632 Manure nitrogen transformations in air, soil and crops on dairy farms. J. M. Powell*[1], K. F. Knowlton[2], M. P. Russelle[3], and M. D. Hanigan[2], [1]*USDA-ARS Dairy Forage Resh. Center*, *Madison, WI*, [2]*Virginia Tech University*, *Blacksburg*, [3]*USDA-ARS Dairy Forage Resh. Center*, *St. Paul, MN*.

Only 25 to 35 % of the crude protein (CP) consumed by dairy cows is converted into milk. Such poor use of dietary CP may be due to inefficiencies associated with forage nitrogen (N) capture and metabolism. Manure N excreted in feces and urine, and the transformation of manure N in air, soil and crops are highly influenced by what dairy cows consume. For example, reducing dietary CP resulted in less total manure N, especially urine N excretion. Ammonia loss from manure from a low CP diet (13.6%) was lower than from a high CP diet (19.4%), representing 9 and 25% of applied manure N, respectively. Increasing condensed tannin content of dietary forage legumes also reduced urine N excretion. Ammonia emissions from barn floors were greater from manure derived from alfalfa silage (AS)-based diets than either birdsfoot trefoil with low or high tannin levels. After application to soil, feces from cows AS-based diets generally lead to higher soil inorganic N (IN) levels than soils amended with feces from corn silage-based diets; feces from AS-based diets increased plant yield and N uptake; feces from high CP diets resulted in greater soil IN levels than feces from low CP diets; and feces from low CP diets did not increase soil IN but decreased plant yield and N uptake. Only a small increase in N efficiency is necessary to make substantial reductions in the dairy industry's contribution to the environmental N load. There appears to be a range of dietary options that satisfy the nutritional requirements of high-producing dairy cows, yet produce manure that has differential effects on post-excretion transformations and environmental losses. Dairy production efficiencies may be gained and manure N losses reduced by incorporating moderate levels of tannins or other protein protection compounds into forages to enhance CP use and reduce dietary CP concentrations, and by developing perennial forages that tolerate manure applications, have improved ammonia absorption and assimilation potential, and are able to assimilate excess soil nitrates.

Key Words: Forages, Manure, Nitrogen cycling

633 Transforming forage plants to increase nitrogen utilization in dairy systems: What are the possibilities? R. Hatfield*[1], J. Grabber[1], M. Sullivan[1], G. Waghorn[2], and M. McCaslin[3], [1]*USDA-ARS*, *Madison, WI*, [2]*Dexcel Limited*, *New Zealand*, [3]*Forage Genetics*, *St. Paul, MN*.

Forages can supply adequate protein to meet the nutritional needs of high producing dairy cows, at least as the crop stands in the field. However proteins are one of the most labile nutritional components in most forages, often being excessively degraded during ensiling and ruminal digestion, leading to depressed amino acid absorption and excessive urea excretion by cattle. Even when forages are grazed, protein-use efficiencies are often low due to rapid plant cytoplasmic protein degradation in the rumen. To maintain high production, dairy diets are frequently supplemented with a protein source to compensate for poor forage protein use. Traditional breeding and molecular approaches can be used to modify forages for improved protein-use by cattle. For example, redesigning alfalfa to produce polyphenol oxidase and *o*-diphenols or condensed tannins would lead to decreased protein degradation during ensiling and ruminal digestion with a likely increase in amino acid absorption by cattle. Production and feeding of such a forage would reduce urea excretion and possibly slow nitrogen release from feces and crop residues, thereby reducing nitrogen losses from farms. Altering specific gene expression in the lignin pathway may allow decreased lignification and increased fiber digestion for improved nitrogen utilization. Genetic selection or molecular alteration of forages to produce greater quantities of rapidly fermented carbohydrates should enhance conversion of non-protein nitrogen to ruminal protein for utilization by cattle. Increasing total biomass production that has good quality remains a challenge for forage production. Exploiting the genetic potential for total biomass production in forages is just now being explored. Redesigning forages to function more efficiently as effective nitrogen sources for dairy cows is not impossible; it could decrease the need for protein supplements, and ultimately decrease nitrogen losses to the environment.

Key Words: Protein, Plant-modification, Nitrogen waste

Physiology and Endocrinology: Endocrinology

634 An erythropoietin receptor (EPOR) gene polymorphism (SNP) alters EPOR mRNA in fetal liver of swine during early gestation. J. L. Vallet* and B. A. Freking, *USDA, ARS, U.S. Meat Animal Research Center*, *Clay Center, NE*.

We previously reported that an EPOR gene C/T SNP was associated with litter size. The T allele created a putative GATA-1 site, which was predicted to increase EPOR gene expression. This experiment determined whether the SNP was associated with: (1) EPOR gene expression by the fetal liver or (2) maturation of the fetal blood supply during early gestation. CC and CT gilts were unilaterally hysterectomized-ovariectomized at 160 d of age, mated to boars of like genotype and slaughtered on d 25 (n = 13 CC, 13 CT), 30 (19, 25) and 40 (14, 15) of gestation. Numbers of corpora lutea (CL) and live fetuses were recorded. For CT gilts only, a blood smear was prepared for each fetus, the fetus was weighed and fetal liver and other tissues were collected. Percentage of nucleated blood cells was assessed on d 30 and 40 (all blood cells were nucleated on d 25). DNA was prepared from fetal tissues to determine EPOR genotype. Total RNA was prepared from fetal liver of one fetus of each genotype for each litter (d 25 and 30), and EPOR mRNA was measured using real time RT-PCR. Number of fetuses decreased ($P < 0.01$) between d 30 (11.7 ± 0.4) and 40 (8.5 ± 0.5) but did not differ between gilt genotypes. Percent nucleated cells decreased significantly between d 30 and 40 but were not affected by fetal genotype. Fetal liver EPOR gene expression was greater ($P < 0.01$) on d 30 compared to d 25 of gestation and a significant additive effect of genotype ($P < 0.01$) was observed (d 25, 3.8 ± 0.7, 4.6 ± 0.7, 5.4 ± 0.7; d 30, 9.8 ± 0.5, 10.3 ± 0.5, 11.3 ± 0.5 relative units; CC, CT, and TT, respectively). Although these results do not indicate an effect of the SNP on litter size, uterine capacity affects litter size on d 40 or later, and the number of gilts on d 40 were likely inadequate. The SNP also did not affect maturation of the fetal blood supply. However, the T allele was associated with increased EPOR gene expression during early pregnancy as predicted and, thus, could influence erythropoiesis and fetal survival.

Key Words: Blood, Erythropoiesis, Fetus

635 Serum constituents and thyroid hormones in sheep fed halophyte forages. A. Riasi*[1], M. Danesh Mesgaran[2], M. J. Zamiri[3], and M. D. Stern[4], [1]*University of Birjand*, *Birjand, Khorasan, Iran*, [2]*Ferdowsi University of Mashad*, *Mashad, Khorasan, Iran*, [3]*University of Shyraz*, *Shyraz, Fars, Iran*, [4]*University of Minnesota*, *St. Paul*.

Halophyte forages originating from central Iranian deserts (*Kochia scoparia*, *Atriplex dimorphostegia*) were harvested at early bloom stage. The potential value of these forages occasionally is constrained by alteration of serum constituents and metabolic hormones of some ruminants. Nine Balouchi ewes (48±2 kg BW) were transferred to metabolism cages and randomly allocated to three dietary treatments [T 1: kochia + alfalfa (1:1), T 2: atriplex + alfalfa (1:1), T 3: alfalfa] in a Latin Square experiment with three periods (28 days periods). During the last 3 days of each period, samples of blood serum and urine were collected. There was no differences between treatments as serum levels of Mg and Na (P>0.05). Whereas, Serum Ca levels for T 1 (8.04 mg/dl) and T 2 (8.29 mg/dl) was lower (P<0.01) than that T 3 (9.19 mg/dl). Halophyte forages elevated serum cholesterol (P<0.01), thyroxine (P<0.001), and bilirubin (P<0.01). Ewes fed T 1 had the highest (P<0.01) blood serum activity of alanine aminotransferase (ALT) (34.8 U/l). The activity of Aspartate aminotransferase (AST) was increased in blood serum of ewes fed halophyte forages (P<0.05). The effect of treatments on blood serum glucose levels were different (P<0.05) and the linear relationship for the sampling time tend to be significant (P<0.08). The effect of treatments on blood serum urea N were completely different (P<0.0001), however there was no linear and quadratic relationship for the time of sampling.

Key Words: Serum constituents, Sheep, Halophyte forages

636 Food deprivation-induced decrease in blood insulin-like growth factor-I is associated with decreased liver growth hormone receptor mRNA and protein in steers. M. Wu[1], R. Akers[1], R. Torres-Diaz[1], S. Frank[2], J. Hall[1], W. Beal[1], and J. Jiang*[1], [1]*Virginia Tech*, *Blacksburg*, [2]*University of Alabama*, *Birmingham*.

The insulin-like growth factor-I (IGF-I) is essential for animal growth and development. Most of the IGF-I in the blood comes from growth hormone (GH)-stimulated IGF-I gene expression in the liver. In a variety of animals including cattle, the IGF-I concentration in the blood is reduced during food deprivation or food restriction. This study was conducted to determine whether the decrease in blood IGF-I was associated with decreased expression of IGF-I mRNA, growth hormone receptor (GHR) mRNA and (or) GHR protein in the liver during food deprivation, to understand the role of liver GHR in the effect of food deprivation on blood IGF-I. Five steers were deprived of food for 60 h while another five steers (control steers) were allowed their usual access to food. A blood sample and subsequently a liver biopsy sample were taken from each steer at the end of the 60-h period. Serum IGF-I concentrations were 46.75% lower (P<0.01) in food-deprived steers than in control steers. Liver IGF-I mRNA, GHR mRNA, GHR1A mRNA (a major GHR mRNA variant in the bovine liver) and liver GHR protein were less abundant (P<0.01) in food-deprived steers than in control steers. Liver expression of non-1A GHR mRNA variants (i.e., the combination of GHR1B, 1C and other minor GHR mRNA forms) was, however, not different (P>0.05) between food-deprived and control steers. Given the relative positions of GHR1A mRNA and GHR protein in the process of IGF-I mRNA production in the liver, that they were all reduced during food deprivation suggests that decreased expression of GHR1A mRNA contributes to decreased IGF-I mRNA expression and hence decreased blood IGF-I during food deprivation.

Key Words: Growth hormone receptor, IGF-I, Cattle

637 Effects of standing estrus and concentrations of estradiol on uterine pH. J. R. Nelson*, B. L. Perry, and G. A. Perry, *South Dakota State University*, *Brookings*.

Research has shown cows in estrus within 24 h of fixed-time AI had elevated concentrations of estradiol and greater pregnancy rates compared to cows not in estrus. Our objective was to determine if estradiol and/or estrus had an effect on uterine pH during a fixed-time AI protocol. Non-lactating beef cows (n = 19) were treated with the CO-Synch protocol (100µg GnRH on d -9; 25 mg PG on d -2; and 100µg GnRH on d 0). Half (n = 10) the cows received an injection of estradiol cypionate (ECP; 1mg) 12 h following PG. Cows detected in standing estrus within 24 h of the second GnRH injection were considered to be in estrus. Cows treated with ECP had greater (P < 0.01) concentrations of estradiol compared to control cows (8.3 ± 0.7 and 5.2 ± 0.7 pg/mL, respectively). A treatment by time interaction (P < 0.01) influenced concentrations of estradiol. All cows had similar (P > 0.15) concentrations of estradiol at time of ECP, but ECP treated cows had elevated (P < 0.02) concentrations of estradiol following the second GnRH injection compared to control cows. Treatment (P = 0.01), time (P < 0.01), and treatment by estrus by time (P = 0.065) influenced uterine pH. Control cows that did not exhibit estrus had a higher uterine pH compared to ECP cows that did not exhibit estrus (P = 0.03) at time of ECP. All cows had a similar uterine pH (P > 0.19) 12 h after ECP. Control cows that did not exhibit estrus had a higher uterine pH compared to control cows that did exhibit estrus (P < 0.01) and ECP cows that exhibited estrus (P = 0.05) at time of the second GnRH injection (time insemination would occur; 7.0 ± 0.1, 6.7 ± 0.1, 6.8 ± 0.1, respectively). ECP cows not exhibiting estrus were intermediate (6.8 ± 0.1). All cows had similar uterine pH 24 h after GnRH through ovulation (P > 0.06). Concentrations of estradiol had no linear (P > 0.21) or quadratic (P > 0.21) relationship with uterine pH. In summary, ECP treatment elevated concentrations of estradiol and lowered uterine pH to a level similar to the uterine pH of control cows that exhibited estrus within 24 h of when insemination would occur.

Key Words: Estradiol, Estrus, Uterine pH

638 Species-specific differences in constitutive androstane receptor (CAR) coding region predicts altered constitutive activity in ruminants. D. L. Greger*[1], C. Morel[2], C. R. Baumrucker[1], and J. W. Blum[2], [1]*Pennsylvania State University*, *University Park*, [2]*University of Bern*, *Bern, Switzerland*.

Constitutive androstane receptor (CAR) is a nuclear receptor that regulates genes involved in detoxification and elimination of potentially toxic foreign and endogenous compounds (xenobiotics and endobiotics). In addition, CAR also regulates genes involved in energy homeostasis, affecting such processes as thyroid hormone metabolism and gluconeogenesis. The constitutive activity and specificity of ligand binding are dependent upon highly conserved amino acids involved in hydrogen bonding that maintain the activated conformation of the CAR molecule. Specifically, computer modeling has shown that a central tyrosine (Y326) is required for maintenance of the activated conformation, likely through the formation of a hydrogen bond with an

asparagine (N165) on the opposite side of the ligand binding domain. Site directed mutagenesis studies have confirmed this prediction. While these residues are highly conserved across mammalian species, we have discovered that ruminant species possess hydrophobic amino acids in these locations that would preclude hydrogen bond formation. In particular, CAR sequences obtained thus far from all ruminant species using multiple individual and pooled DNA samples from several breeds of cattle, as well as individual samples from sheep, red deer, wapiti, roe deer, goat and bison, all possess a phenylalanine substitution at the critical tyrosine residue (Y326F). Interestingly, the Bovidae (Bos taurus, Bos indicus, Bison bison) also have a substitution in the partner amino acid residue asparagine, N165, that is replaced by the hydrophobic residue isoleucine. These changes are not observed in non-ruminant herbivores. Given the importance of CAR as a repressor of hepatic genes encoding gluconeogenic enzymes, and the critical requirement in ruminant species for very high rates of glucose production, the ruminant CAR variants may play a vital role in the regulation of gluconeogenesis.

Key Words: Constitutive androstane receptor, Comparative genomics, Gluconeogenesis

639 Cortisol enhances N-acetylglutamate synthase activity and arginine synthesis in enterocytes of suckling piglets. G. Y. Wu*[1,2], Y. L. Yin[1], and N. E. Flynn[2,3], [1]*The Chinese Academy of Sciences*, *Changsha, Hunan, P.R. China*, [2]*Texas A&M University*, *College Station*, [3]*Angelo State University*, *San Angelo, TX*.

There are marked decreases in both plasma cortisol concentrations and intestinal activity of N-acetylglutamate synthase (NAGS; a key enzyme in arginine synthesis) in piglets during the suckling period. This study was designed to test the hypothesis that cortisol may play an important role in regulating intestinal NAGS expression in sow-reared piglets. Thirty-two 7-day-old suckling pigs were randomly assigned to one of four groups with seven animals each, and received intramuscular injections of vehicle solution (sesame oil) (control group), hydrocortisone-21-acetate (HYD) (25 mg/kg body wt), RU486 (10 mg/kg body wt) (a potent blocker of glucocorticoid receptors), or HYD plus RU486. During the entire experimental period, pigs were nursed by sows. At 14 days of age, pigs were sacrificed for preparation of jejunal enterocytes for measurements of synthesis of citrulline and arginine from glutamine and proline, mitochondrial activity of NAGS, and mitochondrial concentration of N-acetylglutamate (NAG), using established methodologies (Wu, Am J Physiol 272: G1382, 1997; Bush et al. J Nutr 132: 59, 2002). Cortsiol administration had no effect ($P > 0.05$) on body weight but increased ($P < 0.05$) jejunal weight and villus height, compared with the control group. The cortisol treatment enhanced 1) NAGS activity and prevented the postnatal decline in NAGS activity during the suckling period; 2) increased NAG concentration as well as citrulline and arginine synthesis from glutamine and proline in enterocytes ($P < 0.01$), in comparison with the control group. The stimulatory effects of cortisol on intestinal NAGS activity and arginine synthesis were abolished ($P < 0.01$) by co-administration of RU486. Our results indicate that cortisol treatment provides a novel and effective means to prevent the marked declines in intestinal NAGS expression and arginine synthesis in suckling piglets via a glucocorticoid receptor-mediated mechanism. Supported by funds from the Chinese Academy of Sciences, China NSF, and USDA.

Key Words: Cortisol, N-Acetylglutamate, Suckling piglets

640 Adrenal involvement in the biostimulatory effect of bulls. S. A. Tauck*, J. R. Olsen, and J. G. Berardinelli, *Montana State University*, *Bozeman*.

The objective was to evaluate if cortisol concentrations are associated with the biostimulatory effect of bulls in postpartum, primiparous cows. The hypotheses were that interval from start of exposure to resumption of luteal activity; proportions of cows that resumed luteal function during the exposure period; and cortisol concentrations do not differ among cows exposed or not exposed to bulls (Exp. 1), and cows continuously exposed to bull or steer urine (Exp. 2). In Exp. 1, 28 anovular cows were exposed (BE; n=13) or not exposed (NE; n=15) to bulls for 35 d at 58 d after calving. In Exp. 2, 30 anovular cows were fitted with a controlled urine delivery device at 45 d after calving and exposed continuously (24 h/d) to bull (BUE; n=15) or steer (SUE; n=15) urine. Length of exposure was ~64 d. Blood samples were collected from each cow on d 0, and every 3 d throughout exposure periods in both experiments and assayed for progesterone. Cortisol was assayed in samples collected on d 0, 9, 18, and 27 in Exp. 1; and, d 0, 19, 38, and 57 in Exp. 2. In Exp. 1, interval from the start of exposure to resumption of luteal activity was shorter ($P < 0.05$) for BE cows than NE cows, similarly, more ($P < 0.05$) BE cows than NE cows resumed luteal function during the exposure period. In Exp. 2, there was no difference in intervals from the start of exposure to resumption of luteal activity and proportions of cows that resumed luteal function during the exposure period between BUE and SUE cows. In Exp. 1, there was no difference in cortisol concentrations between BE and NE cows at the start of the experiment (d 0), however, cortisol concentrations were greater ($P < 0.05$) in BE cows than NE cows on d 9, 18, and 27. In Exp. 2, cortisol concentrations were higher for BUE than SUE cows on d 0 ($P < 0.05$) thereafter cortisol decreased ($P < 0.05$) but did not differ between BUE and SUE cows. We conclude that continuous physical presence of bulls stimulates resumption of luteal activity and is coincident with increased cortisol concentrations. We hypothesize a possible association between adrenal activation and the biostimulatory effect of bulls.

Key Words: Biostimulation, Cortisol, Postpartum anestrus

641 Localization of Period1 mRNA in the ruminant oocyte and investigations of its role in ovarian function. R. A. Cushman*[1], M. F. Allan[1], S. A. Jones[1], G. P. Rupp[2], and S. E. Echternkamp[1], [1]*U.S. Meat Animal Research Center*, *Clay Center, NE*, [2]*Universtity of Nebraska*, *Clay Center*.

The clock gene Period 1 (Per1) may be a prolificacy gene, because it localized to the mouse oocyte and Per1-null drosophila shed fewer eggs. Because Per1 mapped to a region of mouse chromosome 11 homologous to bovine chromosome 19 where a QTL for ovulation rate existed, we hypothesized that Per1 influenced folliculogenesis and ovulation rate in ruminants. Ovarian cortex was collected at slaughter on Days 5, 12, 15, 17, and 20 after estrus for real-time RT-PCR evaluation of Per1 mRNA expression in Dorset (n = 18); Romanov (n = 10); Romanov/Dorset (n = 21); and Composite (n = 22) ewes. Ovarian cortex was also collected from cows selected for increased ovulation rate (n = 37) or unselected controls (n = 28) on days 4, 5, and 6 of the estrous cycle for *in situ* hybridization and real-time RT-PCR. To examine the role of Per1 in early follicular development, ovarian cortex from neonatal calves (n = 5) was cultured for ten days and Per1 mRNA levels were measured on Day 0 and on Day 10 of culture. The primers generated a 483 bp amplicon with 100% homology to bovine RIGUI-like protein (Per1) which is 20 cM from the QTL. Per1

mRNA expression was unaffected by prolificacy, day of the cycle, or pregnancy status in ewes or cows. The riboprobe hybridized to oocytes of bovine preantral and antral follicles. In bovine ovarian cortical cultures on Day 0, the tissue contained mostly primordial follicles (5.6 ± 0.6 follicles/section); however, after 10 days in culture, the number of primordial follicles per section decreased (0.5 follicles/section) and the number of primary follicles increased as follicles activated (Day 0 = 0.5 ± 0.6 vs. Day 10 = 10.4 ± 0.6 primary follicles/ section; $P < 0.001$). Per1 mRNA did not change over time in culture. We conclude that Per1 mRNA is expressed by ruminant oocytes in preantral and antral follicles; however, its physiological role in mammalian ovarian function remains to be elucidated.

Key Words: Oocyte, Fertility, Gene expression

642 Trace element concentration of bovine ovarian and hepatic tissue. W. S. Swecker, Jr*[1] and D. J. Tomlinson[2], [1]*Virginia Tech*, *Blacksburg*, [2]*Zinpro Corp*, *Eden Praire, MN*.

Adequate provision of nutrients is essential to bovine reproduction. Ovarian tissue in cycling beef and dairy cows must support follicular growth, corpus luteum (CL) formation and lysis. Antioxidant enzymes such as superoxide dismutase and glutathione perioxidase are essential to protect the ovary and may have regulatory functions in hormone synthesis. The objective of this study was to compare concentrations of trace element constituents (Mn, Cu, Zn, Se) of antioxidant enzymes and trace element antagonists (Fe, Mo) in ovarian stroma, luteal tissue, and liver. Liver and ovarian tissue was collected from cows at a commercial abbatoir. Trace element analysis of ovarian stroma, CL, and liver tissue was submitted on a subset of 18 cows that had a CL > 10 mm present. Mineral concentrations were determined by Inductively Coupled Plasma Mass Spectrometry. Manganese concentrations in the CL were similar to liver concentrations and both were 10-fold greater than ovarian Mn concentrations. Copper concentrations were similar between ovarian stoma and CL and were 60-fold lower than liver Cu. Zinc concentrations were similar between CL and ovarian stroma and were 4-fold lower than liver concentrations. Corpus luteum selenium was similar to ovarian concentrations and both were 75% of liver concentrations. For antagonistic trace elements, both Fe and Mo concentrations of the CL were intermediate to liver and ovarian concentrations. In summary, Mn, Fe, and Mo appear to be concentrated in the CL relative to ovarian stroma. Selenium concentrations of ovarian stroma and CL are most similar to liver concentrations as compared to the other elements.

Table 1. Mineral Content of Tissues

Element	Corpus Luteum	Ovarian Stroma	Liver	Pooled SEM
Cu	5.7[a]	3.7[a]	360.6[b]	33.9
Se	1.1[a]	0.9[a]	1.3[b]	0.1
Mn	9.5[a]	0.7[b]	8.1[a]	0.7
Zn	58.1[a]	68.7[a]	262.6[b]	17.6
Fe	182.3[a]	75.0[b]	328.3[c]	23.8
Mo	0.7[a]	0.2[b]	2.8[c]	0.1

Concentrations are ppm [a,b,c] values within row with different superscripts differ $P < 0.05$ DM basis

Key Words: Trace elements, Antioxidant enzymes, Ovary

Ruminant Nutrition: Grazing Nutrition

643 Effects of ruminal fill on bite and grazing dynamics. P. Gregorini*[1,2], S. Gunter[1], C. Masino[2], and P. Beck[1], [1]*University of Arkansas*, *Hope*, [2]*Universidad Nacional de La Plata*, *La Plata, Buenos Aires, Argentina*.

Hunger affects intake rate. Ruminal fill (RF) has been related to such a state; thus, its level may operate in intake regulation, and grazing behavior. This experiment assessed the impact of manipulated ruminal fill on bite traits and grazing dynamics. During 15 d, 3 ruminally cannulated heifers individually grazed bermudagrass pastures. The first 11 d were adaptation to grazing management. New strips were allocated daily at 0800. At 1700 heifers grazed for a session (GS) of 30 min on a new strip. From d 12 to 15, the treatments, ruminal fill 100 (RF100), 66 (RF66) 33 (RF33), and 0% (RF0) of total ruminal contents, were randomly applied in a 3 x 4 Youden-square design. The rumen was emptied and contents weighed at 0700 and 1300, and after each GS to assess morning intake, set treatments (before GS), and estimate bite mass (BM), respectively. All GS were video recorded and analyzed for bite rate (BR), bites/feeding station (BFS), FS/min (FSR), intake/FS (IFS), and time/FS (TFS). Apparent bite depth (ABD), area (ABA), and area grazed/FS (AFS) were calculated. Dependent variables were analyzed by ANOVA. The linear, quadratic and cubic effects of RF were detected using orthogonal contrasts. As RF decreased, BM, BA, BFS, AFS, TFS and IFS increased ($P < 0.01$); while ABD and FSR decreased ($P < 0.01$). Heifers increased IR by changing bite shape and increasing TFS instead of BR. These results support the connection of ingestive and digestive behaviors, and its use in new grazing strategies.

Table 1.

Variable	Treatment				se	Orthogonal contrast[a]		
	RF0	RF33	RF66	RF100		L	Q	C
BM, g	0.77	0.58	0.26	0.22	0.01	<0.01	0.10	0.05
ABA, cm^2	288.48	214.93	93.92	75.84	9.15	<0.010	0.37	0.29
ABD, %	46	48.37	50.62	53	<0.01	<0.01	0.91	0.86
BR, bites/min	49.7	52.1	53.5	51.6	1.27	0.69	0.6	0.89
BFS	9.17	9.04	7.64	5.35	0.25	0.01	0.23	0.92
FSR	4.86	5.91	7.14	9.67	0.17	<0.01	0.25	0.68
Intake/FS, g	6.86	5.20	2.21	0.91	0.13	<0.01	0.67	0.16
TFS, s	11.18	10.17	8.61	6.28	0.23	<0.01	0.41	0.94
AFS, %	96.53	69.01	29.07	10.66	2.54	<0.01	0.6	0.35

[a]Linear (L), Quadratic (Q), and Cubic (C) effects.

Key Words: Ruminal fill, Bite features, Feeding stations