PEER REVIEWED

Evaluating Relationships among Blood Glucose, Plasma Urea Nitrogen, Performance, Morbidity, and Mortality in High-risk Feedlot Heifers

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Abstract

Two experiments were conducted using crossbred beef heifers (Exp. 1: n = 332, 403 lb [183 kg] BW; Exp. 2: n = 334, 456 lb [207 kg] BW) to evaluate relationships between blood glucose, plasma urea nitrogen, average daily gain (ADG), and morbidity and mortality rates. Whole blood glucose (Glc) was measured 24 hours after arrival using a handheld Glc monitor. Plasma was obtained from 179 heifers in Exp. 1 and from 334 heifers in Exp. 2 to determine concentrations of plasma urea nitrogen (PUN) and plasma Glc measured using a spectrophotometer. Heifers were placed into blood Glc categories of high (HGlc) and low (LGlc) Glc and high (HPUN) and low (LPUN) plasma urea nitrogen categories based on whether they had higher or lower levels than the mean of the group with which they arrived. In Exp. 1 ADG was lower for heifers categorized as HGlc and HPUN ($P < 0.05$), but in Exp. 2 ADG was lower ($P < 0.05$) for LPUN than HPUN heifers. Clinical signs (CS), first treatment, and retreatment rates were not different among groups in Exp. 1, but mortality rate was highest for HGlc (10.3 vs 6.0%; $P < 0.03$). In Exp. 2, CS, first treatment, and retreatment rates were lower for heifers categorized as LGlc vs HGlc heifers ($P < 0.03$). Days to first treatment were greater ($P < 0.05$) for heifers categorized as HPUN vs LPUN heifers in Exp. 1 and 2. Elevated PUN may be an indicator of existing catabolism and stress in feedlot calves.

Keywords: bovine, glucose, plasma urea nitrogen, bovine respiratory disease

Résumé

Deux expériences ont été menées avec des taures de boucherie de race mélangée (Exp. 1: n=332, 403 lb [183 kg] masse corporelle; Exp. 2: n=334, 456 lb [207 kg] masse corporelle) afin d'évaluer la relation des taux de mortalité et de morbidité en fonction du glucose sanguin, de l'azote uréique plasmatique et du gain moyen quotidien. La concentration du glucose sanguin était mesurée 24 heures après l'arrivé avec un moniteur de glucose portatif. L'analyse spectrométrique du plasma de 179 taures dans l'expérience 1 et de 334 taures dans l'expérience 2 a servi à déterminer la concentration plasmatique de l'azote uréique et du glucose. Les taures ont été classées dans des catégories hautes ou basses du glucose sanguin ou hautes ou basses de la concentration plasmatique d'azote uréique selon qu'elles avaient des niveaux plus élevés ou moins élevés que la moyenne du groupe dont elles provenaient. Dans l'expérience 1, le gain moyen quotidien était moins élevé chez les taures dans la catégorie haute du glucose sanguin et de l'azote uréique plasmatique ($P<0.05$) mais dans l'expérience 2 le gain moyen quotidien était moins élevé ($P<0.05$) chez les taures dans la catégorie basse de l'azote uréique plasmatique. Les signes cliniques et les taux de traitement et de retraitement ne différaient pas entre les groupes dans l'expérience 1 bien que les taures dans la catégorie haute du glucose sanguin (10.3 v. 6.0%; $P<0.03$) avaient le taux de mortalité le plus élevé. Dans l'expérience 2, les signes cliniques et les taux de traitement et de retraitement étaient moins élevés chez les taures dans la catégorie basse du glucose sanguin ($P<0.03$). Le

nombre de jours avant le premier traitement était plus élevé (P<0.05) chez les taures dans la catégorie haute de l'azote uréique plasmatique dans les expériences 1 et 2. Une concentration plasmatique élevée de l'azote uréique peut être un indicateur du catabolisme actuel et du stress chez les veaux d'engraissement.

Introduction

Newly arrived cattle at feedlots frequently are prone to sickness due to stresses imposed by numerous environmental changes. Responses to stress are an attempt by the animal's body to maintain homeostatic conditions. Disease stress often affects nitrogen kinetics of virus-infected calves by shifting from protein synthesis to protein catabolism.[1] Increases in glucocorticoid secretion associated with stress also result in increased amino acid release from muscle, which retards tissue anabolism and directs nutrient flow toward immune priorities. The immune system's response to a disease is stimulated by the utilization of amino acids as gluconeogenic precursors.[2] Sternbauer *et al*[3] demonstrated that calves given a single dose of a glucocorticoid (flumethasone) had elevated plasma glucose (Glc) concentrations. Hyperglycemia is also commonly associated with endotoxin administration in sheep,[4] cattle,[5] and rats.[6] Similar responses to viral infections are known to cause hyperglycemia.[7,8] The duration of hyperglycemia is dependent upon hepatic glycogen stores and the rate of hepatic gluconeogenesis.[4,6] Hypoglycemia has been shown to follow transient hyperglycemia.[5] It is less understood but thought to be due to the inability of the liver to fulfill Glc demands of peripheral tissues and for repletion of glycogen stores.

Bovine respiratory disease (BRD) accounts for 65 to 79% of the morbidity and 44 to 72% of the mortality in feedlot cattle.[9] While mortality losses are most obvious, high morbidity rates contribute significantly to economic losses associated with BRD. Diagnostic methods and (or) metabolic markers that would enable early detection of BRD would likely prove useful in reducing economic impact of the disease. The objective of this study was to evaluate relationships among blood Glc, plasma urea nitrogen (PUN), health, and growth performance of stressed feeder cattle.

Materials and Methods

Experiment 1

Crossbred beef heifers (n = 332; 403 lb [183 kg] BW) were used in a 36-day receiving trial to evaluate relationships among blood Glc, average daily gain (ADG), and morbidity and mortality rates. Heifers for both experiments were purchased from auction barns in Tennessee and Kentucky, and shipped to Manhattan, KS (approximately 700 miles and 12 to 15 hours in transit). Upon arrival at the Kansas State University Beef Cattle Research Center, cattle were provided grass hay and water and were processed within 24 hours. Cattle were implanted with zeranol,[a] administered a modified-live viral vaccine[b] and clostridial bacterin-toxoid,[c] treated for internal and external parasites,[d] and given a metaphylactic dose of tilmicosin[e] (4.55 mg/lb [10 mg/kg] BW, subcutaneously [SC]) at processing.

Blood Glc concentrations were measured on the day of initial processing using a calibrated hand-held Glc monitor.[f] The monitor provides accurate blood Glc readings over the range of 20 to 600 mg/dL (1.1 to 33.3 m*M*) within a hematocrit range between 20 and 60%. The 3 µL of blood required for testing was obtained from the ear tag placement site. On day 0, blood (10 mL) was also collected from 179 heifers via jugular venipuncture into heparinized vacuum tubes, and immediately placed on ice. Blood was centrifuged for 20 minutes at 1200 × g. Plasma was transferred to a microcentrifuge tube, capped, and stored frozen. Plasma was analyzed for Glc and PUN using a UV/VIS spectrophotometer. To analyze PUN, blood urea nitrogen color reagent (3.2 mL) and blood urea nitrogen acid reagent (2.4 mL) were added to 200 µL of plasma, and then placed in a 212°F (100°C) water bath for 10 minutes. After reacting, samples were placed in a cool water bath for three to five minutes, and absorbance values at 535 nm were promptly measured using a spectrophotometer. Glucose was analyzed using procedures supplied in a commercial test kit.[g] The analysis is based on the concept of oxidizing Glc to gluconic acid and hydrogen peroxide, catalyzed by Glc oxidase, and measuring the guinonimine dye via a spectrophotometer.[10] Guinonimine dye concentration is directly proportional to the Glc concentration.

Heifers (n = 332) were categorized as having whole blood Glc (measured using a hand-held monitor) that was either below or above the mean value of the group (58 mg/dL; LGlc <58 mg/dL; HGlc ≥58 mg/dL). Before the study, the Glc monitor was validated by analyzing whole blood serially spiked with Glc added at rates ranging from 0 to 600 mg/dL.

Concentrations of PUN (179 heifers; results from a concurrent study stimulated interest in PUN after early blocks were already processed so only late arrival blocks were evaluated for PUN) greater than or equal to the mean value of 1.06 m*M* were categorized as high (HPUN), and those below 1.06 m*M* were categorized as low (LPUN).

Heifers were observed daily by fourth year veterinary students and an attending veterinarian from the KSU College of Veterinary Medicine. Heifers were identified as candidates for therapeutic treatment when they exhibited any of the following clinical signs (CS) of BRD: coughing, anorexia, nasal discharge, ocular

discharge, lethargy and (or) depression. Therapeutic treatment (tilmicosin at 4.55 mg/lb BW) was administered SC to heifers when CS were accompanied by a rectal temperature ≥103.5°F (39.7°C) (first treatment). If rectal temperature did not reach 103.5°F, heifers were returned to their pen without treatment. Heifers that exhibited CS after initial observation of and subsequent treatment for respiratory disease (CS relapse, CSR) and had a rectal temperature ≥103.5°F were retreated (retreatment) with 5.9 mg oxytetracycline/lb (13 mg/kg) and 10.0 mg tylosin/lb (22 mg/kg) BW. Days to first treatment (days to first treatment) and days to retreatment after first treatment (days to retreatment) were observed. All cattle that died were necropsied by the attending veterinarian for gross signs of pathology; cause of death for all mortalities was determined to be pneumonia.

Experiment 2

Crossbred beef heifers (n = 334; 456 lb [207 kg] BW) were used in a 35-day receiving trial to evaluate relationships between blood Glc, ADG, morbidity, and mortality rates. Heifers were shipped to the Kansas State University Beef Cattle Research Center, where post-arrival receiving procedures were similar to those used in Exp. 1, except mean values for blood Glc and plasma Glc differed and the procedure for identification of animals requiring retreatment for BRD was modified. Blood Glc values measured with the hand-held monitor were categorized into above or equal to the mean value of 56 mg/dL (HGlc) and below the mean (LGlc). Concentrations of PUN greater than or equal to the population mean of 1.45 m*M* were categorized as high (HPUN), whereas those below 1.45 m*M* were categorized as low (LPUN).

As in Exp. 1, heifers were identified as candidates for therapeutic treatment when they exhibited CS of BRD, and tilmicosin was administered at 4.55 mg/lb BW when CS were accompanied by a rectal temperature ≥ 103.5°F. In Exp. 2, however, heifers that exhibited CS of BRD more than 48 hours following initial therapy (CSR) were retreated regardless of rectal temperature (retreatment); therefore, CSR and retreatments are equal in Exp. 2. Retreatments consisted of 5.9 mg oxytetracycline/lb BW and 10.0 mg tylosin/lb BW. Days to first treatment and days to retreatment after first treatment were observed.

Statistical analysis

Data were analyzed for Exp. 1 and 2 using the General Linear Models procedure.[h] The experimental unit was the individual animal. The model included ADG, CS, first treatment, CSR, retreatment, days to first treatment, days to retreatment, and mortality rate as dependent variables, and Glc category (HGlc or LGlc), PUN category (HPUN or LPUN), and their interaction as independent variables. Main effects of Glc, PUN, and Glc × PUN interaction were evaluated using least square means with a protected F-value of 0.10. The analysis was limited to 179 heifers in Exp. 1. The linear relationship between plasma Glc and whole blood Glc (Exp. 1 and 2) was evaluated using the General Linear Model procedure, with plasma Glc as the dependent variable and blood Glc as the independent variable.

Results and Discussion

Blood Glc values for the 332 heifers in Exp. 1 ranged from 42 to 137 mg/dL, whereas values from the 334 heifers in Exp. 2 ranged from 20 to 111 mg/dL (data not shown). Relationships among blood Glc, PUN, ADG, morbidity, and mortality are shown in Tables 1 (Exp. 1) and 2 (Exp. 2). In Exp. 1 heifers categorized as HGlc had lower ($P<0.10$) ADG (mortalities included) compared to heifers categorized as LGlc (2.67 and 1.81 vs 2.60 and 2.51 lb/day or 1.21 and 0.82 vs 1.30 and 1.14 kg/day, respectively), but there was no relationship between Glc and ADG in Exp. 2. This discrepancy in the relationship between ADG and Glc between Exp. 1 and 2 may be related to differences in severity of disease in the heifers, as shown in numerical differences in mortality between the experiments. It is possible that heifers in Exp. 1 were more dehydrated than those in Exp. 2, resulting in elevated Glc and potentially linked to higher mortality, but hematocrit was not measured. In Exp. 2, heifers categorized as LPUN had lower ($P<0.10$) ADG (mortalities included) than heifers grouped as HPUN, but in Exp. 1 the relationship was reversed for PUN and ADG (mortalities removed). The higher levels of PUN may be indicative of muscle catabolism, therefore resulting in a decrease of tissue accretion. In Exp. 1 there was an interaction of Glc and PUN on ADG (mortalities removed; $P=0.05$), resulting in reduced ADG for heifers in the HGlc/HPUN group, but this interaction was not found in Exp. 2.

Heifers in Exp. 1 appeared lethargic, emaciated, and highly stressed upon arrival, while heifers in Exp. 2 appeared less stressed, which may explain differences in results. In both Exp. 1 and 2, the percentage of heifers exhibiting CS of respiratory disease was greater ($P<0.10$) for heifers demonstrating HPUN levels when compared to LPUN-grouped heifers. Orr *et al*[6] observed that calves infected with infectious bovine rhinotracheitis (IBR) virus, which is commonly isolated from animals with BRD, had greater losses of body nitrogen (N) and increased urinary N losses than healthy calves.

Days to first treatment were greater ($P<0.05$) for heifers grouped as HPUN in Exp. 1 compared to heifers categorized as LPUN (7.2 and 7.9 vs 4.7 and 4.5 days, respectively), but no difference ($P=0.51$ and 0.11 in Exp.

Table 1. Plasma urea nitrogen (PUN) and blood glucose (Glc) and their relationships to ADG, morbidity, and mortality in receiving heifers (Exp. 1).

	LGlc[a]/ LPUN	LGlc/ HPUN	HGlc/ LPUN	HGlc/ HPUN	SEM	Probability[i] Glc	PUN	Glc*PUN
No. heifers	52	38	39	50				
ADG, lb[b]	2.60	2.51	2.67	1.81	0.207	0.12	0.02	0.05
ADG, lb[c]	2.27	2.51	1.74	0.90	1.52	0.01	0.45	0.17
CS[d], %	75.0	94.7	56.4	88.0	11.19	0.26	0.02	0.60
First treatment[e], %	51.9	57.9	46.2	60.0	7.54	0.81	0.19	0.60
CSR[f], %	36.5	44.7	38.5	44.0	9.32	0.94	0.46	0.89
Retreatment[g], %	25.0	23.7	28.2	36.0	7.02	0.27	0.65	0.51
Mortality rate, %	1.9	0.0	10.3	6.0	3.09	0.02	0.32	0.71
Days to first treatment[h]	4.7	7.2	4.5	7.9	0.75	0.75	0.01	0.61
Days to retreatment[h]	0.87	1.08	0.98	1.16	0.28	0.75	0.51	0.98

[a]HGlc ≥58 mg/dL (high glucose), LGlc <58 mg/dLl (low glucose), HPUN ≥1.06 m*M* (high plasma urea nitrogen), LPUN<1.06 m*M* (low plasma urea nitrogen).
[b]Mortalities removed for calculation of average daily gain (ADG).
[c]Mortalities included for calculation of average daily gain (ADG).
[d]Heifers exhibiting clinical signs (CS) of bovine respiratory disease (BRD).
[e]Heifers exhibiting clinical signs of BRD plus a rectal temperature ≥103.5°F and treated for BRD with 4.55 mg tilmicosin/lb BW administered subcutaneously.
[f]Heifers exhibiting a relapse of clinical signs (CSR) of BRD.
[g]Heifers exhibiting a relapse of clinical signs more than 48 hours after initial treatment of BRD plus a rectal temperature ≥103.5°F, and retreated for BRD using 5.9 mg/lb BW oxytetracycline and 10 mg/lb BW tylosin.
[h]Days to first treatment = day 0 to first treatment; days to retreatment = days to retreatment after first treatment.
[i]*P*-values for: Glc = glucose effect; PUN = plasma urea nitrogen effect (PUN); and Glc*PUN = glucose and PUN interaction.

1 and 2, respectively) in days to retreatment after first treatment was observed between heifers categorized as HPUN and LPUN. Elsasser *et al*[7] noted that muscle protein catabolism is increased in stress or infection to provide energy or gluconeogenic substrates. Alterations in protein synthesis and degradation can adversely affect tissue repair after injury or infection, potentially leading to prolonged treatment or recurring disease.[3] This was not evident in our study, as there were no differences in first treatments, CSR, or retreatment rates among the four groups of heifers.

In Exp. 1, mortality rate was greater ($P<0.10$) for heifers categorized as HGlc/LPUN and HGlc/HPUN (10.3% and 6.0%) when compared to heifers grouped as LGlc/LPUN and LGlc/HPUN (1.9% and 0%), and no interaction was observed with PUN ($P=0.71$). In Exp. 2, there was no relationship between Glc or PUN and mortality. Heifers in the HGlc category may have not demonstrated obvious CS of a compromised metabolic state, or heifers had a healthy appetite, and feed intake caused blood Glc levels and PUN concentrations to be greater because of an increase in substrates for gluconeogenesis and protein from the diet. Elevated blood Glc levels in HGlc cattle may be attributable to increased glucocorticoid concentrations, which would be consistent with the stresses that occur with weaning and transportation. Glucocorticoids elevate blood Glc levels as a consequence of decreased insulin sensitivity,[9] thereby impairing Glc uptake by cells. A similar response from calves infused with bacterial lipopolysaccharides (LPS), known to model gram-negative infections, resulted in transient hyperglycemia.[8]

In Exp. 2, the percentage of cattle showing CS of BRD was greater ($P<0.05$) for LGlc heifers as well as HPUN heifers. Heifers classified as LGlc had a higher ($P<0.05$) first treatment rate, CSR, and retreatment rate compared to heifers categorized as HGlc. Elevated Glc levels or extremely depressed Glc concentrations may indicate the animals are compromised. In Exp. 1 and 2, heifers with HPUN levels had a greater percentage demonstrating CS ($P=0.02$), and heifers with HPUN in Exp. 2 also had a greater percentage of first treatments. Heifers with elevated PUN may have exhibited CS more frequently due to the increased muscle protein breakdown. However, heifers categorized as HPUN had greater days to first treatment ($P<0.05$) than heifers grouped as LPUN. Although percentage of heifers demonstrating CS were similar between Exp. 1 and 2,

Table 2. Plasma urea nitrogen (PUN) and blood glucose (Glc) and their relationships to ADG, morbidity, and mortality in receiving heifers (Exp. 2).

	LGlc[a]/ LPUN	LGlc/ HPUN	HGlc/ LPUN	HGlc/ HPUN	SEM	Probability[i]		
						Glc	PUN	Glc*PUN
No. heifers	85	85	95	69				
ADG, lb/d[b]	2.67	2.67	2.40	2.65	0.242	0.20	0.26	0.30
ADG, lb/d[c]	2.47	2.67	2.23	2.65	0.174	0.45	0.08	0.55
CS[d], %	81.3	88.6	68.7	80.3	4.42	0.02	0.03	0.63
First treatment[e], %	71.1	79.7	57.6	71.1	5.02	0.02	0.02	0.62
CSR[f], %	45.0	49.4	27.3	36.8	5.30	0.01	0.19	0.62
Retreatment[g], %	45.0	49.4	27.3	36.8	5.30	0.01	0.19	0.62
Mortality rate, %	1.3	0.0	1.0	0.0	0.85	0.88	0.19	0.88
Days to first treatment[h]	4.2	5.2	4.5	5.4	0.50	0.63	0.05	0.97
Days to retreatment[h]	2.0	2.4	1.4	2.1	0.34	0.22	0.11	0.61

[a]HGlc ≥56 mg/dL (high glucose); LGlc <56 mg/dL (low glucose), HPUN ≥1.45 m*M* (high plasma urea nitrogen) or; LPUN <1.45 m*M* (low plasma urea nitrogen).
[b]Mortalities removed for calculation of average daily gain (ADG).
[c]Mortalities included for calculation of average daily gain (ADG).
[d]Heifers exhibiting clinical signs (CS) of bovine respiratory disease (BRD).
[e]Heifers exhibiting clinical signs of BRD plus a rectal temperature ≥103.5°F and treated for BRD with 4.55 mg tilmicosin/lb BW administered subcutaneously.
[f]Heifers exhibiting a relapse of clinical signs (CSR) of BRD.
[g]Heifers exhibiting a relapse of clinical signs more than 48 hours after initial treatment of BRD plus a rectal temperature ≥103.5°F and retreated for BRD using 5.9 mg/lb BW oxytetracycline and 10 mg/lb BW tylosin.
[h]Days to first treatment = day 0 to first treatment; days to retreatment = days to retreatment after first treatment.
[i]*P*-values for: Glc = glucose effect; PUN = plasma urea nitrogen effect (PUN); and Glc*PUN = glucose and PUN interaction.

mortality rate for Exp. 2 was not different among groups of heifers.

There were apparent differences in the severity of stress between heifers in Exp. 1 and 2. The range for blood Glc was lower in Exp. 2 (20 to 111 mg/dL) compared to Exp. 1 (42 to 137 mg/dL), which may either be indicative of the higher stress levels for cattle used in Exp. 1 or simply be due to increased feed intake. The greater mortality rate for Exp. 1 may suggest that cattle were morbid but did not have sufficiently elevated body temperatures to qualify for treatment. The development of hypothermia is a paradoxical response to extreme endotoxemia. For example, rats given a lethal dose of endotoxin (10 µg, 1 µg, or 0.1 µg/g BW) had significantly depressed colonic temperatures compared to controls, and rats given non-lethal doses of endotoxin (0.01 µg, 0.001 µg, or 0.0001 µg/g BW) had elevated colonic temperatures compared to that of the control.[5] Thus, it is possible that more severely stressed heifers from Exp. 1 had greater numbers with CS of BRD, but did not receive treatment because rectal temperatures did not reach 103.5°F. The incidence of first treatments and retreatments was greater in Exp. 2, which is potentially a function of higher rectal temperatures in these animals rather than differences in CS. For Exp. 1 and 2, heifers with LPUN had less frequent CS. A relationship between Glc and PUN was not observed in the present study; only one instance was observed to have a significant interaction.

Plasma Glc was regressed (Figure 1) on blood Glc concentrations for validation of the hand-held Glc monitor. There was a positive linear relationship ($P<0.09$) with a coefficient of determination of 0.50. Blood packed cell volumes were not measured, so it is impossible to determine whether state of dehydration affected Glc readings. Heifers were allowed 12 to 24 hours to rest and rehydrate, however, heifers which may have already been morbid may not have fully recovered from transport stress. The monitor is incapable of measuring blood Glc values below 20 mg/dL. Determining blood Glc by means of the Glc monitor should be further evaluated, and should potentially include a correction for extent of dehydration.

Conclusions

These studies were conducted on, presumably, high-stress heifers, but differences in mortality levels

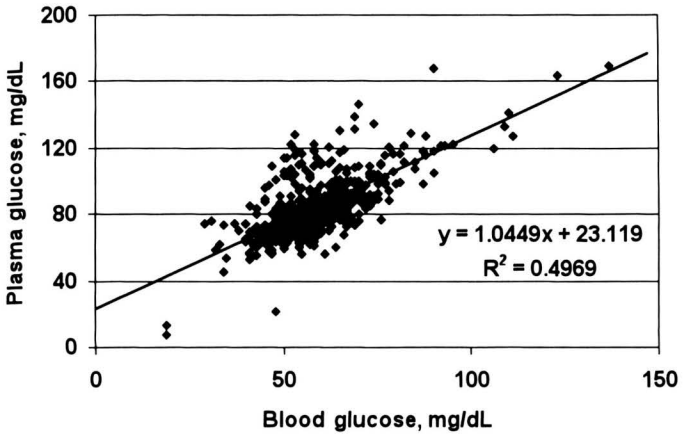

Figure 1. Relationship between blood glucose, measured using a hand-held glucose monitor, and plasma glucose, measured via a colorimetric assay (Experiments 1 and 2).

between studies suggests the level of stress, level of natural disease challenge, or both, between experiments was not equivalent. Elevated PUN was associated with increased incidence of clinical disease in both experiments. Elevated blood Glc was associated with reduced performance and greater mortality, but in only the first experiment. Elevated PUN measured upon arrival may indicate the presence of a catabolic state in long-haul, high-stress feedlot cattle and may, therefore, be an indicator either of existing disease or of susceptibility to disease, but further research is needed to determine a more precise PUN value which could be applied to all classes of cattle.

Acknowledgement

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Endnotes

[a]Ralgro, Intervet/Schering Plough Animal Health, DeSoto, KS

[b]Bovishield 4, Pfizer Animal Health, New York, NY

[c]Ultrabac 7, Pfizer Animal Health, New York, NY

[d]Ivomec, Merial Ltd, Duluth, GA

[e]Micotil, Elanco Animal Health, Greenfield, IN

[f]Glucometer Elite, Bayer Corp, Mishawaka, IN

[g]No. 315 glucose (Trinder), Sigma Diagnostics, St. Louis, MO

[h]SAS (Windows Release 6.12), SAS Inst. Inc., Cary, NC

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1. T.C. Bryant, et al. Effect of Viral Respiratory Vaccine Treatment on Performance, Health and Carcass Traits of Auction-origin Feeder Steers. *The Bovine Practitioner*, Vol. 42, No. 1, Spring 2008, p. 98-103.
2. Calves vaccinated under 6 months of age should be revaccinated at 6 months of age.

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