olsson, 2005; Sullivan et al., 2009). Separation of maternal and fetal blood supplies due to syndesmochorial placentation, prevents the transfer of immunoglobulins in utero (Arthur, 1996; Weaver et al., 2000). Thus, calves are born agammaglobulinemic (Weaver et al., 2000), and characterized as being immunonaive (Barrington and Parish, 2001). Additionally, while we know maternal diet during early lactation can influence milk components (i.e., energy and protein), it is relatively unknown how maternal gestational diets can impact the protein and energy status of the dam during lactation, as well as the quality of her milk.

There is evidence in the ewe, where in spite of similar nutrition during early lactation, milk production was altered when ewes were fed differing nutritional planes during gestation (Meyer et al., 2011). This suggests that mammary gland function and milk quality could be influenced by gestational diets. We hypothesized that DDGS supplementation to cows fed a low-quality forage alters nutrient delivery via the mammary glands, thus improving calf health and growth. Objectives were to investigate arterial blood flow to the mammary glands; colostrum and milk production; incidence of dystocia and metabolic acidosis; passive transfer of immunity; and calf weight gain.

MATERIALS AND METHODS

Cows, Treatments, and Management

All procedures were approved by the North Dakota State University Animal Care and Use Committee (#A14007). Animal handling methods have been previously reported (Kennedy, 2016a). It

should be noted that during the treatment period, average temperature was -10°C and wind speed was 18.3 km/h (min. 16 km/h, max. 21 km/h) which created very cold conditions for cows and certainly could have contributed to weight and body condition loss. The influence of these weather conditions was also difficult to estimate for the NRC model because wind speed was mitigated by open front housing (south facing) where cows could get out of the wind.

Briefly, 27 multiparous beef cows (Angus or Angus $\times$ Simmental; 674 ± 17 kg of BW; 6 ± 5 yr of age) were divided randomly into a control group (CON; $n = 15$) and a treatment group (SUP; $n = 12$). Cows were housed at the Beef Cattle Research Complex in 2 adjacent pens, one for each treatment group. Following a 3-week acclimation period, individual intake was monitored and controlled via RIC feeders (Insentec, Marknesse, Netherlands) beginning on day 201 of gestation for 10 wk. All cows were fitted with radio frequency identification tags to facilitate monitoring of intake and feeding behavior. All cattle had free access to water and trace-mineralized salt blocks (97% NaCl, 3.5 g/kg Zn, 2.0 g/kg Fe, 1.8 g/kg Mn, 350 mg/kg Cu, 100 mg/kg I, 60 mg/kg Co). A basal diet of 90% corn stover and 10% corn silage (5.0% CP on a DM basis, marginally deficient in NE and deficient in rumen degradable protein) was fed for ad libitum intake to both groups, with the supplemented group receiving DDGS at 0.30% of BW (DM basis). Inclusion of corn silage was increased to 20% on day 246 (gestation diet 2; 4.7% CP on DM basis) and again increased to 30% on day 260 (gestation diet 3; 5.5% CP on a DM basis) to meet increased NE demands during pregnancy; supplementation regime remained the same throughout the trial. Each pen contained 8 Insentec feed bunks. During the treatment period, all 8 feed bunks in the CON pen contained the basal diet, while in the SUP pen 6 feed bunks contained the basal diet and 2 contained DDGS. Beginning on day 270 of gestation, close to parturition, all cows were fed the same diet (48% corn stover, 30% corn silage, 22% DDGS; DM basis, 10.8% CP) for ad libitum intake for 10 wk; DDGS supplementation ceased at the start of this diet.

Feed samples of the total mixed diet were collected and analyzed weekly; DDGS samples were analyzed by truckload (2 loads). Forage samples were dried in a 55°C oven for 48 h and ground to pass through a 1-mm screen. Forage and DDGS samples were analyzed for DM, ash, N (Kjeldahl method), Ca, P, and EE by standard procedures

(AOAC, 1990). Crude protein was calculated as N concentration multiplied by 6.25. The NDF (using heat-stable α -amylase and sodium sulfite and expressed inclusive of residual ash) and ADF (expressed inclusive of residual ash) concentrations were analyzed sequentially by methods of Robertson and Van Soest (1981) using a fiber analyzer (Ankom Technology, Fairport, NY).

Mammary Blood Supply Evaluation

To measure blood flow to the mammary glands, color Doppler ultrasonography was performed. Blood flow to the mammary glands, cross-sectional area (CSA), and pulsatility indices of each artery were measured on day 245 (± 5 d) of pregnancy (determined from date of insemination) and day 44 of lactation (calculated from birth; ultrasonography was performed following milk collection; see below). Briefly, a 7.5-MHz finger probe was inserted rectally and used to identify the bifurcation of the internal and external iliac arteries. By following the latter, the external pudendal artery was identified. The external pudendal artery, which branches through the inguinal canal and continues branching to the udder, was measured and considered as representative of blood flow to the mammary glands (Budras et al., 2011; Mordhorst et al., 2017). The arteries were categorized as ipsilateral or contralateral to the pregnant uterine horn (as confirmed with uterine blood flow measurements; Kennedy et al., 2016b). Three separate cardiac cycle waveforms from 2 to 3 separate ultrasonography evaluations were selected for data collection and averaged (i.e., 6 to 9 measurements per artery per cow). Resistance index (RI), pulsatility index (PI), peak systolic velocity, end diastolic velocity, flow time, maternal heart rate (HR), mean velocity, blood flow, CSA, and cross-sectional diameter were recorded. The Doppler software was preprogrammed to calculate $PI = (\text{peak systolic velocity} - \text{end diastolic velocity}) / \text{mean velocity}$; $RI = (\text{peak systolic velocity} - \text{end diastolic velocity}) / \text{peak systolic velocity}$; and blood flow (mL/min) = mean velocity (cm/s) $\times$ CSA (cm²) $\times$ 60 s/min. Finally, total mammary blood flow was calculated as the sum of ipsilateral and contralateral mammary blood flow measurements.

Parturition Procedures and Measurements

During calving, cows were allowed to remain in their pens with the group until signs of labor were observed. If it was possible to move the cow inside the barn without causing undue stress, she was

brought indoors and put in an individual pen for calving. Otherwise, she was allowed to calve outside with the group and the cow–calf pair was immediately brought inside. Cows had ad libitum access to hay and water while inside the barn. All cows in the experiment had singleton calves.

Upon parturition, second stage labor time was recorded; time started when amniotic sac appeared at the vulva and ended when calf was expelled from the dam. A calving ease score was assigned post labor (1 = no assistance; 5 = caesarian section; NAAB, 2002). The calf was removed from the pen to collect a blood sample via jugular venipuncture. Whole blood was analyzed immediately for blood gas and pH (see below). Excess amniotic fluid was removed and calf was weighed. While the calf was being processed, the cow was removed and placed into a squeeze chute. Cow BW was determined, the right rear quarter was manually milked completely, and weight of colostrum was recorded. A sample was frozen at -20°C for IgG analysis. An udder score (1 = very pendulous with a broken floor; 9 = very tight) and teat size scores (1 = very large, balloon-shaped; 9 = very small) were then assigned to each dam (Rasby, 2011).

After completion of sample collection from the dam, the cow–calf pair was placed back into their individual pen. Time to stand was recorded; time started when calf showed first signs of movement and concluded when the calf could stand and take a step without falling. Length of first nursing was recorded; starting when calf was actively nursing and obtaining colostrum and ending when calf showed little interest, moved away, and laid down. A calf vigor score (1 = normal, 2 = weak and nursed unassisted, 3 = weak, nursed assisted and lived, 4 = weak, nursed assisted and expired, 5 = stillborn) was assigned to each calf and a proprietary mothering score (1 = the dam was up within ten minutes after delivery, is actively licking the calf to stimulate standing and is vocalizing to the calf encouraging it to nurse; 4 = cow shows aggression towards calf, will not let calf nurse, and does not vocalize) was assigned to each dam. Each cow–calf pair was monitored for signs of general health. At 24 h after calving, calves were once more removed from the individual pen, BW was recorded and a second blood sample was collected via jugular venipuncture and analyzed immediately for blood gas. Cows were weighed and body condition was scored (9-point scale; 1 = emaciated; 9 = very obese; NRC, 2000). Finally, cow–calf pairs were returned to original treatment pens following the 24 h data collection period, unless weather or health status was of concern.

Calves were weighed every 2 wk until day 56. Cow–calf pairs were then transported to pasture and managed similarly. At weaning, calf BW was recorded. On each weigh day, blood samples were collected via jugular venipuncture for serum and plasma. Blood samples were centrifuged within 4 h of collection at $1,380 \times g$ to separate serum and plasma, which was then stored at -20°C until analysis.

Milk Collection

On day 44 of lactation, milk production was determined for each cow using a portable milking machine (InterPuls, Albinea, Italy). Briefly, each cow was weighed, milked completely, housed separately from her calf in an individual pen for a 5-h period with access to her normal diet and water, and then finally milked completely again for 5-h milk production and calculation of daily milk production. Time required to completely milk each cow was recorded, total milk was weighed, and a sample was collected for component analysis (Dairy Lab Services, Inc., Dubuque, IA). Samples were analyzed for fat, true protein, lactose, other solids, total solids, urea, and somatic cell count.

Blood, Colostrum, and Milk Analyses

Blood samples for gas analysis were collected in heparin-treated vacuum tubes. Immediately following collection, blood pH, partial pressure of carbon dioxide (pCO_2), partial pressure of oxygen (pO_2), base excess, HCO_3^- , total CO_2 , soluble O_2 , and lactate were measured and blood Na, K, Cl, hematocrit, and hemoglobin were measured with a handheld analyzer (CG4+ and E3+Cl cartridge; Vetscan i-STAT 1; Abaxis North America, Union City, CA).

Analyses of serum triiodothyronine (T3), thyroxine (T4), and cortisol concentrations were determined by chemiluminescence immunoassay using the Immulite 1000 system with components of commercial kits (Siemens, Los Angeles, CA) as described previously (Lekatz et al., 2010). Within each assay, samples were assayed in duplicate. Intra-assay CV were 6.6% and 3.9% and interassay CV were 2.3% and 2.3% for T3 and T4, respectively. The intra-assay cortisol CV was 17.5%.

Plasma glucose concentrations were measured in duplicate using a hexokinase-based assay (Infinity Glucose Hexokinase; Thermo Trace, Louisville, CO). Urea concentration in plasma samples was measured by absorption of a urea-specific chromogen at 520 nm (QuantiChrom Urea Assay,

BioAssay Systems, Hayward, CA) according to the manufacturer's specifications. Nonesterified fatty acid (NEFA) concentration in plasma samples was measured using an enzymatic colorimetric assay (HR Series NEFA-HR, Wako Chemicals USA, Richmond, VA).

Plasma (0 and 24 h) and colostrum was analyzed for IgG by radial immunodiffusion (Saskatoon Colostrum Co., Saskatoon, Sask.).

Statistical Analysis

Data were analyzed with generalized least squares with repeated measures (mixed model procedure; SAS Institute, Cary, NC) or ordinary least squares (general linear model procedure; SAS Institute). Models included fixed effects of maternal diet (SUP vs. CON), day of gestation or lactation, and the interaction of day and maternal diet when appropriate. Random effects were cow and calf sex. The effect of calf sex and the interaction of diet and calf sex were also included in the model when $P < 0.20$. Covariance structures for repeated measures were selected based on best-fit statistic of information criteria.

RESULTS

Cows

Weight and condition. As previously reported by Kennedy et al. (2016a), SUP cows gained BW (1.27 kg/d, $P < 0.01$) and maintained body condition (0.0003 score/d; $P = 0.79$), while CON cows tended to lose BW (0.23 kg/d, $P = 0.06$) and lost body condition (-0.102 score/d; $P < 0.01$) from day 201 to day 270 of gestation.

Dam BW at parturition was greater in supplemented dams (695 vs. 610 ± 24 kg, $P = 0.01$) compared to CON dams. Dam BW at 24 h after parturition remained greater in SUP dams (694 vs. $597 \text{ kg} \pm 23$, $P = 0.004$) compared to CON dams. Control fed dams lost 13 kg in 24 h while SUP dams only lost 1 kg. Dam BCS was also greater in SUP dams at parturition (5.81 vs. 4.96 ± 0.15 , $P = 0.001$) compared to CON dams.

Parturition and colostrum. Supplemented cows gave birth to 7 males and 5 females, while CON cows produced 8 males and 6 females (1 calf died shortly after birth due to extreme low temperatures). No differences ($P > 0.38$) between treatment groups were seen for gestation length, time of second stage labor, or calving ease (Table 1). Colostrum weight

Table 1. Parturition statistics of beef cows that were fed control or control plus supplementation from day 201 to 270 of gestation

Variable	Diet			P-value
	CON ¹	SUP ¹	SEM ²	
Gestation length, days	277	276	1.1	0.43
Second stage labor length, minutes	48	57	15	0.66
Calving ease ³	1.87	1.44	0.36	0.39
Colostrum weight ⁴ , g	614	837	95	0.10
Colostrum IgG, mg/mL	119.1	130.2	6.6	0.23
Colostrum IgG, g	79	107	14	0.18

¹Maternal diets; CON ($n = 15$), control group consuming basal diet; SUP ($n = 12$), supplemented group consuming basal diet + DDGS at 0.30% BW.

²SEM for $n = 12$.

³Calving ease score (1 = no assistance; 5 = caesarian section).

⁴Colostrum weight from right hindquarter only.

(right hindquarter) tended to be greater ($P = 0.10$) in SUP dams compared to CON dams. There was an effect of sex on colostrum production where dams with female offspring produced more colostrum (891 vs. 561 ± 102 g, $P = 0.02$) than dams that had male offspring. Colostral IgG concentration and quantity was not different ($P \geq 0.18$) between treatment groups.

Mammary gland hemodynamics. On day 245 of gestation, maternal heart rate was increased ($P < 0.01$) in SUP vs. CON cows (Table 2). There was no effect of treatment on mammary gland blood flow ($P = 0.71$) or CSA ($P = 0.60$) measured contralateral to the conceptus. Contralateral PI and RI were not influenced ($P \geq 0.15$) by the effect of treatment. On the ipsilateral side, SUP cows had increased ($P = 0.02$) mammary gland blood flow, reduced ($P = 0.01$) PI, but similar ($P = 0.11$) RI compared to CON cows. When totaled, mammary gland blood flow on day 245 of gestation did not differ ($P = 0.12$) between treatment groups. On day 44 of lactation, measurements of mammary arterial hemodynamics were not altered ($P \geq 0.32$) by previous gestational diet.

Milk production. Supplemented cows took longer ($P = 0.05$) than CON cows to finish milking (12.92 vs. 10.69 ± 0.76 min). There was a tendency ($P = 0.07$) for SUP cows to produce more milk compared to CON cows (Table 3). Additionally, when included in the model, no main effect ($P = 0.42$) of calf sex was observed for milk production (data not reported). Analysis of milk components revealed no differences ($P \geq 0.33$) between treatment groups.

Table 2. Mammary blood flow in beef cows that were fed control or control plus supplementation from day 201 to 270 of gestation

Variable	Diet		SEM ²	P-value
	CON ¹	SUP ¹		
Day 245 of gestation				
Heart rate, beats/min	63.7	75.4	2.4	<0.01
Total blood flow, L/min	4.20	5.36	0.56	0.14
Ipsilateral to the gravid horn				
Blood flow, L/min	1.77	2.76	0.30	0.02
Cross-sectional area, cm ²	0.42	0.49	0.05	0.28
Pulsatility index ³	1.87	1.49	0.10	0.01
Resistance index ⁴	0.76	0.73	0.02	0.11
Contralateral to the gravid horn				
Blood flow, L/min	2.43	2.60	0.34	0.71
Cross-sectional area, cm ²	0.50	0.46	0.05	0.60
Pulsatility index ³	1.82	1.58	0.12	0.15
Resistance index ⁴	0.77	0.74	0.02	0.16
Day 44 of lactation				
Heart rate, beats/min	76.1	75.0	1.8	0.67
Total blood flow, L/min	13.59	14.44	0.89	0.49
Ipsilateral to the gravid horn				
Blood flow, L/min	6.89	7.65	0.64	0.40
Cross-sectional area, cm ²	0.73	0.79	0.05	0.39
Pulsatility index ³	1.04	0.95	0.06	0.32
Resistance index ⁴	0.61	0.59	0.02	0.56
Contralateral to the gravid horn				
Blood flow, L/min	6.39	7.07	0.52	0.36
Cross-sectional area, cm ²	0.72	0.72	0.06	0.98
Pulsatility index ³	1.02	0.97	0.06	0.54
Resistance index ⁴	0.62	0.60	0.02	0.56

¹Maternal diets; CON ($n = 15$), control group consuming basal diet; SUP ($n = 12$), supplemented group consuming basal diet + DDGS at 0.3% BW.

²SEM for $n = 12$.

³Pulsatility index = (peak systolic velocity - end diastolic velocity)/mean velocity.

⁴Resistance index = (peak systolic velocity - end diastolic velocity)/peak systolic velocity.

Calves

Parturition measures. Calf birth weight and 24 h BW was greater ($P = 0.02$) in offspring from SUP dams (Table 4; Kennedy et al., 2016b) compared to offspring of CON dams. Calves were similar ($P \geq 0.22$) in heart girth and crown rump length. Calf vigor score, time to first stand, and the length of the first nursing was also similar ($P \geq 0.23$).

Blood gas, metabolites, and hormones. There was a tendency for an interaction of treatment and time in base excess ($P = 0.09$), plasma glucose ($P = 0.08$), and lactate ($P = 0.08$) in neonatal blood samples from calves (Table 5). Base excess was not different ($P = 0.55$) at 0 h in calves from SUP and CON. By

Table 3. Milk production and components from day 44 of lactation in beef cows fed control or control plus supplementation from day 201 to 270 of gestation

Variable	Diet		SEM ²	P-value
	CON ¹	SUP ¹		
Milk production, kg/d	10.2	13.5	1.2	0.07
Fat, %	4.11	4.21	0.33	0.83
Protein, %	3.08	2.98	0.07	0.33
Lactose, %	5.08	5.13	0.06	0.64
Total solids, %	13.18	13.22	0.32	0.95
Urea nitrogen, mg/dL	8.4	9.3	0.9	0.48
Somatic cells ³ , no./mL	151	249	65	0.30

¹Maternal diets; CON (*n* = 15), control group consuming basal diet; SUP (*n* = 12), supplemented group consuming basal diet + DDGS at 0.3% BW.

²SEM for *n* = 12.

³Two outliers (cows likely with mastitis) were removed; when included in analysis means were CON = 16,669; SUP = 429; SEM = 1,127; *P* = 0.44.

24 h, calves from CON cows increased their base excess compared to 0 h (*P* = 0.04) and SUP calf 24 h (*P* = 0.08) concentrations. While glucose was similar (*P* = 0.55) in calves at 0 h, calves from SUP cows tended to have greater (*P* = 0.08) circulating glucose at 24 h compared to CON. Lactate decreased (*P* = 0.03) in calves from CON cows from 0 to 24 h, whereas, lactate in calves from SUP cows was unchanged (*P* = 0.75) over the 24-h period.

There were no other treatments by time interactions (*P* > 0.11) for blood measurements (Table 5). Blood pH was not different (*P* = 0.11) in calves between treatment groups but did increase (*P* < 0.01) from 0 to 24 h. Blood pCO₂ was greater (*P* = 0.04) in calves born to SUP dams. Blood pCO₂ decreased (*P* < 0.01) from birth to 24 h. Blood pO₂ was similar (*P* ≥ 0.12) in calves regardless of day or treatment. Hemoglobin concentrations tended to be greater (*P* = 0.07) in calves born to SUP vs. CON dams and decreased (*P* = 0.02) from 0 to 24 h.

Neonatal IgG concentrations did not differ (*P* = 0.21) between treatment groups, but as expected increased (*P* < 0.001) from 0 to 24 h. Blood protein concentrations were greater (*P* = 0.05) in calves born to SUP vs. CON calves (53.3 ± 1.2 and 50.0 ± 1.1, respectively). Protein concentrations increased (*P* < 0.001) from 0 to 24 h. Concentrations of plasma NEFA were not affected (*P* = 0.94) by treatment but did decrease (*P* < 0.001) from birth to 24 h. Plasma urea was not affected (*P* > 0.25) by treatment or time. Maternal diet did not alter (*P* > 0.25) birth concentrations of calf cortisol, T3, and T4. Serum cortisol decreased (*P* < 0.001) from birth to 24 h.

Table 4. Calf birth measures from beef cows that were fed control or control plus supplementation from day 201 to 270 of gestation

Variable	Diet		SEM ²	P-value
	CON ¹	SUP ¹		
Calf BW, kg				
Birth	39.8	43.2	1.0	0.02
24 h	40.4	44.0	1.1	0.02
Time to stand, minutes	38.9	50.8	8.2	0.30
Length of first nursing, minutes	34.0	23.4	6.5	0.23
Heart girth circumference, mm	821	841	12	0.22
Crown-rump length, mm	847	831	20	0.57
Calf vigor ³	1.00	1.15	0.12	0.36

¹Maternal diets; CON (*n* = 15), control group consuming basal diet; SUP (*n* = 12), supplemented group consuming basal diet + DDGS at 0.3% BW.

²SEM for *n* = 12.

³Calf vigor score (1 = normal; 5 = stillborn).

When hormones and metabolites were assessed from day 14 to 56 (Fig. 1), there were no treatment by day interactions (*P* > 0.16), nor any main effect of treatment (*P* > 0.31). Cortisol and NEFA were not affected (*P* > 0.14) by day, whereas urea tended to decrease (*P* = 0.06), and glucose, T3, and T4 decreased (*P* < 0.01) as calves aged.

There was no interaction of day by treatment, nor main effect of treatment on calf weight from birth to 56 days (*P* > 0.38), although, as expected calves gained (*P* < 0.01) weight from birth to day 56 of lactation (Fig. 2). By weaning, however, calves from SUP dams weighed more (*P* = 0.04) than those of the CON group (309.7 vs. 292.0 ± 6.0 kg).

DISCUSSION

In this study, there appears to be advantages of supplementation of DDGS to the dam on mammary gland function. In the current study, we tended to or were close to reaching significance in late gestational mammary gland blood flow, colostrum weight, as well as milk production. We did see weight advantages in calf birth and weaning weights that help convince us that mammary physiology may be augmented by late gestational maternal nutrition.

Blood flow through the pudendoepigastric trunk of the external pudendal artery has been demonstrated to be representative of blood flow to the mammary glands (Götze et al., 2010). However, little work has been done to characterize mammary blood flow in beef cattle or its potential influence on calf postnatal performance. Our

Table 5. Blood statistics in offspring of beef cows that were fed control or control plus supplementation from day 201 to 270 of gestation

Variable	CON ¹		SUP ¹		SEM ²	P-value		
	0 h	24 h	0 h	24 h		Diet	Time	Diet × time
pH	7.38	7.52	7.34	7.44	0.41	0.11	0.002	0.71
pCO ₂ , kPa	6.84	5.16	7.93	5.71	0.41	0.04	<0.001	0.48
pO ₂ , kPa	4.72	5.93	3.35	5.60	1.16	0.44	0.12	0.64
Hemoglobin, g/L	125.0	112.8	131.6	122.2	4.6	0.07	0.02	0.75
Base excess, mM	4.1	7.8	5.2	4.4	1.4	0.40	0.28	0.09
Plasma IgG, g/L	0.34	31.5	0.30	37.9	2.7	0.21	<0.001	0.21
Protein, g/L	41.5	58.5	42.2	64.5	1.8	0.05	<0.001	0.12
Plasma glucose, mM	3.27	6.04	2.87	6.84	0.33	0.55	<0.001	0.08
Blood lactate, mM	4.69	2.59	3.56	3.90	0.74	0.90	0.21	0.08
Plasma NEFA, μM	537	405	605	328	57	0.94	<0.001	0.21
Plasma urea, mM	6.88	8.03	7.71	7.88	0.58	0.56	0.26	0.40
Serum cortisol, nM	212	72	197	90	15	0.91	<0.001	0.26
Serum T3, nM	7.20	--	8.34	--	1.69	0.62	--	--
Serum T4, nM	138	--	138	--	15	0.96	--	--

¹Maternal diets; CON ($n = 15$), control group consuming basal diet; SUP ($n = 12$), supplemented group consuming basal diet + DDGS at 0.3% BW.

²SEM for $n = 12$.

laboratory has previously reported no difference in mammary blood flow in cows supplemented DDGS to restricted hay intake during late gestation (Mordhorst et al., 2017), but no analysis of colostrum or milk components was done at that time. In dairy cattle, mammary blood flow has been strongly correlated with milk yield (Götze et al., 2010). Additionally, gestational diet can alter systemic blood flow via changes in circulating hormones and growth factors during pregnancy that facilitate nutrient delivery and transport of vasoactive compounds to the still developing mammary gland (i.e., the udder parenchyma and surrounding vessels) in anticipation of colostrum and milk production (Svennersten-Sjaunja and Olsson, 2005). To explain why we had mammary gland advantages in blood flow in the current study compared to that of Mordhorst et al. (2017), we postulate that there is a link between blood flow to the mammary gland and uterine blood flow. In the present experiment, blood flow to the mammary gland on ipsilateral side was increased during late gestation. In the same animals as the current study, uterine blood flow was increased during late gestation (Kennedy et al., 2016b) when cows were supplemented with DDGS and allowed ad libitum forage access. When cows were provided DDGS, but limit fed low-quality hay, uterine blood flow was reduced, and no alterations in mammary blood flow were detected (Mordhorst et al., 2017). Perhaps there are factors produced by the conceptus influencing not only the gravid uterine horn, but also vasculature in the surrounding area, such as that would influence

mammary blood flow. Enhanced nutrient delivery via increased blood flow to the mammary gland in turn can certainly influence milk production (Sullivan et al., 2009, McSweeney et al., 1993) and subsequently postnatal performance of beef calves.

When ipsi- and contralateral blood flows were summed, the total mammary gland blood flow was just out of a statistical significance range. Alongside notable decreases in measurements of resistance and increases in heart rate, there clearly exists the potential for increased blood flow and perfusion in supplemented dams. Although we did not reach significance for colostrum weight in the current study, it should be noted that only one quarter of the total colostrum supply was sampled for weight in order to allow the calf to suckle naturally. Perhaps if the entire colostrum pool was sampled, SUP cows would have achieved greater colostral weight at birth.

The tendency for the increased milk production during early lactation is not explained by mammary gland hemodynamic measurements. It should be noted that hemodynamic measurements were obtained after the mammary gland had been emptied. Therefore, we hypothesize that mammary gland blood flow may differ prior to milking compared to postmilking, but unfortunately those hemodynamics were not measured in this study. When ewes were nutrient restricted during mid to late gestation, but fed adequately during early lactation, milk production was reduced (Meyer et al., 2011) and alveolar number was decreased (Neville et al., 2013) compared to ewes that were

Figure 1. Metabolite and hormone concentrations in offspring of beef cows that were fed control or control plus supplementation from day 201 to 270 of gestation.

adequately fed throughout gestation. Perhaps our gestational diets altered the cellularity of the bovine mammary gland.

Despite the birth weight advantage to calves from SUP cows, viability of the calves did not differ as measured by time to stand and suckle and consequently it is assumed that the ability for the calves to obtain colostrum did not differ. While SUP cows approached significance to produce more colostrum, calf sex did have an influence (there was no sex by treatment effect), with dams carrying female

Figure 2. Body weight of offspring of beef cows that were fed control or control plus supplementation from day 201 to 270 of gestation.

calves producing more than those carrying males. This phenomenon has been reported in dairy cows (Hinde et al., 2014; Joaquin et al., 2015). Joaquin et al. (2015) found that both total immunoglobulin concentration and colostrum volume were affected by the sex of the neonate. Hinde et al. (2014) found that dams with female offspring produced 5% more milk during lactation than dams with male offspring. Moreover, Gionbelli et al. (2017) reported that female bovine fetuses have a greater intestinal mass and villi length compared to their male counterparts, indicating the female fetus appears to have developed to receive these additional nutrients in the colostrum/milk. While an influence of calf sex on growth performance to weaning was not observed, the potential for daughter-biased colostrum and milk production clearly could impart advantages on early growth and survival of calves in a typical cow-calf operation.

There is little evidence of a direct link between gestational cow nutrition and passive immune transfer in calves (Perino, 1997). The tendency for protein supplemented dams to produce more colostrum has been reported in ewes (O'Doherty and Crosby, 1996). However, in the present study there were no differences between IgG concentrations in colostrum from SUP and CON dams, which agrees with experiments performed by others (Olson et al., 1981; Blecha et al., 1981; Hough et al., 1990). Olson et al. (1981) further reported no effect of protein or ME restriction on serum IgG concentration in dams. While Burton et al. (1984) did not see an influence on colostrum IgG concentration, they did observe reduced serum IgG concentration in calves from protein-restricted dams. In

the current study, CON cows were deficient in protein (Kennedy et al., 2016a), and gave birth to lighter calves. As no differences in calf IgG concentrations 24 h after birth were seen, perhaps calves from CON cows were either more efficient in their IgG uptake by transfer across the gut or they had greater ingestion of colostrum, which had similar IgG concentrations. When lambs are born from nutrient restricted ewes, they have greater IgG uptake within the first 24 h of life (Hammer et al., 2011).

We have too few observations to truly measure responses in calving difficulty but most indices of dystocia (i.e., length of second-stage labor, time to stand, time to first nursing, calving ease, and calf vigor) were not different, while blood parameters typically associated with dystocia and measures of respiratory and metabolic acidosis (i.e., $p\text{CO}_2$, lactate, and base excess) did differ. Szenci (1983) noted prolonged parturition can cause the development of respiratory or metabolic acidosis, and calves with prolonged calving are more likely to become clinically acidotic than calves born from shorter parturition. Fetal asphyxia is characterized by mixed respiratory-metabolic acidosis (Bleul and Götz, 2013). The respiratory component of acidosis is caused by an accumulation of $p\text{CO}_2$ in fetal blood due to diminished removal by the placenta (Szenci, 1983). Metabolic acidosis in neonatal calves is primarily caused by lactate, but $p\text{CO}_2$ does contribute via the formation of carbonic acid (Bleul and Götz, 2013). Moreover, plasma lactate is greater in calves delivered after calving assistance compared to unassisted calves (Diesch et al., 2004; Sorge et al., 2009; Bleul and Götz, 2013). Increased incidences of dystocia also increases serum cortisol and glucose in both cows and calves, suggesting that the stress of parturition affects metabolic changes peripartum (Civelek et al., 2008).

The elevated $p\text{CO}_2$ concentration observed at 0 h, and the lack of clearing of lactate by 24 h in calves born to SUP dams may infer that the larger calf weight, albeit not enough to classify or change dystocia measures, affected metabolism of the calf. In addition, plasma glucose in SUP calves was greater at 24 h and blood lactate was maintained from 0 to 24 h, despite no differences in cortisol. Perhaps these patterns can be explained by an enhancement of the Cori cycle in the SUP calves or greater ingestion of glucose or other metabolites in the first 24 h of life. Numerous studies in sheep have demonstrated effects of IUGR on the Cori cycle and the resulting downstream metabolic shifts in offspring (Yates et al., 2012); it is possible that SUP calves' metabolism could have been altered in a similar fashion.

Although calves from SUP cows were born heavier than those from CON cows, their growth performance was similar through the first 2 mo of life. Mirroring this were similar metabolite and metabolic hormone profiles. Between 56 and 200 d, there must have been a stimulus for growth of which we are unaware. First, the tendency observed at day 44 for greater milk production could be a key player. It is possible that SUP dams simply experienced greater milk production until weaning. Additionally, the indicators of acidosis observed in the neonates could have set back the calves of SUP dams during the first months of life. Finally, there may have been alterations in colostral or milk components (that were not measured) that were ingested early which may have later influenced calf metabolism and growth. Transmission of milk-borne bioactive factors has been demonstrated to have influences on postnatal development in mouse and livestock models (Bartol et al., 2013); perhaps an aspect of lactocrine signaling may have played a role in our calves' efficiency or growth performance. Regardless of what the signal(s) may have been, the resultant increased weaning weight of calves from SUP cows allow for an economic advantage to the producer.

In conclusion, DDGS supplementation's influence on mammary blood flow, colostrum, and milk production offers potential benefits in terms of both nutrient and passive immunity delivery to the calf, with clear effects from late gestation to weaning. Health and growth strongly predispose the offspring for success, both of which can be greatly influenced by advantages in colostrum and milk production in dams or increased efficiency of the offspring. These findings warrant further investigation as they imply the leverage that the maternal nutrition during late gestation has on calf performance from uterine to postnatal life. Further investigations on how gestational diet affects calf performance due to greater nutrient and/or immunity delivery, be it placental or mammary, are decidedly of merit.

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