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Effects of supplement type on performance, reproductive, and physiological responses of Brahman-crossbred females¹

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ABSTRACT: Two experiments were conducted to compare the performance and physiological responses of forage-fed beef females supplemented with either a molasses-based (ML) or a citrus pulp-based (CT) supplement. In Exp. 1, BW gain, reproductive performance, and concentrations of blood urea N (BUN), plasma glucose, insulin, IGF-I, and progesterone (P4) were assessed in 60 Brahman × Angus heifers supplemented 3 times weekly with either ML or CT. Supplement intakes were formulated to be isocaloric and isonitrogenous. Reproductive performance was not affected by treatments, but mean BW gain was greater ($P < 0.01$) for heifers fed CT than for those fed ML (0.40 vs. 0.30 kg/d). Mean plasma concentrations of glucose, insulin, and IGF-I were greater ($P < 0.05$) for heifers fed CT, whereas BUN was greater ($P < 0.05$) for heifers fed ML. Mean plasma P4 concentration did not differ between treatments, but both groups had lower plasma P4 concentrations during days that supplements were offered

($P < 0.01$). In Exp. 2, forage DMI and concentrations of BUN, plasma glucose, insulin, IGF-I, and P4 were assessed in 24 Brahman × British mature cows supplemented with the same treatments described in Exp. 1. Overall forage DMI did not differ between treatments, but a day effect and a treatment × day interaction were detected ($P < 0.05$). Both groups consumed less forage during the days on which the supplements were offered ($P < 0.01$), and forage DMI for cows fed CT was less ($P < 0.05$) than for cows fed ML during those days. No differences were detected in any blood or plasma measurement. In addition, no differences in concentrations of P4 were detected between CT- and ML-fed cows. We concluded that CT-supplemented heifers had greater BW gain compared with ML-supplemented heifers, but no differences in reproductive performance were observed. We also observed that CT-supplemented cows had a greater variability in forage DMI compared with ML-supplemented cows.

Key words: heifer growth, pregnancy, supplementation

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INTRODUCTION

Energy supplementation is often beneficial, if not essential, for grazing cow-calf operations, particularly those based on subtropical and tropical forages, because energy is the primary nutritional consideration for optimum reproductive performance of beef females (Mass, 1987; Moore et al., 1991). With greater energy intake, heifers attain puberty earlier (Schillo et al., 1992), whereas cows have shortened postpartum anestrus

(Roberts et al., 1997). Conversely, inadequate energy intake is associated with delayed onset of puberty, extended postpartum intervals, and decreased conception and pregnancy rates (Santos and Amstalden, 1998).

Liquid and dry feedstuffs are options for supplementation, with molasses and citrus pulp as respective examples. Citrus pulp and molasses differ in their carbohydrate profile (NRC, 2001), which may affect forage intake, diet digestibility, energy utilization, and consequent animal performance. Sucrose is the main carbohydrate of molasses (Pate, 1983), whereas pectin is the main carbohydrate of citrus pulp (Arthington et al., 2002). In addition, previous research by our group (Arthington et al., 2004) reported that beef heifers supplemented with dry feed consumed the entire amount of supplement faster than heifers supplemented with an equivalent amount of CP and TDN from a liquid supplement. Although no differences in BW gain were de-

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tected, heifers fed the liquid supplement had greater pregnancy rates. Supported by studies indicating that consumption of large amounts of feed in a short period is associated negatively with blood progesterone (P4) concentrations (Sangsritavong et al., 2002; Vasconcelos et al., 2003), we hypothesized that heifers supplemented with dry feed would have decreased circulating P4 and consequently impaired pregnancy rates due to increased rate of feed intake.

The objective of the present experiments was to investigate the effects of feeding behavior associated with supplement type on performance, plasma metabolites and hormones associated with energy intake, and reproductive efficiency of Brahman-crossbred females.

MATERIALS AND METHODS

The animals utilized in these experiments were cared for in accordance with acceptable practices as outlined in the Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching (FASS, 1999).

Two experiments were conducted at the University of Florida, Institute of Food and Agricultural Sciences, Range Cattle Research and Education Center, Ona. The first experiment was conducted from September to December 2004 and was divided into a sampling phase (September and October) and a breeding phase (November and December). The second experiment was conducted during the months of October and November 2004.

Animals

Exp. 1. Sixty Brahman \times Angus crossbred heifers (BW \pm SD = 246 \pm 23 kg; age \pm SD = 312 \pm 20 d) were utilized in this experiment. For the sampling phase (d 0 to 45), heifers were stratified by initial BW and age and randomly allocated to 12 pens (5 heifers/pen). Pens were randomly assigned to 1 of 2 treatments: 1) molasses-based supplement (ML) or 2) citrus pulp-based supplement (CT). Pen was considered the experimental unit (6 pens/treatment), and each pen consisted of 1.3 ha of bahiagrass (*Paspalum notatum*). For the breeding phase (d 46 to 107), heifers were grouped by treatment into 2 bahiagrass pastures and exposed to Angus bulls for 60 d. For both sampling and breeding phases, all heifers remained in their initial assigned pasture throughout the period.

Exp. 2. Twenty-four nonlactating and nonpregnant multiparous Brahman \times British crossbred cows (BW \pm SD = 502 \pm 55 kg) were stratified by BW and randomly allocated to 12 feedlot pens (2 cows/pen). Pens were assigned randomly to 1 of the 2 treatments: 1) molasses-based supplement (ML) or 2) citrus pulp-based supplement (CT). Pen was considered the experimental unit (6 pens/treatment). Before the beginning of the experiment, with the purpose of acquiring animals with similar and substantial plasma P4 concentrations, 6 ani-

mals from each treatment, corresponding to 3 pens/treatment, received a 100- μ g injection of GnRH (Cystorelin, Merial Ltd., Duluth, GA) on d -9 followed 7 d later by treatment with PGF2 α (25 mg; Lutalyse, Pfizer Animal Health, New York, NY) and insertion of 2 controlled internal device release inserts (CIDR) containing 1.38 g of P4 (d -2; Pfizer Animal Health), which remained in the cows throughout the experimental period (d 1 to 37).

Diets

Exp. 1. Pasture quality during the sampling phase was estimated to be 56% TDN and 7.4% CP (DM basis) from samples collected at the beginning of the trial and analyzed by a commercial laboratory (Dairy One Forage Laboratory, Ithaca, NY). The pastures utilized in this experiment were not fertilized before or during the experimental period. A complete, commercial mineral-vitamin mix (14% Ca, 9% P, 24% NaCl, 0.20% K, 0.30% Mg, 0.20% S, 0.005% Co, 0.15% Cu, 0.02% I, 0.05% Mn, 0.004% Se, 0.3% Zn, 0.08% F, and 82 IU/g of vitamin A) and water were offered for ad libitum consumption throughout the experiment. Stargrass (*Cynodon nlemfuensis*) hay was offered in amounts to ensure ad libitum access when pasture availability was limited. Hay quality was estimated to be 52% TDN and 6.5% CP (DM basis) from samples also collected at the beginning of the trial and analyzed by commercial laboratory (Dairy One Forage Laboratory). Treatments consisted of 2 energy supplements (Table 1), fed 3 times weekly (Mondays, Wednesdays, and Fridays at 0700) at a rate of 2.1 and 2.3 kg of DM per heifer daily for ML and CT, respectively. Supplement intakes were formulated to be isocaloric and isonitrogenous (Table 1) and also balanced for Ca concentration, given the high concentration of Ca in citrus pulp.

Exp. 2. Limpograss (*Hemarthria altissima*) hay was offered for ad libitum consumption throughout the experiment, and hay quality was estimated to be 54% TDN and 9.1% CP (DM basis) from samples analyzed by commercial laboratory (Dairy One Forage Laboratory). Cows had free access to a complete mineral mix (similar to Exp. 1) and water. Treatments consisted of 2 energy supplements (Table 1), fed 3 times weekly (Mondays, Wednesdays, and Fridays at 0800) at a rate of 4.1 and 4.5 kg of DM per cow daily for ML and CT, respectively. Supplement intakes were formulated to be isocaloric and isonitrogenous (Table 1) and balanced for Ca concentration, given the high concentration of Ca in citrus pulp.

Sampling

Exp. 1. One week before the beginning (d -7 and d -6) and at the end (d 107 and d 108) of the experiment, heifers were weighed on 2 consecutive days to determine both full and shrunk (after 16 h of feed and water restriction) BW. Heifers also were weighed every other

Table 1. Ingredient composition, nutrient profile, and intake rate of supplements fed to heifers during Exp. 1 and to cows during Exp. 2

Item	Exp. 1		Exp. 2	
	CT ¹	ML ²	CT	ML
Ingredients, % as-fed				
Blackstrap sugarcane molasses	0.0	78.3	0.0	79.1
Citrus pulp	74.7	0.0	75.3	0.0
Cottonseed meal	25.3	20.2	24.7	19.8
Calcium carbonate	0.0	1.5	0.0	1.1
Nutrient profile, % of DM				
DM	87.0	76.0	86.5	75.4
TDN ³	70.0	77.0	70.0	78.0
CP ³	19.0	20.7	18.7	20.5
Rumen degradable protein ⁴	14.3	16.5	13.7	15.9
Rumen undegradable protein ⁴	4.7	4.2	5.0	4.6
NPN ⁴	1.2	7.7	1.2	7.8
Ether extract ⁴	2.7	0.5	2.7	0.5
Ca ³	1.7	1.8	1.5	1.4
P ³	0.6	0.5	0.4	0.4
Daily supplement intake, ⁵ kg/heifer				
DM	2.30	2.10	4.50	4.10
TDN	1.61	1.61	3.15	3.19
CP	0.43	0.43	0.84	0.84
Ether extract	0.06	0.01	0.12	0.02
Ca	0.04	0.04	0.06	0.06
P	0.01	0.01	0.02	0.02

¹CT = citrus pulp-based supplement fed 3 times weekly (Mondays, Wednesdays, and Fridays).

²ML = molasses-based supplement fed 3 times weekly (Mondays, Wednesdays, and Fridays).

³Values obtained from a commercial laboratory analysis (Dairy One Forage Laboratory, Ithaca, NY). The TDN was calculated as described by Weiss et al. (1992).

⁴Values estimated using the NRC model (2001).

⁵Estimated from the supplement consumption of the pen.

week to monitor ADG, but overall ADG was calculated using initial and final shrunk BW values. Blood samples were collected weekly (on Wednesdays) throughout the experiment to determine onset of puberty using plasma P4 concentrations. A heifer was considered pubertal if plasma P4 concentration was greater than 1.5 ng/mL for 2 consecutive weeks. Although several publications (Lalman et al., 1993; Yelich et al., 1996; Imwalle et al., 1998) use 1.0 ng/mL of blood P4 as the puberty criteria for *Bos taurus* heifers, prepubertal heifers with high Brahman influence may have P4 concentrations above this level proceeding from alternate sources such as the adrenal gland, which may be a confounding factor for determining the establishment of puberty. Cattle with Brahman breeding influence have increased behavioral stress compared with *B. taurus* breeds (Hearnshaw and Morris, 1984; Fordyce et al., 1988; Voisin et al., 1997), and stressor elements are known to increase the production of P4 by the adrenal gland (Roman-Ponce et al., 1981; Hollenstein et al., 2005).

During the sampling phase, in addition to the weekly collections, blood samples were obtained once per day during 4 consecutive days, every other week, beginning at 4 h after the supplements were offered, to determine

concentrations of glucose, blood urea N (BUN), insulin, IGF-I, and P4. These samples were collected from d 0 to 3, d 14 to 17, d 28 to 31, and d 42 to 45, which were classified as periods (PR) 1, PR2, PR3, and PR4, respectively. Periods always began on Mondays and ended on Thursdays.

During the breeding phase (d 46 to 107), heifers were exposed to mature Angus bulls. Each group was exposed to 2 bulls at the same time (1:15, bull-to-heifer ratio), and bulls were rotated weekly between groups to account for potential effects of bull. Heifer pregnancy status was verified by the presence of a fetus using transrectal ultrasonography (5.0 MHz transducer, Aloka 500V, Wallingford, CT) 70 d after the end of the experiment.

Random samples of the feedstuffs were collected before the beginning of the trial and analyzed for nutrient composition at commercial laboratories (Dairy One Forage Laboratory for cottonseed meal and citrus pulp samples and SDK Laboratories, Hutchinson, KS, for molasses samples).

Exp. 2. During the first 3 wk of the experiment (d 1 to 21), blood samples were collected immediately before and 4, 8, 24, 32, and 48 h after the first supplement feeding of the week (d 1, 8, and 15) for determination of glucose, BUN, insulin, IGF-I, and P4 concentrations. Weeks were classified as follows: wk 1 = PR1; wk 2 = PR2; and wk 3 = PR3.

During the second part of the experiment (d 22 to 37), daily forage DMI was recorded. Hay was offered for ad libitum consumption, and forage refusal was collected and weighed daily. A sample of the offered hay was collected twice during this period (d 23 and 30) to determine DM content and nutrient composition (Dairy One Forage Laboratory), whereas samples of refusal were collected daily from each pen to determine only DM content. Hay samples were dried for 96 h at 50°C in forced-air ovens. Random samples of the feedstuffs also were collected before the beginning of the trial and analyzed for nutrient composition at commercial laboratories (Dairy One Forage Laboratory for cottonseed meal and citrus pulp samples and SDK Laboratories for molasses samples).

Blood Analysis

Blood samples were collected via jugular venipuncture during Exp. 1 and from the coccygeal vein or artery during Exp. 2 into commercial blood collection tubes (Vacutainer, 10 mL; Becton Dickinson, Franklin Lakes, NJ) containing sodium heparin, placed on ice immediately, and centrifuged at 855 × g for 30 min for plasma collection. Plasma was frozen at -20°C on the same day of collection.

A Technicon AutoAnalyzer (Technicon Instruments Corp., Chauncey, NY) was used to determine glucose (Coulombe and Favreau, 1963; modified and described by Bran + Luebbe Industrial Method number 339-01) and BUN (Gochman and Schmitz, 1972; modified and

described by Bran + Luebbe Industrial Method number 339-19) concentrations. A double antibody RIA was used to determine concentrations of insulin (Malven et al., 1987; Badinga et al., 1991) and IGF-I (Badinga et al., 1991). The extraction procedure used in the IGF-I assay was modified from Badinga et al. (1991) by using an ethanol:acetone:acetate ratio of 6:3:1. Concentrations of P4 were determined using a Coat-A-Count Kit (DPC Diagnostic Products Inc., Los Angeles, CA) solid phase ^{125}I RIA. The intra- and interassay CV for Exp. 1 were, respectively, 9.7 and 10.0% for insulin, 8.1 and 5.9% for IGF-I, and 4.5 and 8.1% for P4. The intra- and interassay CV for Exp. 2 were, respectively, 12.9 and 15.9% for insulin, 9.9 and 11.0% for IGF-I, and 2.4 and 11.2% for P4. For both experiments, the minimum detectable concentrations of insulin, IGF-1, and P4 were 0.02, 10, and 0.1 ng/mL, respectively.

Statistical Analysis

Exp. 1. Performance and physiological data were analyzed using the MIXED procedure (SAS Inst. Inc., Cary, NC). The model statement used for hormone and metabolite analysis contained the effects of treatment, period, day(period), and the interactions of treatment \times period and treatment \times day(period). Data were analyzed using animal(pen) and pen(treatment) as random variables. The model statement used for ADG, pregnancy rates, puberty rate, and age at puberty analysis contained only the effect of treatment. Data were analyzed using pen(treatment) as a random variable. Results are reported as least squares means. Means were separated using LSD. Survival analysis (PROC LIFETEST; Wilcoxon test) and logistic regression (PROC LOGISTIC) from SAS were used to further analyze puberty and pregnancy rates. In addition, Pearson correlation coefficients among hormones, metabolites, and ADG were generated using the CORR procedure of SAS. Significance was set at $P \leq 0.05$, and tendencies were determined if $P > 0.05$ and ≤ 0.10 . Only significant interactions are reported.

Exp. 2. Data were analyzed using PROC MIXED of SAS. The model used for analysis of forage DMI contained the effects of treatment and day and the interaction of treatment \times day. The random variable was pen(treatment). The model statement used for hormone and metabolite analysis contained the effects of treatment, period, time(period), and the interactions of treatment \times period and treatment \times time(period). The random variables were animal(pen) and pen (treatment). Only data from cows inserted with CIDR were utilized to compare P4 concentrations between treatments. Results are reported as least squares means. Means were separated using LSD. Significance was set at $P \leq 0.05$, and tendencies were determined if $P > 0.05$ and ≤ 0.10 . Only significant interactions are reported.

RESULTS AND DISCUSSION

Exp. 1

Heifers fed ML required approximately 48 h to completely consume the supplement, whereas heifers fed

Table 2. Average daily gain and reproductive performance of heifers offered citrus pulp (CT)- or molasses (ML)-based supplements during Exp. 1

Item	CT	ML	SEM	P-value
ADG, ¹ kg/d	0.40	0.30	0.025	<0.01
Pregnancy rate, ² %	60	58	8.1	0.83
Puberty rate, ^{3,4} %	80	77	9.9	0.81
Age at puberty, d	379	369	6.0	0.22

¹Calculated using initial (d -6) and final (d 108) shrunk BW.

²Pregnant heifers/total heifers, expressed as a percentage.

³Estrous cycling heifers/total heifers, expressed as a percentage.

⁴Puberty = progesterone concentration greater than 1.5 ng/mL for 2 consecutive weeks. Achievement of puberty was declared at the first week.

CT consumed the whole amount of supplement in approximately 30 h after supplements were offered. In addition, the majority of supplement was consumed during feeding days for both treatments. This intake behavior was unexpected, because previous research by our group (Arthington et al., 2004) reported that heifers reared in similar management and environment conditions consumed entire amounts of dry supplement within 2 to 3 h after feeding, whereas liquid supplement was entirely consumed in approximately 48 h. However, in this previous study, the dry ingredients were compacted and offered in a range cube form, whereas in the current study, the dry supplement consisted in a poorly pelletized citrus pulp mixed with cottonseed meal. Consequently, the physical density of the dry supplement offered in the current study may have accounted for this unexpected intake behavior. We concluded that intake behavior did not differ between treatments as expected, and the results obtained for heifer performance and reproductive efficiency in this experiment should be attributed primarily to the nutritional differences between CT and ML.

Period and day(period) effects were detected ($P < 0.01$) for all metabolites and hormones. The day(period) effect is likely explained by the daily variability in supplement consumption, considerably affected by the environment and management activities, although supplement feeding and blood samplings always began at the same time throughout the experiment. The period effect may be attributed to the adaptation of the animals to the experimental procedures such as feeding and handling management, because it was observed that both energy and protein status of the animals, suggested by concentrations of hormones and metabolites, improved with the advance of the experiment without any modification in the treatments.

Heifers fed CT had greater ADG compared with heifers fed ML (0.40 vs. 0.30 kg/d, respectively; $P < 0.01$; Table 2), concurring with previous data reporting that animals fed molasses-based supplements usually have inferior BW gain compared with animals fed supplements based on other energy sources, such as corn or soybean hulls (Pate, 1983; Royes et al., 2001). However,

Table 3. Blood urea N (BUN) and plasma glucose concentrations (mg/dL) of heifers offered citrus pulp (CT)- or molasses (ML)-based supplements during Exp. 1¹

Item	Period 1			Period 2			Period 3				Period 4				Pooled SEM	
	Tu	Wed ²	Th	Mo ²	Tu	Wed ²	Th	Mo ²	Tu	Wed ²	Th	Mo ²	Tu	Wed ²		Th
BUN																
CT	1.2	2.7	1.6	0.5	2.5	1.7	2.9	4.7	8.3	5.1	6.4	4.1	6.9	5.7	10.4	0.24
ML	2.7	3.7	4.7	2.7	2.7	2.7	4.3	6.7	6.9	6.5	7.1	7.1	6.5	6.4	7.9	0.24
<i>P</i> -value ³	<0.01	0.07	<0.01	<0.01	0.77	0.08	0.01	<0.01	0.01	0.01	0.18	<0.01	0.46	0.22	<0.01	
Glucose																
CT	91.2	81.1	83.6	80.3	85.4	83.6	87.7	77.4	84.4	88.8	81.8	76.6	88.9	73.6	82.8	2.5
ML	80.6	76.9	81.3	72.9	77.6	77.1	78.1	68.6	73.5	70.5	71.2	69.4	74.7	71.9	70.6	2.5
<i>P</i> -value ³	0.01	0.31	0.58	0.07	0.06	0.12	0.02	0.03	0.01	<0.01	0.01	0.08	<0.01	0.69	<0.01	

¹Mo = Monday; Tue = Tuesday; Wed = Wednesday; and Th = Thursday. Blood samples were not collected on Mo during the first period.

²Days at which supplements were offered. Supplements were offered at 0700.

³Treatment comparison within period and day.

the superiority in performance of CT-fed heifers was not reflected in reproductive efficiency. There were no treatment effects on pregnancy rate, puberty rate, and age at puberty (Table 2).

Heifers fed ML had greater mean BUN concentrations ($P < 0.05$) compared with heifers fed CT (5.17 vs. 4.17 mg/dL, respectively; Table 3). However, this effect was mainly observed during the first 2 periods of the experiment (treatment \times period interaction; $P < 0.01$). During periods 3 and 4, treatment differences in BUN concentration were not significant ($P = 0.07$ and $P = 0.60$, respectively). A treatment \times day(period) interaction also was detected for BUN ($P < 0.01$). Heifers fed ML had greater ($P < 0.01$ or < 0.05 ; Table 3) concentrations of BUN compared with CT-fed heifers on Tuesday and Thursday during PR1, Monday and Thursday during PR2, Monday and Wednesday during PR3, and Monday during PR4, whereas CT-fed heifers had greater BUN concentrations on Tuesday during PR3 and Thursday during PR4. In addition, ML-fed heifers tended ($P < 0.10$) to have greater BUN concentrations on Wednesday during PR1 and PR2 (Table 3). Compared with CT, ML had a greater rumen degradable protein content (Table 1), which is likely due to the greater NPN content of blackstrap molasses compared with citrus pulp (100 and 26% of CP, respectively; NRC, 1996). Therefore, the protein fraction of ML possibly had greater ruminal degradation rate compared with the protein fraction of CT, which may have led to greater ruminal ammonia production and consequently greater BUN concentrations of ML-fed heifers during most part of the sampling period (Hammond, 1997). Concentrations of BUN should range from 11 to 15 mg/dL for growing animals to ensure maximum rates of gain (Byers and Moxon, 1980). In the current study, both supplement treatments appeared to be deficient in protein according to the BUN concentrations observed, because they were consistently below the optimum (Table 3). However, using the NRC (1996) model, supplements were formulated to supply the protein requirements of developing heifers gaining BW similar to that observed in this experiment. In addition, when diets

from both treatments (supplement intake + estimated forage intake) were analyzed with the NRC software (NRC, 1996), protein requirements were observed to be satisfied when actual ADG was used in the model. Therefore, BUN might not be the most accurate indicator of protein status in our experiment.

Mean glucose (83.3 vs. 74.7 mg/dL for CT and ML, respectively; Table 3), insulin (0.89 vs. 0.75 ng/mL for CT and ML, respectively; Figure 1), and IGF-I (121.5 vs. 108.9 ng/mL for CT and ML, respectively) concentrations were greater ($P < 0.05$) for heifers fed CT compared with heifers fed ML. The differences between the treatments increased with the advance of the experiment for glucose and insulin concentrations; therefore, treatment \times period interactions were detected ($P < 0.01$). A treatment \times day(period) interaction was observed only for glucose ($P < 0.01$). Although CT-fed heifers always had numerically greater glucose concentrations during the sampling period, statistical differences ($P < 0.01$ or < 0.05 ; Table 3) were only observed on Tuesday during PR1, Thursday during PR2, throughout PR3, and on Tuesday and Thursday during PR4. Statistical tendencies ($P < 0.10$) were observed on Monday and Tuesday during PR2 and Monday during PR4. This interaction may be attributed to the differences in supplement intake behavior and ruminal degradability, factors that influence energy availability and absorption (NRC, 2001). The VFA synthesized in the rumen account for approximately 70% of the caloric requirements of ruminants (Bergman, 1990). According to Royes et al. (2001), cattle supplemented with a high-fiber supplement have greater total ruminal VFA concentrations compared with cattle supplemented with molasses. In addition, several researchers have reported that feeding diets with high molasses or sucrose inclusion rates resulted in increased butyric acid concentration in the rumen of cattle (Owen et al., 1967; Kellogg and Owen, 1969; Hatch and Beeson, 1972), whereas diets rich in citrus pulp, and consequently pectin, produced a greater proportion of acetic acid (Hentges et al., 1966; Drude et al., 1971; Schaibly and Wing, 1974). Both butyric acid and acetic acid are VFA that are oxidized for energy

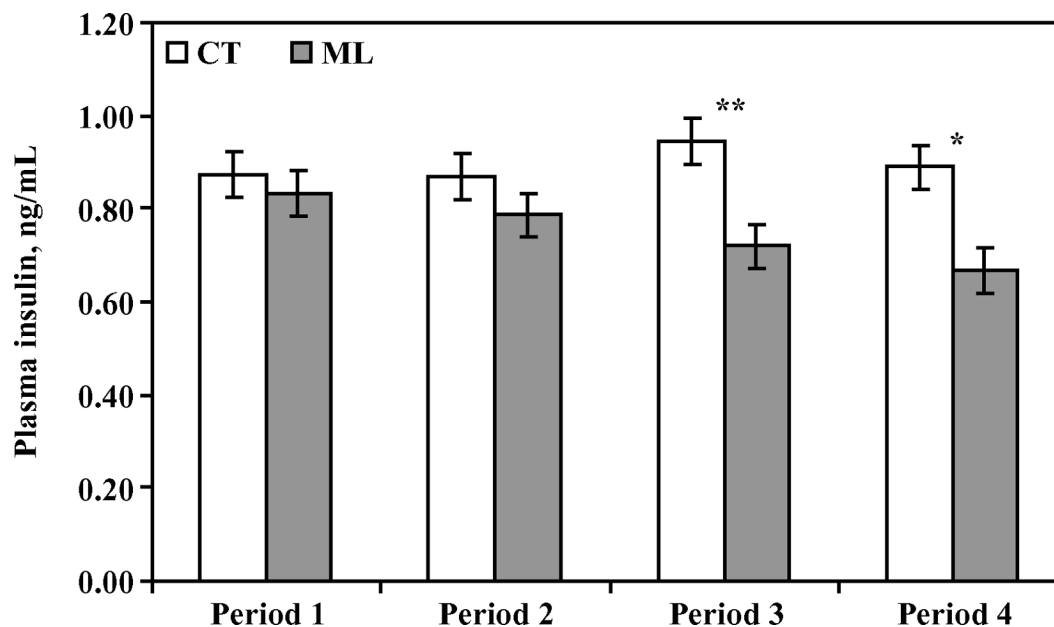


Figure 1. Insulin concentrations, pooled within period, of heifers offered citrus pulp (CT)- or molasses (ML)-based supplements during Exp. 1. Heifers fed CT had greater insulin concentrations ($P < 0.05$, SEM = 0.044). A treatment \times period interaction was detected ($P < 0.01$). * $P < 0.01$; ** $P < 0.001$.

production (Bergman, 1990). The epithelial tissue of the rumen retains approximately 90% of the butyrate produced and converts it to ketone bodies and carbon dioxide, whereas only 30% of acetate is metabolized by ruminal epithelium, and the remaining is oxidized throughout most of the body tissues to generate ATP (Bergman, 1990). In addition, butyrate may have a detrimental effect on VFA metabolism by restraining hepatic propionate utilization, thus partially inhibiting gluconeogenesis and restraining glucose synthesis, availability, and absorption (Aiello et al., 1989). Therefore, perhaps ML-fed heifers had less total VFA synthesis in the rumen along with a greater ruminal butyrate:acetate ratio compared with CT-fed heifers. As a result, CT-fed heifers had a superior energy status and improved gluconeogenesis, which resulted in increased concentrations of glucose, insulin, IGF-I, and consequent greater ADG compared with ML-fed heifers.

The positive relationship observed in this experiment among glucose, insulin, and IGF-I concentrations with ADG is supported also by others (Vizcarra et al., 1998; Lapierre et al., 2000; Hersom et al., 2004). Correlation coefficients among glucose, insulin, IGF-I (all pooled across days and periods), and ADG were determined among all heifers ($n = 58$) using Pearson correlation coefficients (Table 4). Significant positive correlations were observed for IGF-I with ADG ($P < 0.01$) and for IGF-I with insulin ($P < 0.05$).

To determine if P4 concentrations would be influenced by treatments and consequent intake behavior, only samples from heifers declared pubertal during the sampling phase were used. Because the heifers were at random stages of the estrous cycle, and concentra-

tions of P4 varied significantly across heifers, P4 concentrations were analyzed on a percentage basis, comparing the values from days that supplements were fed to values from days that supplements were not fed (set as 100%). Both treatments had lesser P4 concentration during feeding days (average decrease = 11.0%; $P < 0.01$), but no differences were observed between treatments (89.5 and 90.3% for CT and ML, respectively; $P = 0.66$; data not shown). Elevated or infrequent feed intake may decrease circulating concentrations of P4 and therefore impair reproductive performance, given that P4 is essential for the onset of puberty (Gonzalez-Padilla et al., 1975) and for the recognition and maintenance of early pregnancy (Spencer and Bazer, 2002). Vasconcelos et al. (2003) fed pregnant lactating Holstein cows either 100% of the daily diet at once, half of the diet every 12 h, a quarter of diet every 6 h, or left animals unfed. The authors reported that the first 2 treatments decreased circulating concentrations of P4 by 1 h after feeding, and concentrations remained de-

Table 4. Correlations between plasma measurements and ADG of heifers offered citrus pulp (CT)- or molasses (ML)-based supplements during Exp. 1¹

Item	ADG	Glucose	Insulin
Glucose	-0.065		
Insulin	0.62	-0.140	
IGF-I	0.137	0.29	0.329
	0.414	0.003	
	<0.01	0.98	<0.05

¹Upper row = correlation coefficients; lower row = P -values.

pressed until 8 to 9 h after feeding. An inverse relationship between feed intake and blood P4 concentrations also was reported by others (Rabiee et al., 2001; Sangsritavong et al., 2002; Pescara et al., 2005). In the current experiment, the differences observed in feed intake behavior (approximately 30 vs. 48 h for total supplement consumption for CT- and ML-fed heifers, respectively) were not as substantial as expected, and this might explain why no differences between treatments were found in P4 concentrations or in reproductive performance.

Nutritional status, and consequently BW gain and composition, is typically considered the primary determinant of puberty attainment, because it is highly correlated with frequency and amplitude of LH pulses, which stimulate development of ovarian follicles to pre-ovulatory stages (Schillo et al., 1992). Furthermore, blood metabolites and hormones such as glucose, insulin, and IGF-I have been associated with reproductive performance. These substances appear to be the connection between nutritional status and the increase in LH secretion and activity, by influencing hypothalamic-hypophyseal secretory activity (Butler and Smith, 1989; Schillo et al., 1992) and also amplifying the effects of LH in ovarian follicular cells (Spicer and Echterkamp, 1995). Concentrations of LH were not assessed in this experiment because of its pulsatile release pattern (Schillo et al., 1992), which requires frequent blood samplings to obtain accurate measurements of basal concentrations, pulse amplitude, and pulse frequency of this hormone. However, in the current experiment, neither BW gain nor the glucose-insulin-IGF-I axis was associated with reproductive performance within treatments. Heifers fed CT had greater ($P < 0.05$) ADG and blood concentrations of these metabolites and hormones but similar reproductive performance compared with heifers fed ML. When variables were pooled across treatments, and heifers were divided by puberty attainment and pregnancy status, IGF-I was the only measurement significantly associated with reproductive performance (Table 5). Concentrations of IGF-I are correlated positively with insulin concentrations (Table 4; Keisler and Lucy, 1996; Webb et al., 2004). Therefore, to increase blood IGF-I concentrations to enhance BW gain and reproductive performance, glucose synthesis should be maximized. Feeding level is associated positively with blood glucose concentration (Vizcarra et al., 1998; Lapierre et al., 2000; Hersom et al., 2004). In addition, because propionate accounts for up to 76% of the glucose synthesized in the liver (Reynolds et al., 1994), supplements containing ingredients that maximize the amount of propionate synthesized in the rumen should be fed to growing cattle.

Exp. 2

The same supplement intake behavior from Exp. 1 was observed in Exp. 2. Cows fed ML required approximately 48 h to consume the entire amount of supple-

Table 5. Relationships of ADG and plasma concentration of metabolites and hormones to reproductive performance of heifers offered citrus pulp (CT)- or molasses (ML)-based supplements during Exp. 1

Item	Attainment of puberty ¹		SEM	P-value
	Pubertal (n = 45)	Nonpubertal (n = 13)		
ADG, kg/d	0.35	0.33	0.061	0.60
Glucose, mg/dL	78.1	79.1	3.0	0.77
Insulin, ng/mL	0.83	0.80	0.052	0.64
IGF-I, ng/mL	120.1	99.1	4.2	<0.01
Item	Pregnancy status ²		SEM	P-value
	Pregnant (n = 33)	Nonpregnant (n = 12)		
ADG, kg/d	0.37	0.31	0.033	0.21
Glucose, mg/dL	79.7	75.0	2.5	0.12
Insulin, ng/mL	0.83	0.83	0.059	0.98
IGF-I, ng/mL	123.4	111.4	4.4	0.05

¹Puberty = progesterone concentration greater than 1.5 ng/mL for 2 consecutive weeks. Puberty achievement was declared at the first week.

²Pubertal heifers only.

ment, whereas cows fed CT required approximately 30 h. The majority of supplement was consumed during feeding days for both treatments.

Average daily DMI of forage did not differ between treatments (1.16 vs. 1.20% of BW for CT and ML groups, respectively; $P = 0.52$; Figure 2). However, a day effect and also a treatment \times day interaction were detected ($P < 0.01$). Forage DMI was less for both groups on feeding vs. nonfeeding days (0.65 and 1.55% of BW for cows fed CT vs. 0.96 and 1.38% of BW for cows fed ML during feeding and nonfeeding days, respectively; $P < 0.01$; Figure 2). This effect can be attributed to the increased consumption of supplements during feeding days, because forage DMI is associated negatively with energy supplement intake (Caton and Dhuyvetter, 1997; Kunkle et al., 1999; Bodine and Purvis, 2003). Furthermore, because CT-fed cows consumed their entire amount of supplement faster and consequently had greater supplement consumption during feeding days compared with cows fed ML, their depression in forage DMI was greater ($P < 0.05$; Figure 2) on these days.

A treatment \times time(period) interaction was detected ($P < 0.01$; Table 6) for BUN concentrations. Cows fed CT had greater ($P < 0.01$) BUN concentrations 32 h after feeding during PR2, before and 48 h after supplement feeding during PR3, and tended to have ($P < 0.10$) greater BUN concentrations 24 and 48 h after supplement feeding during the second period (Table 6). Although CT-fed cows had greater supplement consumption during feeding days and consequently during the first hours after feeding, CT had lower NPN and rumen degradable protein content compared with ML (Table 1), which may have delayed the increase in BUN associated with the ammonia production from ruminal pro-

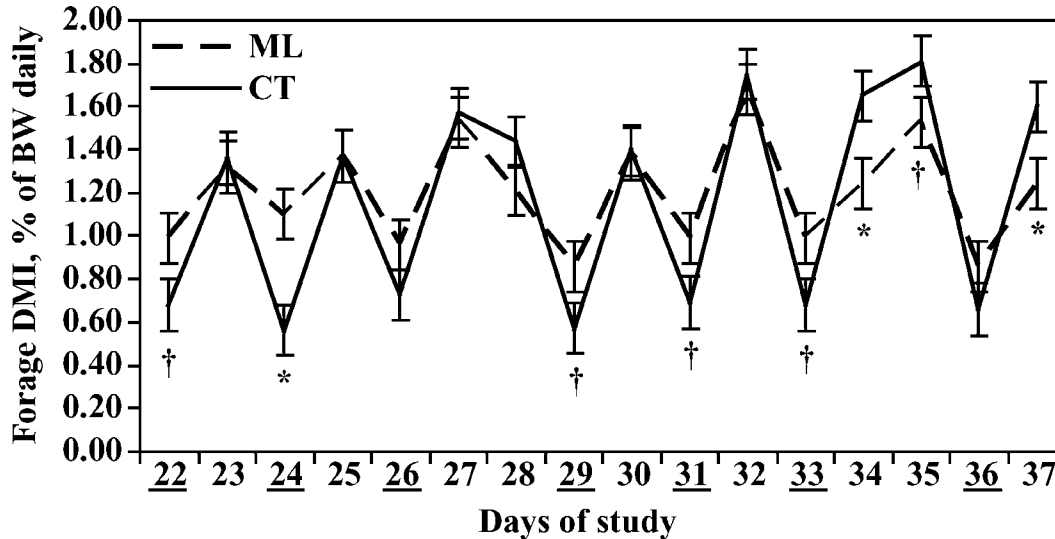


Figure 2. Forage DMI, of cows offered citrus pulp (CT)- or molasses (ML)-based supplements, as a percentage of full BW daily. Days on which supplements were offered are underlined. No significant differences were detected (1.16 vs. 1.20% of BW daily for CT- and ML-fed cows, respectively; $P = 0.52$, SEM = 0.086). A day effect and treatment \times day interaction were detected ($P < 0.01$). * $P < 0.05$; † $P < 0.10$.

tein degradation (Hammond, 1997). A treatment \times period interaction also was observed for BUN ($P < 0.01$; Table 6); during the first period, cows fed ML tended to have greater BUN concentrations compared with CT-fed cows (3.87 vs. 3.02 mg/dL; $P = 0.06$), but cows fed CT had greater BUN concentrations during the third period (4.79 vs. 3.86 mg/dL; $P < 0.05$). A time(period) effect was also detected ($P < 0.01$) due to increased BUN concentrations for both treatments after supplements were offered (Table 6). According to Hammond (1997), BUN concentrations should range from 7 to 8 mg/dL for mature beef cows. Therefore, cows on both treatments appeared to be deficient in protein intake according to their BUN concentrations, because they were often below this optimal range (Table 6). However, both supplements were formulated to supply the protein requirements of mature cows using the NRC model (1996).

No significant differences between treatments were observed for concentrations of glucose (84.9 vs. 86.0 mg/dL for CT and ML, respectively; $P = 0.88$; data not shown), insulin (1.77 vs. 1.51 ng/mL for CT and ML, respectively; $P = 0.33$; Table 7), or IGF-I (110 vs. 100

ng/mL for CT and ML, respectively; $P = 0.49$; Table 7). A treatment \times period interaction ($P < 0.05$) was detected for insulin and IGF-I and, similar to Exp. 1, can be explained by the observed pattern of an increase in the differences between treatments with the advance of the experiment for both hormones (Table 7). During the second period, CT-fed cows tended to have greater insulin concentrations compared with ML-fed cows. Similar to the results observed in Exp. 1, insulin and IGF-I concentrations were numerically greater for cows fed CT compared with cows fed ML. The lack of statistical significance is most likely related to fewer animals utilized in this experiment. In addition, according to the NRC (1996), the cows utilized in this experiment required approximately 8.0 Mcal/d of NE_m . Given that overall forage DMI was similar and treatments were isocaloric, cows from both treatments consumed approximately 14.0 Mcal/d (175% of the NE_m requirements). Supplements were fed at the same percentage of animal BW during Exp. 1 and Exp. 2. Because energy requirements for growing heifers and mature cows are different, the heifers in Exp. 1 were supplemented with moderate amounts of energy, whereas the cows in Exp.

Table 6. Blood urea N (BUN; mg/dL) concentration of cows offered citrus pulp (CT)- or molasses (ML)-based supplements during Exp. 2¹

Item	Period 1, h						Period 2, h						Period 3, h						SEM
	0	4	8	24	32	48	0	4	8	24	32	48	0	4	8	24	32	48	
CT	0.8	1.2	1.8	3.7	4.3	6.1	4.3	3.1	3.2	7.0	8.3	7.5	4.6	2.7	1.7	4.7	6.3	8.5	0.29
ML	1.7	2.3	2.6	4.8	5.5	6.1	3.6	3.7	4.1	5.7	5.7	6.3	2.7	2.8	2.7	4.4	5.1	5.3	0.28
P-value ²	0.21	0.14	0.24	0.13	0.12	0.96	0.35	0.47	0.24	0.07	<0.01	0.10	<0.01	0.90	0.17	0.64	0.11	<0.01	

¹Supplements were offered after blood was sampled at 0 h.

²Treatment comparison within period and hour.

Table 7. Insulin and IGF-I concentrations (ng/mL) of cows offered citrus pulp (CT)- or molasses (ML)-based supplements during Exp. 2

Item	Period 1	Period 2	Period 3	SEM
Insulin				
CT	1.27	1.97	2.10	0.19
ML	1.15	1.51	1.87	0.18
<i>P</i> -value ¹	0.66	0.09	0.42	
IGF-I				
CT	93	112	126	10.0
ML	91	101	108	9.7
<i>P</i> -value ¹	0.86	0.45	0.22	

¹Treatment comparison within period.

2 were supplemented with excessive amounts of supplemental energy. These differences in supplemental energy, relative to cow requirement, might be an additional explanation for the lack of statistical significance observed in the current experiment.

For cows provided CIDR, mean P4 concentration did not differ between treatments (4.08 vs. 3.14 ng/mL for CT- and ML-fed cows, respectively; $P = 0.12$; data not shown). A period effect was detected ($P < 0.01$), because P4 concentrations decreased linearly for both groups from the first period to the last period, and this effect can be associated with the decrease in P4 release from the CIDR with advancing time (average P4 concentration = 5.27, 3.18, and 2.39 ng/mL for periods 1, 2, and 3, respectively). A time(period) effect also was observed ($P < 0.01$). Throughout the experiment, P4 concentrations decreased for both groups after the supplements were offered (Figure 3). The greatest decrease in P4 was observed in the first 4-h interval after supplements

were offered and was likely caused by an increased hepatic clearance of blood P4 associated with metabolic body rate in response to acute feed intake (Sangsritavong et al., 2002).

General Discussion

The objective of the first experiment was to investigate if yearling heifers fed a molasses-based supplement would experience greater reproductive performance compared with heifers fed with supplements based on dry feed (citrus pulp), as previously observed by our group. To further test our main hypothesis, we conducted a second experiment with mature cows, where blood was collected with greater frequency to verify whether P4 was being metabolized faster in cows supplemented with citrus pulp. However, the expected supplement intake behavior was not observed in either experiment and, consequently, no major differences between treatments were observed in P4 concentrations of heifers and cows.

In the first experiment, heifers supplemented with CT had greater concentrations of glucose, insulin, and IGF-I, which resulted in greater ADG compared with ML-supplemented heifers; however, reproductive performance was not affected by treatments. In addition, concentration of IGF-I was the only measurement significantly correlated with BW gain and positively associated with attainment of puberty and pregnancy.

In the second experiment, differences between treatments for plasma metabolites and hormones were not as substantial as in Exp. 1. However, numeric differences relative to treatment effects on insulin and IGF-I were similar to Exp. 1. We concluded that cows were

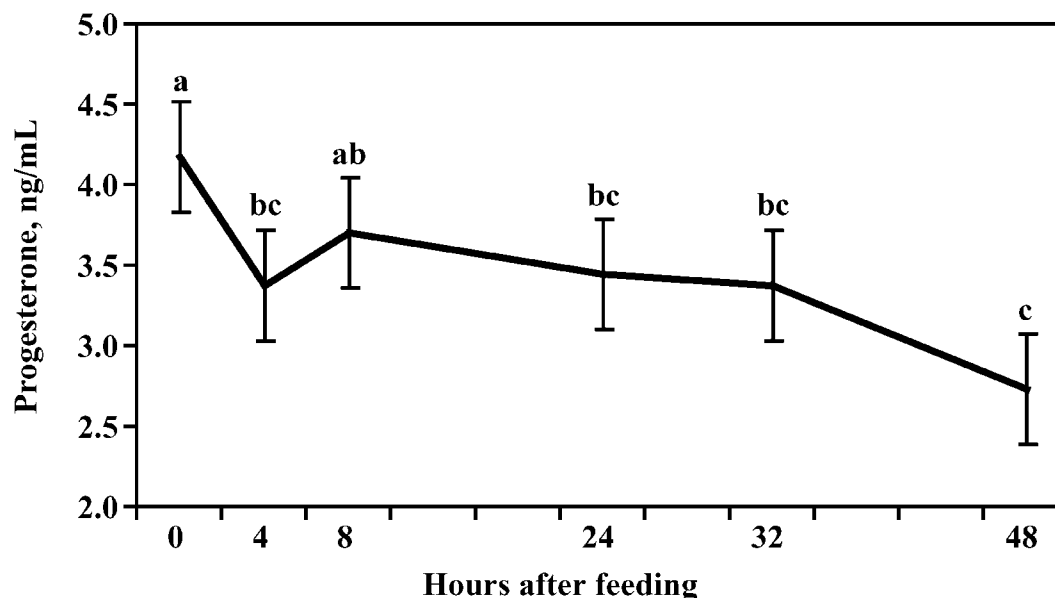


Figure 3. Progesterone concentrations, pooled within treatments and time within periods, of cows offered citrus pulp (CT)- or molasses (ML)-based supplements in Exp. 2. Supplements were offered after blood was sampled at 0 h. A time (period) effect was detected ($P < 0.01$). ^{a-c}Hours not bearing a common letter differ ($P < 0.05$).

provided excessive supplemental energy, and any potential responses to the treatments were suppressed. In addition, although no differences were observed for overall forage DMI, cows supplemented with citrus pulp had a greater daily oscillation in forage intake compared with cows supplemented with molasses.

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